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(54) Title: ANGPTL8-BINDING AGENTS AND METHODS OF USE THEREOF

FIG. 1A

Heavy chain variable region sequence alignments

Kabat	1	10	22	31--35	40	50--a-----60---65
AbM	1	10	22	26-----35	40	50--a----58 65
Chothia	1	10	22	26-----32	40	a-55 65
Contact	1	10	22	30---35	40	47-----a----58 65
IMGT	1		23	27-----38	41	56-----65 74
AHon	1		23	27	42	57 76

1E5	QVQLQQSGAELVKPGTSVRLSCKAS	GYFTTDYTIH	WVKLRLSGQGLEWIG	WFYPGSDNIKYNNAKFKD
1E9	QVQLQQSGTELVKPGASVVLSCCKAS	GYFTTDYTIH	WVKQRLSGQGLEWIG	WFYPGSDNIKFNNAKFRD
1A8	QVQLQQSGAELVKPGASVVLSCCKAS	GYFTTDYTIH	WVKQRLSGQGLEWIG	WFYPGSDNIKYNNEKFKD
Consensus		GYFTTDYTIH	WFYPGSDNIKXNXKFXD	
	(SEQ ID NO:11)		(SEQ ID NO:58)	

Kabat	70	80	abc	90	95-----102	110
AbM	70	80	abc	90	95-----102	110
Chothia	70	80	abc	90	96-----101	110
Contact	70	80	abc	90	93-----101	110
IMGT	75			89	105-----117	
AHon					109	138

1E5	KATLTADKSSSTVYMDLGRLTSEDSAVYFCAR	HEAFSYYDVAWFAY	WGQGTLVTVSA	(SEQ ID NO:35)
1E9	KATLTADKSSSTVYMELSLRTSEDSAVYFCAR	HEAFYVYDVAWFAN	WGQGTLVTVST	(SEQ ID NO:44)
1A8	KATLTADKSSSIVYMELSLRTSEDSAVYFCAR	HEAYYYDVAWFAY	WGQGTLVTVSA	(SEQ ID NO:56)
Consensus		HEAXXXYDVAWFAX		
		(SEQ ID NO:59)		

(57) Abstract: The present disclosure provides binding agents, such as antibodies, that specifically bind angiopoietin-like protein 8 (ANGPTL8), including human ANGPTL8, and methods of their use.

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[Continued on next page]

TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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ANGPTL8-BINDING AGENTS AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of priority to U.S. Serial No. 62/584,637 filed November 10, 2017, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present disclosure generally relates to agents that bind angiopoietin-like protein 8 (ANGPTL8), particularly antibodies that bind human ANGPTL8, as well as therapeutic methods of using the agents.

BACKGROUND

[0003] Angiopoietin-like protein 8 (ANGPTL8) is an ANGPTL family member that has been implicated in both triglyceride and glucose metabolism. ANGPTL8 is also referred to in the literature as betatrophin, lipasin, TD26, RIFL (refeeding-induced fat and liver protein), and C19orf80. ANGPTL8 is produced by the liver and in white adipose tissue and circulates in the blood. ANGPTL8 is considered a mediator of post-prandial trafficking of fatty acids to adipose tissue and a regulator of postprandial glucose metabolism, but its mechanism of action remains unclear. Overexpression of ANGPTL8 has been associated with elevated triglyceride levels. (See, e.g., Santulli G, 2014, *Frontiers in Endocrinology*, 5:1-6; Zhang R and Abou-Samra AB, 2014, *Cardiovascular Diabetology*, 13:133).

[0004] Elevated blood triglyceride levels and hypertriglyceridemia are significant contributing factors to cardiovascular diseases. Elevated triglyceride levels can also be a sign of, and contributing factor to, conditions that increase a person's overall risk of heart disease, these conditions may include obesity, metabolic syndrome, excess body fat, high blood pressure, high blood sugar, and abnormal cholesterol levels. In addition, high triglyceride levels also contribute to decreased health status in subjects with poorly controlled type 2 diabetes, hypothyroidism, liver disease, kidney disease, and genetic metabolic disorders. Further complicating treatment for associated conditions, elevated triglyceride levels are a common side effect of routinely prescribed medications such as beta blockers, birth control, diuretics, steroids, and certain cancer treatments, such as tamoxifen.

[0005] Although there are therapeutics available that may reduce elevated triglyceride levels in a subject, there is a need for new agents that effectively reduce elevated triglyceride levels. In addition, there is a need for agents that may be used in methods of treatment for a variety of diseases and disorders associated with elevated triglycerides levels, high LDL-cholesterol levels, and/or low HDL-cholesterol levels.

BRIEF SUMMARY

[0006] The present disclosure provides agents that bind to and/or interact with ANGPTL8. The agents may include, but are not limited to, polypeptides such as antibodies that specifically bind ANGPTL8. Such agents may bind ANGPTL8, an ANGPTL8 fragment, an ANGPTL8 peptide, and/or an ANGPTL8 epitope. These agents may be referred to herein as “ANGPTL8-binding agents.” In some embodiments, the agent is an ANGPTL8 antagonist. In some embodiments, the agent is an ANGPTL8 agonist. The disclosure provides methods of using the agents. In some embodiments, the agents are used for lowering triglyceride levels. In some embodiments, the agents are used for treating a disease or disorder associated with elevated triglyceride levels. In some embodiments, the disease or disorder includes, but is not limited to, hypertriglyceridemia, metabolic syndrome, atherosclerosis, obesity, diabetes, hypothyroidism, acute pancreatitis, liver disease, or kidney disease. In some embodiments, the agents are used for increasing HDL-cholesterol levels. In some embodiments, the agents are used for lowering LDL-cholesterol levels. In some embodiments, the agents are used in combination with at least one additional therapeutic agent.

[0007] The disclosure also provides compositions, such as pharmaceutical compositions, comprising the agents described herein. Polynucleotides and/or vectors encoding the agents and methods of making the agents are also provided. Cells comprising or producing the agents described herein are provided as well as cells comprising the polynucleotides and/or the vectors described herein.

[0008] In one aspect, the present disclosure provides agents that bind ANGPTL8. In some embodiments, the agent binds human ANGPTL8. In some embodiments, the agent binds cynomolgus monkey (“cyno”) ANGPTL8. In some embodiments, the agent binds human ANGPTL8 and cyno ANGPTL8. In some embodiments, the agent is an antibody. In some embodiments, the agent is an antibody that binds human ANGPTL8. In some embodiments, the agent is an antibody that binds cyno ANGPTL8. In some embodiments, the agent is an antibody that binds human ANGPTL8 and cyno ANGPTL8. In some embodiments, the agent is an antibody that binds human ANGPTL8 and does not bind mouse ANGPTL8.

[0009] In some embodiments, an antibody or antigen-binding fragment thereof specifically binds an N-terminal region of human ANGPTL8, wherein the N-terminal region comprises amino acids 25-60 of SEQ ID NO:1 and wherein the antibody (i) lowers triglyceride levels, (ii) lowers LDL-cholesterol, and/or (iii) increases HDL-cholesterol in a subject. In some embodiments, an antibody or antigen-binding fragment thereof specifically binds human ANGPTL8 and comprises: (a) a heavy chain CDR1 comprising GYTFTDVTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y

or V, and X₄ is Y or N (SEQ ID NO:59); and/or (b) a light chain CDR1 comprising X₁SSQSLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61). In some embodiments, the antibody or antigen-binding fragment thereof comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:16, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:21, 22, 23, or 24, and (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 28, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:32, 33, or 34. In some embodiments, the antibody or antigen-binding fragment thereof comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69, and (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, the antibody or antigen-binding fragment thereof comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, or 51, and (b) a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, the antibody or antigen-binding fragment thereof further comprises (a) a heavy chain FR1, a heavy chain FR2, a heavy chain FR3, and a heavy chain FR4; and/or (b) a light chain FR1, a light chain FR2, a light chain FR3, and a light chain FR4.

[0010] In some embodiments, an antibody or antigen-binding fragment thereof, comprises: (a) a heavy chain variable region having at least 90% sequence identity to SEQ ID NOs:35, 44, 56, or 62; and/or (b) a light chain variable region having at least 90% sequence identity to SEQ ID NOs:36, 45, 57, or 63. In some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:35 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:36. In some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:44 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:45. In some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:56 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:57. In some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:62 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:63. In

some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising SEQ ID NO:35, and a light chain variable region comprising SEQ ID NO:36. In some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45. In some embodiments, an antibody or antigen-binding fragment thereof, comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57. In some embodiments, an antibody or antigen-binding fragment thereof of comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63.

[0011] In some embodiments, an antibody or antigen-binding fragment thereof that specifically binds ANGPTL8 comprises the heavy chain CDR1, CDR2, and CDR3, and/or the light chain CDR1, CDR2, and CDR3 from: (a) the antibody designated 1E5 that comprises a heavy chain variable region comprising SEQ ID NO:35 and a light chain variable region comprising SEQ ID NO:36; (b) the antibody designated Hz1E5 that comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63; (c) the antibody designated 1E9 that comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45; or (d) the antibody designated 1A8 that comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57. In some embodiments, the antibody or antigen-binding fragment thereof, comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:35 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:36. In some embodiments, the antibody or antigen-binding fragment thereof, comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:62 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:63. In some embodiments, the antibody or antigen-binding fragment thereof, comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:44 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:45. In some embodiments, the antibody or antigen-binding fragment thereof, comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:56 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:57.

[0012] In some embodiments, an antibody or antigen-binding fragment thereof that specifically binds ANGPTL8 comprises: (a) a heavy chain variable region comprising a CDR1, a CDR2, and a CDR3 amino acid sequence depicted in Tables 1-3; and/or (b) a light chain variable region comprising a CDR1, a CDR2, and a CDR3 amino acid sequence depicted in Tables 1-3.

[0013] In some embodiments, an antibody that specifically binds ANGPTL8 comprises a heavy chain of SEQ ID NO:64 and/or a light chain of SEQ ID NO:65.

[0014] In another aspect of the disclosure, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises: (a) a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y or V, and X₄ is Y or N (SEQ ID NO:59); and/or (b) a light chain CDR1 comprising X₁SSQSLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61). In some embodiments, an antibody or antigen-binding fragment thereof binds an epitope on ANGPTL8 recognized by a reference antibody, wherein the reference antibody comprises: (a) a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y or V, and X₄ is Y or N (SEQ ID NO:59); and/or (b) a light chain CDR1 comprising X₁SSQSLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61). In some embodiments, the reference antibody further comprises (a) a heavy chain variable region FR1, a heavy chain variable region FR2, a heavy chain variable region FR3, and a heavy chain variable region FR4, and (b) a light chain variable region FR1, a light chain variable region FR2, a light chain variable region FR3, and a light chain variable region FR4.

[0015] In some embodiments, the reference antibody comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:16, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:21, 22, 23, or 24, and (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 28, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:32, 33, or 34. In some embodiments, the reference antibody comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69, and (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, the reference antibody comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, or 51, and (b) a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, the reference

antibody further comprises (a) a heavy chain variable region FR1, a heavy chain variable region FR2, a heavy chain variable region FR3, and a heavy chain variable region FR4, and (b) a light chain variable region FR1, a light chain variable region FR2, a light chain variable region FR3, and a light chain variable region FR4.

[0016] In some embodiments, the reference antibody comprises a heavy chain variable region comprising SEQ ID NOS:25, 44, 56, or 62; and a light chain variable region comprising SEQ ID NOS:26, 45, 57, or 63. In some embodiments, the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:25 and a light chain variable region comprising SEQ ID NO:26. In some embodiments, the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45. In some embodiments, the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57. In some embodiments, the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63.

[0017] In some embodiments, the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:3. In some embodiments, the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:6. In some embodiments, the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:7. In some embodiments, the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:8.

[0018] In some embodiments of each of the aforementioned aspects and embodiments, as well as other aspects and embodiments described herein, the antibody is a monoclonal antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In some embodiments, the antibody is a human chimeric antibody. In some embodiments, the antibody or antibody fragment thereof is a Fab, Fab', F(ab')₂, Fv, scFv, (scFv)₂, single chain antibody, dual variable region antibody, single variable region antibody, linear antibody, a V region, a bispecific antibody, or a multispecific antibody.

[0019] In some embodiments of each of the aforementioned aspects and embodiments, as well as other aspects and embodiments described herein, an antibody or antigen-binding fragment thereof described herein is conjugated to a detectable marker. In some embodiments, the detectable marker is selected from a radioisotope, a metal chelator, an enzyme, a fluorescent compound, a bioluminescent compound, and a chemiluminescent compound.

[0020] In some embodiments, an antibody or antigen-binding fragment thereof described herein is conjugated to a cytotoxic agent.

[0021] In some embodiments of each of the aforementioned aspects and embodiments, as well as other aspects and embodiments described herein, an antibody or antigen-binding fragment thereof described herein is an antagonist of ANGPTL8. In some embodiments, the antibody or antigen-binding fragment thereof reduces or decreases ANGPTL8 levels. In some embodiments, the antibody or antigen-binding fragment thereof inhibits ANGPTL8 activity. In some embodiments, the antibody or antigen-binding fragment thereof modulates lipoprotein lipase (LPL) activity. In some embodiments, the antibody or antigen-binding fragment thereof increases or enhances LPL activity. In some embodiments, the antibody or antigen-binding fragment thereof lowers or reduces triglyceride levels. In some embodiments, the antibody or antigen-binding fragment thereof lowers or reduces LDL-cholesterol levels. In some embodiments, the antibody or antigen-binding fragment thereof increases HDL-cholesterol levels. In some embodiments, the antibody or antigen-binding fragment thereof lowers triglyceride levels and increases HDL-cholesterol levels.

[0022] In some embodiments of each of the aforementioned aspects and embodiments, as well as other aspects and embodiments described herein, an agent described herein specifically binds ANGPTL8 and (i) lowers triglyceride levels in a subject; (ii) increases HDL-cholesterol levels in a subject; and/or (iii) lowers LDL-cholesterol levels in a subject.

[0023] In another aspect, the disclosure provides compositions comprising an ANGPTL8-binding agent (e.g., an antibody or antigen-binding fragment thereof) described herein. Methods of using a composition comprising an ANGPTL8-binding agent (e.g., an antibody or antigen-binding fragment thereof) described herein are also provided.

[0024] In another aspect, the disclosure provides pharmaceutical compositions comprising an ANGPTL8-binding agent (e.g., an antibody or antigen-binding fragment thereof) described herein and a pharmaceutically acceptable carrier.

[0025] In some embodiments of each of the aforementioned aspects, as well as other aspects and/or embodiments described elsewhere herein, the ANGPTL8-binding agent (e.g., an antibody or antigen-binding fragment thereof) is isolated. In some embodiments, the ANGPTL8-binding agent (e.g., an antibody or antigen-binding fragment thereof) is substantially pure.

[0026] In another aspect, the disclosure provides polynucleotides comprising a polynucleotide that encodes an ANGPTL8-binding agent described herein. In some embodiments, the polynucleotide is isolated. In some embodiments, a vector comprises a polynucleotide that encodes an ANGPTL8-binding agent described herein. In some embodiments, an isolated cell comprises a polynucleotide that encodes an ANGPTL8-binding agent described herein. In some embodiments, an isolated cell comprises a vector comprising a polynucleotide that encodes an ANGPTL8-binding agent described herein. In some embodiments, the disclosure also provides cells comprising or producing an ANGPTL8-binding agent

described herein. In some embodiments, the cell is a monoclonal cell line. In some embodiments, the cell is a hybridoma. In some embodiments, the cell produces an anti-ANGPTL8 antibody described herein. In some embodiments, a transgenic animal produces an ANGPTL8-binding agent described herein.

[0027] In another aspect, the disclosure provides a binding agent that binds to essentially the same epitope on ANGPTL8 as an antibody described herein. In some embodiments, the binding agent is a heterodimeric molecule comprising an anti-ANGPTL8 antibody or antigen-binding fragment thereof described herein. In some embodiments, the binding agent comprises a scaffold protein comprising one or more of the CDRs shown in Tables 1-3. In some embodiments, the binding agent competes with one or more of the anti-ANGPTL8 antibodies described herein for binding to ANGPTL8 in a competitive binding assay.

[0028] In another aspect, the disclosure provides methods of using the ANGPTL8-binding agents (e.g., an antibody or antigen-binding fragment thereof) described herein. In some embodiments, a method of lowering triglyceride levels in a subject with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein. In some embodiments, a method of treating a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein. In some embodiments, the disease or disorder is metabolic syndrome, obesity, diabetes, atherosclerosis, cardiovascular disease, hyperlipidemia, hypertriglyceridemia, pancreatitis, renal disease, liver disease, or hypothyroidism. In some embodiments, the elevated triglyceride levels are elevated plasma triglyceride levels. Those of skill in the art generally consider a “normal” plasma triglyceride level to be less than 150 mg/dL. In some embodiments, the elevated plasma triglyceride level is 150 mg/dL or higher. In some embodiments, the elevated plasma triglyceride level is about 150 to about 200 mg/dL. In some embodiments, the elevated plasma triglyceride level is about 200 to about 500 mg/dL. In some embodiments, the elevated plasma triglyceride level is about 500 mg/dL or higher. In some embodiments, after administration of the ANGPTL8-binding agent to the subject, the elevated triglyceride level is reduced by at least 5%, by at least 10%, by about 10-15%, by about 10-20%, by about 20-30%, or by more than 25% as compared to triglyceride levels in the subject prior to the administration of the agent.

[0029] In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein. In some embodiments, a method of reducing LDL-cholesterol levels in a subject comprises administering to the subject a therapeutically

effective amount of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein.

[0030] In some embodiments of the methods described herein, the subject has metabolic syndrome, has been diagnosed with metabolic syndrome, has symptoms of metabolic syndrome, or is at risk for developing metabolic syndrome. In some embodiments, the subject has hyperlipidemia or dyslipidemia, has been diagnosed with hyperlipidemia or dyslipidemia, has symptoms of hyperlipidemia or dyslipidemia, or is at risk for developing hyperlipidemia or dyslipidemia. In some embodiments, the subject has cardiovascular disease, has been diagnosed with cardiovascular disease, has symptoms of cardiovascular disease, or is at risk for developing cardiovascular disease. In some embodiments, the subject has diabetes (e.g., type 2 diabetes), has been diagnosed with diabetes (e.g., type 2 diabetes), has symptoms of diabetes (e.g., type 2 diabetes), or is at risk for developing diabetes (e.g., type 2 diabetes). In some embodiments, the subject is overweight or obese or is at risk of becoming overweight or obese.

[0031] In some embodiments of the methods described herein, the subject is administered one or more additional therapeutic agents. In some embodiments, the additional therapeutic agent(s) is selected from the group consisting of: a fibrate, a statin, omega-3 fatty acids, and niacin.

[0032] Also disclosed herein is the use of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein in the manufacture of a medicament for lowering triglyceride levels. The use of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein in the manufacture of a medicament for treatment of a disease or disorder associated with elevated triglyceride levels. The use of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein in the manufacture of a medicament for increasing HDL-cholesterol levels. The use of an ANGPTL8-binding agent (e.g., antibody or antigen-binding fragment thereof) described herein in the manufacture of a medicament for lowering LDL-cholesterol levels.

[0033] In some embodiments of each of the aforementioned aspects and embodiments, as well as other aspects and embodiments described herein, the subject is human.

[0034] Where aspects or embodiments of the disclosure are described in terms of a Markush group or other grouping of alternatives, the present disclosure encompasses not only the entire group listed as a whole, but also each member of the group individually and all possible subgroups of the main group, and also the main group absent one or more of the group members. The present disclosure also envisages the explicit exclusion of one or more of any of the group members in the claimed disclosure.

BRIEF DESCRIPTION OF THE FIGURES

[0035] Figs. 1A-1B. Amino acid sequence alignments for exemplary anti-ANGPTL8 antibodies. Fig. 1A. Heavy chain variable region sequence alignment with consensus CDR sequences. Fig. 1B. Light chain variable region sequence alignment with consensus CDR sequences.

[0036] Figs. 2A-2B. Fig. 2A. Representative diagrams of the human ANGPTL8 deletion variants V2-V5. Fig. 2B. Plasma triglyceride levels in mice injected with rAAVs expressing human ANGPTL8, or one of variants V2, V3, V4, and V5. Control animals were injected with a control rAAV.

[0037] Fig. 3. Plasma triglyceride levels in mice injected with a rAAV expressing human ANGPTL8 and treated with anti-hANGPTL8 antibodies. Control animals were (i) injected with rAAV expressing hANGPTL8 and no antibody treatment; (ii) injected with rAAV expressing hANGPTL8 and treated with a control anti-KLH antibody; or (iii) injected with a rAAV expressing GFP.

[0038] Fig. 4. Binding affinity measurement of 1E5 antibody to NusA-hANGPTL8 fusion protein.

[0039] Fig. 5A-5B. Fig. 5A. Binding affinity measurements of chimeric 1E5 antibody and humanized antibody Hz1E5 to human SE1 peptide. Fig. 5B. Plasma triglyceride levels in mice injected with a rAAV expressing human ANGPTL8 and treated with anti-hANGPTL8 antibodies (i) parental 1E5 from hybridoma, (ii) chimeric antibody 1E5, or (iii) humanized antibody Hz1E5. Control animals were (i) injected with a rAAV expressing hANGPTL8 and treated with a control anti-KLH antibody, or (ii) injected with a rAAV expressing GFP.

[0040] Fig. 6A-6B. Fig. 6A. Plasma triglyceride levels in cynomolgus monkeys treated with anti-hANGPTL8 antibody Hz1E5 or a vehicle control. Fig. 6B. Plasma HDL-cholesterol levels in cynomolgus monkeys treated with anti-hANGPTL8 antibody Hz1E5 or a vehicle control.

DETAILED DESCRIPTION

[0041] The present disclosure provides novel agents, including but not limited to polypeptides such as antibodies, that bind ANGPTL8. The ANGPTL8-binding agents include, but are not limited to, polypeptides, antibodies, scaffold proteins, and heterodimeric molecules. ANGPTL8-binding agents include, but are not limited to, antagonists of ANGPTL8 activity and/or agents that modulate ANGPTL8 activity. Related polypeptides, polynucleotides, compositions comprising the agents, and methods of making the agents are also provided. Methods of using the novel agents, such as methods of treating an ANGPTL8-related disorder or disease are provided. Methods of lowering elevated triglyceride levels, of increasing HDL-cholesterol levels, and/or lowering LDL-cholesterol levels are also provided.

I. Definitions

[0042] Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[0043] The term “binding agent” as used herein refers to a molecule which binds a specific antigen or target (e.g., ANGPTL8). A binding agent may comprise a protein, peptide, nucleic acid, carbohydrate, lipid, or small molecular weight compound. In some embodiments, a binding agent comprises an antibody or an antigen-binding fragment thereof. In some embodiments, a binding agent is an antibody or an antigen-binding fragment thereof. In some embodiments, a binding protein comprises an alternative protein scaffold or artificial scaffold and an antigen-binding site comprising CDRs or CDR derivatives. In some embodiments, a binding protein is a fusion protein comprising an antigen-binding site. In some embodiments, a binding protein is a bispecific or multispecific molecule comprising at least one antigen-binding site.

[0044] The term “antibody” as used herein refers to an immunoglobulin molecule that recognizes and binds a target through at least one antigen-binding site. “Antibody” is used herein in the broadest sense and encompasses various antibody structures, including but not limited to, polyclonal antibodies, recombinant antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, human antibodies, bispecific antibodies, multispecific antibodies, diabodies, tribodies, tetrabodies, single chain Fv (scFv) antibodies, and antibody fragments as long as they exhibit the desired antigen-binding activity.

[0045] The term “intact antibody” or “full-length antibody” refers to an antibody having a structure substantially similar to a native antibody structure. This includes an antibody comprising two light chains each comprising a variable region and a light chain constant region (CL) and two heavy chains each comprising a variable region and at least heavy chain constant regions CH1, CH2, and CH3.

[0046] The term “antibody fragment” as used herein refers to a molecule other than an intact antibody that comprises a portion of an antibody and generally an antigen-binding site. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')2, Fv, disulfide-linked Fv (sdFv), Fd, linear antibodies, single chain antibody molecules (e.g., scFv), diabodies, tribodies, tetrabodies, minibodies, dual variable domain antibodies (DVD), single variable domain antibodies, and multispecific antibodies formed from antibody fragments.

[0047] The term “variable region” as used herein refers to the region of an antibody light chain or the region of an antibody heavy chain that is involved in binding the antibody to antigen. The variable region of an antibody heavy chain and an antibody light chain have similar structures, and generally comprise

four framework regions and three complementarity determining regions (CDRs) (also known as hypervariable regions).

[0048] The term “framework region” refers to amino acid residues other than the CDR residues within a variable region. The framework region (FR) generally comprises four domains, FR1, FR2, FR3, and FR4.

[0049] The term “monoclonal antibody” as used herein refers to a substantially homogenous antibody population involved in the highly specific recognition and binding of a single antigenic determinant or epitope. The individual antibodies comprising the population are identical, except for possible naturally occurring mutations that may be present in minor amounts. The term “monoclonal antibody” encompasses intact and full-length monoclonal antibodies as well as antibody fragments (e.g., Fab, Fab', F(ab')₂, Fv), single chain (scFv) antibodies, fusion proteins comprising an antibody fragment, and any other modified immunoglobulin molecule comprising an antigen-binding site. Furthermore, “monoclonal antibody” refers to such antibodies made by any number of techniques, including but not limited to, hybridoma production, phage library display, recombinant expression, and transgenic animals.

[0050] The term “chimeric antibody” refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0051] The term “humanized antibody” as used herein refers to a chimeric antibody that includes human immunoglobulins (e.g., recipient antibody) in which the native CDR residues are replaced by residues from corresponding CDRs from a nonhuman species (e.g., donor antibody) such as mouse, rat, rabbit, or nonhuman primate, wherein the donor antibody has the desired specificity, affinity, and/or activity. In some instances, one or more framework region residues of the human immunoglobulin are replaced by corresponding nonhuman residues. Furthermore, humanized antibodies can comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine and/or optimize antibody characteristics. A humanized antibody may comprise variable regions containing all or substantially all of the CDRs that correspond to those of a nonhuman immunoglobulin and all or substantially all of the framework regions that correspond to those of a human immunoglobulin. In some embodiments, the humanized antibody will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin.

[0052] The term “human antibody” as used herein refers to an antibody that possesses an amino acid sequence that corresponds to an antibody produced by a human and/or an antibody that has been made using any of the techniques that are known to those of skill in the art for making human antibodies. These techniques include, but not limited to, phage display libraries, yeast display libraries, transgenic animals, and B-cell hybridoma technology.

[0053] The terms “epitope” and “antigenic determinant” are used interchangeably herein and refer to that portion of an antigen or target capable of being recognized and bound by a particular antibody. When the antigen or target is a polypeptide, epitopes can be formed both from contiguous amino acids and noncontiguous amino acids juxtaposed by tertiary folding of the protein. Epitopes formed from contiguous amino acids (also referred to as linear epitopes) are typically retained upon protein denaturing, whereas epitopes formed by tertiary folding (also referred to as conformational epitopes) are typically lost upon protein denaturing. An epitope typically includes at least 3, and more usually, at least 5, 6, 7, or 8-10 amino acids in a unique spatial conformation. In some cases, X-ray crystallography is used to predict potential epitopes on a target protein. In some cases, X-ray crystallography is used to characterize an epitope on a target protein by analyzing the amino acid residue interactions of an antigen/antibody complex.

[0054] The term “specifically binds” as used herein refers to an agent (e.g., an antibody) that interacts more frequently, more rapidly, with greater duration, with greater affinity, or with some combination of the above to a particular antigen, epitope, protein, or target molecule than with alternative substances. In some embodiments, an agent (e.g., an antibody) that specifically binds an antigen (e.g., human ANGPTL8) may bind related antigens (e.g., cyno ANGPTL8). An antibody that specifically binds an antigen can be identified, for example, by immunoassays, ELISAs, BIACORE assays, or other techniques known to those of skill in the art.

[0055] The terms “polypeptide” and “peptide” and “protein” are used interchangeably herein and refer to polymers of amino acids of any length. The polymer may be linear or branched, it may comprise modified amino acids, and it may be interrupted by non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification. Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid, including but not limited to, unnatural amino acids, as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure may be based upon antibodies, the term “polypeptide” encompasses polypeptides as a single chain and polypeptides of two or more associated chains.

[0056] The terms “polynucleotide” and “nucleic acid” and “nucleic acid molecule” are used interchangeably herein and refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase.

[0057] The terms “identical” or percent “identity” in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified

percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity may be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that may be used to obtain alignments of amino acid or nucleotide sequences are well-known in the art. These include, but are not limited to, BLAST, ALIGN, Megalign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two nucleic acids or polypeptides of the disclosure are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, and in some embodiments at least 95%, 96%, 97%, 98%, 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the sequences that is at least about 10, at least about 20, at least about 40-60 nucleotides or amino acid residues, at least about 60-80 nucleotides or amino acid residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 nucleotides or amino acid residues, such as at least about 80-100 nucleotides or amino acid residues, and in some embodiments the sequences are substantially identical over the full length of the sequences being compared, for example, (i) the coding region of a nucleotide sequence or (ii) an amino acid sequence.

[0058] The phrase “conservative amino acid substitution” as used herein refers to a substitution in which one amino acid residue is replaced with another amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is considered to be a conservative substitution. Generally, conservative substitutions in the sequences of polypeptides and/or antibodies do not abrogate the binding of the polypeptide or antibody to the target binding site. Methods of identifying nucleotide and amino acid conservative substitutions that do not eliminate binding are well-known in the art.

[0059] The term “vector” as used herein means a construct, which is capable of delivering, and usually expressing, one or more gene(s) or sequence(s) of interest in a host cell. Examples of vectors include, but are not limited to, viral vectors, naked DNA or RNA expression vectors, plasmid, cosmid, or phage vectors, DNA or RNA expression vectors associated with cationic condensing agents, and DNA or RNA expression vectors encapsulated in liposomes.

[0060] The term “isolated” as used herein refers to a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition which is in a form not found in nature. An “isolated” antibody is substantially free of material from the cellular source from which it is derived. In some embodiments, isolated polypeptides, soluble proteins, antibodies, polynucleotides, vectors, cells, or compositions are those which have been purified to a degree that they are no longer in a form in which they are found in nature. In some embodiments, a polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition which is isolated is substantially pure. A polypeptide, soluble protein, antibody, polynucleotide, vector, cell, or composition may be isolated from a natural source or from a source such as an engineered cell line.

[0061] The term “substantially pure” as used herein refers to material which is at least 50% pure (i.e., free from contaminants), at least 90% pure, at least 95% pure, at least 98% pure, or at least 99% pure.

[0062] The term “subject” refers to any animal (e.g., a mammal), including, but not limited to, humans, non-human primates, canines, felines, rabbits, rodents, and the like, which is to be the recipient of a treatment or therapy. Generally, the terms “subject” and “patient” are used interchangeably herein in reference to a human subject.

[0063] The term “pharmaceutically acceptable” as used herein refers to a substance approved or approvable by a regulatory agency or listed in the U.S. Pharmacopeia, European Pharmacopeia, or other generally recognized pharmacopeia for use in animals, including humans.

[0064] The terms “pharmaceutically acceptable excipient, carrier, or adjuvant” or “acceptable pharmaceutical carrier” as used herein refer to an excipient, carrier, or adjuvant that can be administered to a subject, together with at least one therapeutic agent (e.g., an antibody), and which does not destroy the pharmacological activity of the therapeutic agent. In general, those of skill in the art and the U.S. FDA consider a pharmaceutically acceptable excipient, carrier, or adjuvant to be an inactive ingredient of any formulation.

[0065] The term “pharmaceutical formulation” as used herein refers to a preparation which is in such form as to permit the biological activity of the agent (e.g., an antibody) to be effective. A pharmaceutical formulation generally comprises additional components, such as a pharmaceutically acceptable excipient, carrier, adjuvant, buffers, etc.

[0066] The term “effective amount” or “therapeutically effective amount” as used herein refers to the amount of an agent (e.g., an antibody) which is sufficient to reduce and/or ameliorate the severity and/or duration of a disease, disorder or condition and/or a symptom in a subject. The term also encompasses an amount of an agent necessary for the (i) reduction or amelioration of the advancement or progression of a given disease, disorder, or condition, (ii) reduction or amelioration of the recurrence, development, or onset of a given disease, disorder, or condition, and/or (iii) the improvement or enhancement of the

prophylactic or therapeutic effect(s) of another agent or therapy (e.g., an agent other than the binding agents provided herein).

[0067] The term “therapeutic effect” as used herein refers to the effect and/or ability of an agent (e.g., an antibody) to reduce and/or ameliorate the severity and/or duration of a disease, disorder, or condition and/or a symptom in a subject. The term also encompasses the ability of an agent to (i) reduce or ameliorate the advancement or progression of a given disease, disorder, or condition, (ii) reduce or ameliorate the recurrence, development, or onset of a given disease, disorder, or condition, and/or (iii) to improve or enhance the prophylactic or therapeutic effect(s) of another agent or therapy (e.g., an agent other than the binding agents provided herein).

[0068] The term “treat” or “treatment” or “treating” or “to treat” or “alleviate” or “alleviation” or “alleviating” or “to alleviate” as used herein refers to both (1) therapeutic measures that aim to cure, slow down, lessen symptoms of, and/or halt progression of a pathologic condition or disorder and (2) prophylactic or preventative measures that aim to prevent or slow the development of a targeted pathologic condition or disorder. Thus, those in need of treatment include those already with the disorder, those at risk of having/developing the disorder, and those in whom the disorder is to be prevented.

[0069] The term “prevent” or “prevention” or “preventing” as used herein refers to the partial or total inhibition of the development, recurrence, onset, or spread of a disease, disorder, or condition, or a symptom thereof in a subject.

[0070] The term “prophylactic agent” as used herein refers to an agent that can partially or totally inhibit the development, recurrence, onset, or spread of a disease, disorder or condition, or a symptom thereof in a subject.

[0071] As used herein, reference to “about” or “approximately” a value or parameter includes (and describes) embodiments that are directed to that value or parameter. For example, a description referring to “about X” includes description of “X.”

[0072] The phrase “substantially all” as used herein refers to at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, 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%.

[0073] As used in the present disclosure and claims, the singular forms “a,” “an” and “the” include plural forms unless the context clearly dictates otherwise.

[0074] It is understood that wherever embodiments are described herein with the term “comprising” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided. It is also understood that wherever embodiments are described herein with the phrase “consisting essentially of” otherwise analogous embodiments described in terms of “consisting of” are also provided.

[0075] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

II. ANGPTL8-binding agents

[0076] Angiopoietin-like protein 8 (ANGPTL8) belongs to a family of proteins that is structurally similar to the angiopoietins, and are referred to as angiopoietin-like proteins or ANGPTLs. To date, the ANGPTL family includes eight proteins, ANGPTL1 to ANGPTL8. ANGPTL proteins have a similar biological structure and generally contain an amino-terminal coiled-coil domain, a linker region, and a carboxyl-terminal fibrinogen-like domain. In contrast to other members of the family, ANGPTL8 does not have a fibrinogen-like domain. The amino acid (aa) sequences for human ANGPTL8 (UniProtKB No. Q6UXH0), cynomolgus monkey (“cyno”) ANGPTL8 (NCBI Ref. No. XP_005588064.1) and mouse ANGPTL8 (UniProtKB No. Q8R1L8) are provided herein as SEQ ID NO:1, SEQ ID NO:9, and SEQ ID NO:10, respectively. As used herein, reference to amino acid positions of ANGPTL8 refer to the numbering of amino acid sequences including the signal sequence.

[0077] The present disclosure provides agents that bind ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds a fragment of ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds within a specific region of ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds an epitope on ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds mouse ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds human ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds human ANGPTL8 and cyno ANGPTL8.

[0078] In some embodiments, the ANGPTL8-binding agent binds within amino acids 22-83 of human ANGPTL8 and/or amino acids 22-83 of cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds within amino acids 84-138 of human ANGPTL8 and/or amino acids 84-138 of cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds within amino acids 139-198 of human ANGPTL8 and/or amino acids 139-198 of cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds within amino acids 25-60 of human ANGPTL8 and/or amino acids 25-60 of cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds within amino acids 22-46 of human ANGPTL8 and/or amino acids 22-46 of cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent binds within amino acids 32-46 of human ANGPTL8 and/or amino acids 32-46 of cyno ANGPTL8.

[0079] In some embodiments, the ANGPTL8-binding agent binds within amino acids 22-83 of SEQ ID NO:1. In some embodiments, the ANGPTL8-binding agent binds within amino acids 84-138 of SEQ ID NO:1. In some embodiments, the ANGPTL8-binding agent binds within amino acids 139-198 of SEQ ID NO:1. In some embodiments, the ANGPTL8-binding agent binds within amino acids 25-60 of SEQ ID NO:1. In some embodiments, the ANGPTL8-binding agent binds within amino acids 22-46 of SEQ ID NO:1. In some embodiments, the ANGPTL8-binding agent binds within amino acids 32-46 of SEQ ID NO:1.

[0080] In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:2. In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:3. In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:4. In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:5. In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:6. In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:7. In some embodiments, the ANGPTL8-binding agent binds an epitope comprising amino acids within SEQ ID NO:8. In some embodiments, the ANGPTL8-binding agent binds at least one amino acid within SEQ ID NO:3. In some embodiments, the ANGPTL8-binding agent binds at least one amino acid within SEQ ID NO:4. In some embodiments, the ANGPTL8-binding agent binds at least one amino acid within SEQ ID NO:5. In some embodiments, the ANGPTL8-binding agent binds at least one amino acid within SEQ ID NO:6. In some embodiments, the ANGPTL8-binding agent binds at least one amino acid within SEQ ID NO:7. In some embodiments, the ANGPTL8-binding agent binds at least one amino acid within SEQ ID NO:8.

[0081] In some embodiments, the ANGPTL8-binding agent is an antibody. In some embodiments, the antibody is a recombinant antibody. In some embodiments, the antibody is a monoclonal antibody. In some embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is a humanized antibody. In some embodiments, the antibody is a human antibody. In some embodiments, the antibody is an IgA, IgD, IgE, IgG, or IgM antibody. In some embodiments, the antibody is an IgG1 antibody. In some embodiments, the antibody is an IgG2 antibody. In some embodiments, the antibody is an IgG4 antibody. In some embodiments, the antibody is an antibody fragment comprising an antigen-binding site. In some embodiments, the antibody is a bispecific antibody or a multispecific antibody. In some embodiments, the antibody is a monovalent antibody. In some embodiments, the antibody is a monospecific antibody. In some embodiments, the antibody is a bivalent antibody.

[0082] In some embodiments, the antibody is isolated. In some embodiments, the antibody is substantially pure.

[0083] In some embodiments, the ANGPTL8-binding agents are polyclonal antibodies. Polyclonal antibodies can be prepared by any known method. In some embodiments, polyclonal antibodies are produced by immunizing an animal (e.g., a rabbit, rat, mouse, goat, donkey) with an antigen of interest (e.g., a purified peptide fragment, a recombinant protein, or a fusion protein) using multiple subcutaneous or intraperitoneal injections. In some embodiments, the antigen is conjugated to a carrier such as keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor. The antigen (with or without a carrier protein) is diluted in sterile saline and usually combined with an adjuvant (e.g., Complete or Incomplete Freund's Adjuvant) to form a stable emulsion. After a sufficient period of time, polyclonal antibodies are recovered from the immunized animal, usually from blood or ascites. The polyclonal antibodies can be purified from serum or ascites according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[0084] In some embodiments, an ANGPTL8-binding agent is a monoclonal antibody. In some embodiments, monoclonal antibodies are prepared using hybridoma methods known to one of skill in the art. For example, using the hybridoma method, a mouse, rat, rabbit, hamster, or other appropriate host animal, is immunized as described above to elicit the production of antibodies that specifically bind the immunizing antigen. In some embodiments, lymphocytes are immunized *in vitro*. In some embodiments, the immunizing antigen is a human protein or a fragment thereof. In some embodiments, the immunizing antigen is a mouse protein or a fragment thereof.

[0085] Following immunization, lymphocytes are isolated and fused with a suitable myeloma cell line using, for example, polyethylene glycol. The hybridoma cells are selected using specialized media as known in the art and unfused lymphocytes and myeloma cells do not survive the selection process. Hybridomas that produce monoclonal antibodies directed specifically against a chosen antigen can be identified by a variety of methods including, but not limited to, immunoprecipitation, immunoblotting, and *in vitro* binding assays (e.g., flow cytometry, FACS, ELISA, and radioimmunoassay). Once hybridoma cells that produce antibodies of the desired specificity, affinity, and/or activity are identified, the clones may be subcloned by limiting dilution techniques. The hybridomas can be propagated either in *in vitro* culture using standard methods or *in vivo* as ascites tumors in an animal. The monoclonal antibodies can be purified from the culture medium or ascites fluid according to standard methods in the art including, but not limited to, affinity chromatography, ion-exchange chromatography, gel electrophoresis, and dialysis.

[0086] In some embodiments, monoclonal antibodies are made using recombinant DNA techniques as known to one skilled in the art. For example, the polynucleotides encoding a monoclonal antibody are isolated from mature B-cells or hybridoma cells, such as by RT-PCR using oligonucleotide primers that

specifically amplify the genes encoding the heavy and light chains of the antibody, and their sequence is determined using standard techniques. The isolated polynucleotides encoding the heavy and light chains are then cloned into suitable expression vectors which produce the monoclonal antibodies when transfected into host cells such as *E. coli*, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin proteins.

[0087] In some embodiments, recombinant monoclonal antibodies, or fragments thereof, are isolated from phage display libraries expressing variable domains or CDRs of a desired species. Screening of phage libraries can be accomplished by various techniques known in the art.

[0088] In some embodiments, a monoclonal antibody is modified, for example, by using recombinant DNA technology to generate alternative antibodies. In some embodiments, the constant domains of the light chain and heavy chain of, for example, a mouse monoclonal antibody are substituted for constant regions of, for example, a human antibody to generate a chimeric antibody, or for a non-immunoglobulin polypeptide to generate a fusion antibody. In some embodiments, the constant regions are truncated or removed to generate a desired antibody fragment of a monoclonal antibody. Site-directed or high-density mutagenesis of the variable region(s) can be used to optimize, for example, specificity and affinity of a monoclonal antibody.

[0089] In some embodiments, an ANGPTL8-binding agent is a humanized antibody. Various methods for generating humanized antibodies are known in the art. In some embodiments, a human antibody comprises one or more amino acid residues that have been introduced into it from a source that is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. In some embodiments, humanization is performed by substituting one or more non-human CDR sequences for the corresponding CDR sequences of a human antibody. In some embodiments, the humanized antibodies are constructed by CDR grafting, in which the amino acid sequences of all six CDRs of the parent non-human antibody (e.g., rodent) are grafted onto a human antibody framework.

[0090] The choice of which human heavy chain variable region and/or light chain variable region to be used in generating humanized antibodies can be made based on a variety of factors and by a variety of methods. In some embodiments, the “best-fit” method is used where the sequence of the variable region of a non-human (e.g., rodent) antibody is screened against the entire library of known human variable region sequences. The human sequence that is most similar to that of the rodent sequence is selected as the human variable region framework for the humanized antibody. In some embodiments, a method is used wherein a particular variable region framework derived from a consensus sequence of all human antibodies of a particular subgroup of light or heavy chains is selected. In some embodiments, the

framework is derived from the consensus sequences of the most abundant human subclasses. In some embodiments, human germline genes are used as the source of the variable region framework sequences.

[0091] Other methods for humanization include, but are not limited to, a method called “superhumanization” which is described as the direct transfer of CDRs to a human germline framework, a method termed Human String Content (HSC) which is based on a metric of “antibody humanness,” methods based on generation of large libraries of humanized variants (including phage, ribosomal, and yeast display libraries), and methods based on framework region shuffling.

[0092] In some embodiments, an ANGPTL8-binding agent is a human antibody. Human antibodies can be directly prepared using various techniques known in the art. In some embodiments, human antibodies are generated from immortalized human B lymphocytes immunized *in vitro*. In some embodiments, human antibodies are generated from lymphocytes isolated from an immunized individual. In any case, cells that produce an antibody directed against a target antigen can be generated and isolated. In some embodiments, a human antibody is selected from a phage library, where that phage library expresses human antibodies. Alternatively, phage display technology may be used to produce human antibodies and antibody fragments *in vitro*, from immunoglobulin variable region gene repertoires from unimmunized donors. Techniques for the generation and use of antibody phage libraries are well known in the art. Once antibodies are identified, affinity maturation strategies known in the art, including but not limited to, chain shuffling and site-directed mutagenesis, may be employed to generate higher affinity human antibodies.

[0093] In some embodiments, human antibodies are produced in transgenic mice that contain human immunoglobulin loci. Upon immunization these mice are capable of producing the full repertoire of human antibodies in the absence of endogenous immunoglobulin production.

[0094] In some embodiments, the ANGPTL8-binding agent is a bispecific antibody. Bispecific antibodies are capable of recognizing and binding at least two different antigens or epitopes. The different epitopes can either be within the same molecule (e.g., two epitopes on ANGPTL8) or on different molecules (e.g., one epitope on ANGPTL8 and one epitope on a different target). In some embodiments, a bispecific antibody has enhanced potency as compared to an individual antibody or to a combination of more than one antibody. In some embodiments, a bispecific antibody has reduced toxicity as compared to an individual antibody or to a combination of more than one antibody. It is known to those of skill in the art that any therapeutic agent may have unique pharmacokinetics (PK) (e.g., circulating half-life). In some embodiments, a bispecific antibody has the ability to synchronize the PK of two active binding agents wherein the two individual binding agents have different PK profiles. In some embodiments, a bispecific antibody has the ability to concentrate the actions of two agents in a common area (e.g., tissue) in a subject. In some embodiments, a bispecific antibody has the ability to concentrate

the actions of two agents to a common target (e.g., a specific cell type). In some embodiments, a bispecific antibody has the ability to target the actions of two agents to more than one biological pathway or function. In some embodiments, a bispecific antibody has the ability to target two different cells and bring them closer together.

[0095] In some embodiments, a bispecific antibody has decreased toxicity and/or side effects. In some embodiments, a bispecific antibody has decreased toxicity and/or side effects as compared to a mixture of the two individual antibodies or the antibodies as single agents. In some embodiments, a bispecific antibody has an increased therapeutic index. In some embodiments, a bispecific antibody has an increased therapeutic index as compared to a mixture of the two individual antibodies or the antibodies as single agents.

[0096] Several techniques for making bispecific antibodies are known by those skilled in the art. In some embodiments, the bispecific antibodies comprise heavy chain constant regions with modifications in the amino acids which are part of the interface between the two heavy chains. In some embodiments, the bispecific antibodies are generated using a knobs-into-holes (KIH) strategy. In some embodiments, the bispecific antibodies comprise variant hinge regions incapable of forming disulfide linkages between the heavy chains. In some embodiments, the bispecific antibodies comprise heavy chains with changes in amino acids that result in altered electrostatic interactions. In some embodiments, the bispecific antibodies comprise heavy chains with changes in amino acids that result in altered hydrophobic/hydrophilic interactions.

[0097] Bispecific antibodies can be intact antibodies or antibody fragments comprising antigen-binding sites.

[0098] Antibodies with more than two valencies are also contemplated. For example, trispecific or tetraspecific antibodies can be prepared. Thus, in some embodiments the antibodies to ANGPTL8 are multispecific.

[0099] In some embodiments, the ANGPTL8-binding agent is an antibody that binds ANGPTL8. In some embodiments, the ANGPTL8-binding agent is an antibody that binds human ANGPTL8. In some embodiments, the ANGPTL8-binding agent is an antibody that binds cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent is an antibody that binds human and cyno ANGPTL8. In some embodiments, the ANGPTL8-binding agent is an antibody that binds mouse ANGPTL8. In some embodiments, the ANGPTL8-binding agent is an antibody that binds a portion or fragment of ANGPTL8. In some embodiments, the ANGPTL8-binding agent is an antibody that binds an ANGPTL8 peptide. In some embodiments, the ANGPTL8-binding agent is an antibody that binds the SE1 peptide. In some embodiments, the ANGPTL8-binding agent is an antibody that binds an ANGPTL8 epitope.

[00100] In some embodiments, the binding agent is an anti-ANGPTL8 antibody or antigen-binding fragment thereof that comprises one, two, three, four, five, and/or six CDRs of any one of the antibodies described herein. In some embodiments, an anti-ANGPTL8 antibody or antigen-binding fragment thereof comprises (i) one, two, and/or three heavy chain CDRs from Tables 1-3, and/or (ii) one, two, and/or three light chain CDRs from Tables 1-3.

[00101] In some embodiments, an antibody or antigen-binding fragment thereof that specifically binds ANGPTL8 comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, CDR3 from Table 1. In some embodiments, an antibody or antigen-binding fragment thereof that specifically binds ANGPTL8 comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, CDR3 from Table 2. In some embodiments, an antibody or antigen-binding fragment thereof that specifically binds ANGPTL8 comprises a heavy chain CDR1, CDR2, and CDR3 and a light chain CDR1, CDR2, CDR3 from Table 3.

Table 1. Antibody 1E5 CDR and Variable Region Sequences

	Exemplary	IMGT	Kabat	Chothia	Contact	AbM
Heavy Chain CDR1	GYFTTDYTH (SEQ ID NO:11)	GYFTTDY (SEQ ID NO:12)	DYTH (SEQ ID NO:13)	GYFTTDY (SEQ ID NO:14)	TDYTH (SEQ ID NO:15)	GYFTTDYTH (SEQ ID NO:11)
Heavy Chain CDR2	WYYPGSDNIKYNA KFKD (SEQ ID NO:16)	FYPGSDNI (SEQ ID NO:17)	WYYPGSDNIKYNA KFKD (SEQ ID NO:16)	PGSD (SEQ ID NO:18)	WIGWYEGSDNIK (SEQ ID NO:19)	WYYPGSDNIK (SEQ ID NO:20)
Heavy Chain CDR3	HEAFSYDDVAY FAY (SEQ ID NO:21)	ARHEAFSYDDVAY FAY (SEQ ID NO:22)	HEAFSYDDVAY FAY (SEQ ID NO:21)	EAFSYDDVAYFA (SEQ ID NO:23)	ARHEAFSYDDVAYFA (SEQ ID NO:24)	HEAFSYDDVAYFA (SEQ ID NO:21)
Light Chain CDR1	RSSQSLVHSNGNT YLH (SEQ ID NO:25)	QSLVHSNGNT YLH (SEQ ID NO:26)	RSSQSLVHSNGNT YLH (SEQ ID NO:25)	SQSLVHSNGNT YLH (SEQ ID NO:27)	VHSNGNTYLHWY (SEQ ID NO:28)	RSSQSLVHSNGNT YLH (SEQ ID NO:25)
Light Chain CDR2	TVSNRFS (SEQ ID NO:29)	TVS (SEQ ID NO:30)	TVSNRFS (SEQ ID NO:29)	TVS (SEQ ID NO:30)	LLIYTYSNRF (SEQ ID NO:31)	TVSNRFS (SEQ ID NO:29)
Light Chain CDR3	SQTHFPT (SEQ ID NO:32)	SQTHFPT (SEQ ID NO:32)	SQTHFPT (SEQ ID NO:32)	TTHFPT (SEQ ID NO:33)	SQTHFPT (SEQ ID NO:34)	SQTHFPT (SEQ ID NO:32)
1E5 Heavy Chain Variable Region						
QQQLOQSGAEILVKPGTTSVRLSCKASGYTFDYTHIHWVKIRSGQGLEWIGWYYPGSDNIKYNAFKDKATLTADKSTSTAYMELSSL TSEDSAVYFCARHEAFSYDDVAYWQGQTLTVVSA (SEQ ID NO: 35)						
1E5 Light Chain Variable Region						
DVVNTOPLSLEVSLDQASISCRSSQLVHSNGNTYLHWYLQKPGQSPKLLIYTSHWVQAPGQLEWMGWYYPGSDNIKYNAFKDKATLTADKSTSTAYMELSSL DLGVYFCQOTTTHFPTTGGTGTLEIK (SEQ ID NO: 36)						
H21E5.A1 Heavy Chain Variable Region						
EVQLVQSGAEVKPGSSVVKVSCKASGYTFDYTHIHWVKIRSGQGLEWIGWYYPGSDNIKYNAFKDKATLTADKSTSTAYMELSSL RSEDTAVYFCARHEAFSYDDVAYWQGQTLTVVSS (SEQ ID NO: 62)						
H21E5.A1 Light Chain Variable Region						
DVVNTOPLSLEVSLDQASISCRSSQLVHSNGNTYLHWYLQKPGQSPKLLIYTSHWVQAPGQLEWMGWYYPGSDNIKYNAFKDKATLTADKSTSTAYMELSSL DVGVYFCQOTTTHFPTTGGTGTLEIK (SEQ ID NO: 63)						

Table 2. Antibody 1E9 CDR and Variable Region Sequences

	Exemplary	IMGT	Kabat	Chothia	Contact	AbM
Heavy Chain CDR1	GTYTDXTHH (SEQ ID NO:11)	GYTFDXT (SEQ ID NO:12)	DYTTH (SEQ ID NO:13)	GYTDX (SEQ ID NO:14)	TDXTIH (SEQ ID NO:15)	GYTFDXTIH (SEQ ID NO:11)
Heavy Chain CDR2	WTFPGSDNIKFNKA KFRD (SEQ ID NO:37)	FYFGSDNI (SEQ ID NO:17)	WTFPGSDNIKFNKA KFRD (SEQ ID NO:37)	PGSD (SEQ ID NO:18)	WIGNFTYFGSDNIK (SEQ ID NO:19)	WTFPGSDNIK (SEQ ID NO:20)
Heavy Chain CDR3	HEAFYVYDVAWEAN (SEQ ID NO:38)	ARHEAFYVYDVAWEAN FAN (SEQ ID NO:39)	HEAFYVYDVAWEAN (SEQ ID NO:38)	EAFYVYDVAWEAN (SEQ ID NO:68)	ARHEAFYVYDVAWEAN (SEQ ID NO:69)	HEAFYVYDVAWEAN (SEQ ID NO:38)
Light Chain CDR1	RSSQSLVHSNGNT YLH (SEQ ID NO:25)	QSLVHSNGNT YLH (SEQ ID NO:26)	RSSQSLVHSNGNT YLH (SEQ ID NO:25)	SQSLVHSNGNT YLH (SEQ ID NO:27)	VHSNGNTYLHWF (SEQ ID NO:40)	RSSQSLVHSNGNT YLH (SEQ ID NO:25)
Light Chain CDR2	TVSNRFS (SEQ ID NO:29)	TVS (SEQ ID NO:30)	TVSNRFS (SEQ ID NO:29)	TVS (SEQ ID NO:30)	LLIYTTSNRF (SEQ ID NO:31)	TVSNRFS (SEQ ID NO:29)
Light Chain CDR3	SQSTHFPYT (SEQ ID NO:41)	SQSTHFPYT (SEQ ID NO:41)	SQSTHFPYT (SEQ ID NO:41)	SITHFPY (SEQ ID NO:42)	SQSTHFPY (SEQ ID NO:43)	SQSTHFPYT (SEQ ID NO:41)
1E9 Heavy Chain Variable Region						
QVQLQQSGTPELVKPGASVKLSCASGYTFDXTIHWVKQRSQGLEWIGWYYPGSDNIKENAKFRDKATLTDKSSSTVYIMELSRL TSEDSAVYFCARHEAFYVYDVAWEANWQGQTLVTVST (SEQ ID NO: 44)						
1E9 Light Chain Variable Region						
DVVMQTPLSLFVSLGQASISCRSSSQSLVHSNGNTYLHWFLQKGQSPNLLIYTWSNRFSGVPDFRSGSGSGTDFTLKISRVEAG DLGVYFCQSSTHFPYTFEGGTKLEIK (SEQ ID NO: 45)						

Table 3. Antibody 1A8 CDR and Variable Region Sequences

	Exemplary	IMGT	Kabat	Chothia	Contact	AbM
Heavy Chain CDR1	GTYTEDYTIH (SEQ ID NO:11)	GYTFEDYTI (SEQ ID NO:12)	DYTIIH (SEQ ID NO:13)	GYTETDY (SEQ ID NO:14)	TDYTIH (SEQ ID NO:15)	GTYTEDYTIH (SEQ ID NO:11)
Heavy Chain CDR2	WTFPGSDNIKYNE KFKD (SEQ ID NO:46)	TYFGSDNI (SEQ ID NO:17)	WTFPGSDNIKYNE KFKD (SEQ ID NO:46)	PGSD (SEQ ID NO:18)	WIGWTFPGSDNIK YNEKFKD (SEQ ID NO:47)	WTFPGSDNIK (SEQ ID NO:20)
Heavy Chain CDR3	HEAXXXYDVAYFAY (SEQ ID NO:48)	ARHEAXXXYDVAY FAY (SEQ ID NO:49)	HEAXXXYDVAYFAY FAY (SEQ ID NO:48)	EAXYYDVAYFAY (SEQ ID NO:50)	ARHEAXXXYDVAYFAY (SEQ ID NO:51)	HEAXXXYDVAYFAY (SEQ ID NO:48)
Light Chain CDR1	SSSQSILVHSNGNT FLH (SEQ ID NO:52)	QSLVHSNGNT FLH (SEQ ID NO:53)	SSSQSILVHSNGNT FLH (SEQ ID NO:52)	SQSLVHSNGNT FLH (SEQ ID NO:54)	VHSNGNTFLHWF FLH (SEQ ID NO:55)	SSSQSILVHSNGNT FLH (SEQ ID NO:52)
Light Chain CDR2	TVSNRFS (SEQ ID NO:29)	TVS (SEQ ID NO:30)	TVSNRFS (SEQ ID NO:29)	TVS (SEQ ID NO:30)	LLIYTTSNRF (SEQ ID NO:31)	TVSNRFS (SEQ ID NO:29)
Light Chain CDR3	SQSTHFPYT (SEQ ID NO:41)	SQSTHFPYT (SEQ ID NO:41)	SQSTHFPYT (SEQ ID NO:41)	SQSTHFPY (SEQ ID NO:42)	SQSTHFPY (SEQ ID NO:43)	SQSTHFPYT (SEQ ID NO:41)

1A8 Heavy Chain Variable Region

QVQLOQSGAEILVKPGASVKLSCRAKASGYTFDYTIHWWKQRSQGGLEWIGWYYPGSDNIKYNEKFDDKATLTADKSSSIVYMEELSRLI
TSEDSAVYFCARHEAXXXYDVAYFAYWQGQTLTVSA. (SEQ ID NO: 56)

1A8 Light Chain Variable Region

DVVMTOQPLSLPVSLGDQASISCSQSLVHSNGNTFLHWFQRPQSPKLLIYTTSNRFSGVPDFSGSGSTDFTLKISRVEAE
DLGVYFCFSQSTHFPYTGGTLEIK (SEQ ID NO: 57)

[00102] CDRs are defined by a variety of methods/systems by those skilled in the art. These systems and/or definitions have been developed and refined over a number of years and include Kabat, Chothia, IMGT, AbM, and “contact.” The Kabat definition is based on sequence variability and generally is the most commonly used. The Chothia definition is based on the location of the structural loop regions. The IMGT system is based on sequence variability and location within the structure of the variable domain. The AbM definition is a compromise between Kabat and Chothia. The “Contact” definition is based on analyses of the available antibody crystal structures. An Exemplary system, as included in Tables 1-3, is a combination of Kabat and Chothia. A comparison of these various definitions and numbering systems is shown below. These are also depicted in Fig. 1 which shows variable region sequence alignments of exemplary antibodies.

	Exemplary	IMGT	Kabat	AbM	Chothia	Contact
HC CDR1	26-35	27-38	31-35	26-35	26-32	30-35
HC CDR2	50-65	56-65	50-65	50-58	53-55	47-58
HC CDR3	95-102	105-117	95-102	95-102	96-101	93-101
LC CDR1	24-34	27-38	24-34	24-34	26-32	30-36
LC CDR2	50-56	56-65	50-56	50-56	50-52	46-55
LC CDR3	89-97	105-117	89-97	89-97	91-96	89-96

[00103] In some embodiments, an antibody that specifically binds ANGPTL8 comprises (a) a heavy chain CDR1 from Table 1, a heavy chain CDR2 from Table 1, and a heavy chain CDR3 from Table 1; and/or (b) a light chain CDR1 from Table 1, a light chain CDR2 from Table 1, and a light chain CDR3 from Table 1.

[00104] In some embodiments, an antibody that specifically binds ANGPTL8 comprises a heavy chain CDR1 comprising SEQ ID NOS:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOS:16, 17, 18, 19, or 20; and a heavy chain CDR3 comprising SEQ ID NOS:21, 22, 23, or 24. In some embodiments, an anti-ANGPTL8 antibody further comprises a light chain CDR1 comprising SEQ ID NOS:25, 26, 27, or 28; a light chain CDR2 comprising SEQ ID NOS:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOS:32, 33, or 34. In some embodiments, an anti-ANGPTL8 antibody comprises a light chain CDR1 comprising SEQ ID NOS:25, 26, 27, or 28; a light chain CDR2 comprising SEQ ID NOS:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOS:32, 33, or 34. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NOS:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOS:16, 17, 18, 19, or 20; a heavy chain CDR3 comprising SEQ ID NOS:21, 22, 23, or 24; a light chain CDR1 comprising SEQ ID NOS:25, 26, 27, or 28; a light chain CDR2 comprising SEQ ID NOS:29, 30, or 31; and a light chain CDR3 comprising

SEQ ID NOs:32, 33, or 34. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NO:11; a heavy chain CDR2 comprising SEQ ID NO:16; a heavy chain CDR3 comprising SEQ ID NO:21; a light chain CDR1 comprising SEQ ID NO:25; a light chain CDR2 comprising SEQ ID NO:29; and a light chain CDR3 comprising SEQ ID NO:32. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NO:13; a heavy chain CDR2 comprising SEQ ID NO:16; a heavy chain CDR3 comprising SEQ ID NO:21; a light chain CDR1 comprising SEQ ID NO:25; a light chain CDR2 comprising SEQ ID NO:29; and a light chain CDR3 comprising SEQ ID NO:32.

[00105] In some embodiments, an antibody that specifically binds ANGPTL8 comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, 15, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR2 comprising SEQ ID NOs:16, 17, 18, 19, 20, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR3 comprising SEQ ID NOs:21, 22, 23, 24, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, 28, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a light chain CDR2 comprising SEQ ID NOs:29, 30, 31, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and a light chain CDR3 comprising SEQ ID NOs:32, 33, 34, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In some embodiments, the amino acid substitutions are conservative substitutions. In some embodiments, the substitutions are made as part of a humanization process. In some embodiments, the substitutions are made as part of a germline humanization process. In some embodiments, the substitutions are made as part of an affinity maturation process. In some embodiments, the substitutions are made as part of an optimization process.

[00106] In some embodiments, an antibody that specifically binds ANGPTL8 comprises (a) a heavy chain CDR1 from Table 2, a heavy chain CDR2 from Table 2, and a heavy chain CDR3 from Table 2; and/or (b) a light chain CDR1 from Table 2, a light chain CDR2 from Table 2, and a light chain CDR3 from Table 2.

[00107] In some embodiments, an antibody that specifically binds ANGPTL8 comprises a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20; and a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69. In some embodiments, an anti-ANGPTL8 antibody further comprises a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an anti-ANGPTL8 antibody comprises a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NOs:11,

12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20; a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69; a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NO:11; a heavy chain CDR2 comprising SEQ ID NO:37; a heavy chain CDR3 comprising SEQ ID NO:38; a light chain CDR1 comprising SEQ ID NO:25; a light chain CDR2 comprising SEQ ID NO:29; and a light chain CDR3 comprising SEQ ID NO:41. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NO:13; a heavy chain CDR2 comprising SEQ ID NO:37; a heavy chain CDR3 comprising SEQ ID NO:38; a light chain CDR1 comprising SEQ ID NO:25; a light chain CDR2 comprising SEQ ID NO:29; and a light chain CDR3 comprising SEQ ID NO:41.

[00108] In some embodiments, an antibody that specifically binds ANGPTL8 comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, 15, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, 20, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, 69, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, 40, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a light chain CDR2 comprising SEQ ID NOs:29, 30, 31, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and a light chain CDR3 comprising SEQ ID NOs:41, 42, 43, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In some embodiments, the amino acid substitutions are conservative substitutions. In some embodiments, the substitutions are made as part of a humanization process. In some embodiments, the substitutions are made as part of a germline humanization process. In some embodiments, the substitutions are made as part of an affinity maturation process. In some embodiments, the substitutions are made as part of an optimization process.

[00109] In some embodiments, an antibody that specifically binds ANGPTL8 comprises (a) a heavy chain CDR1 from Table 3, a heavy chain CDR2 from Table 3, and a heavy chain CDR3 from Table 3; and/or (b) a light chain CDR1 from Table 3, a light chain CDR2 from Table 3, and a light chain CDR3 from Table 3.

[00110] In some embodiments, an antibody that specifically binds ANGPTL8 comprises a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20; and a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, 51. In some embodiments, an anti-ANGPTL8 antibody further comprises a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an anti-ANGPTL8 antibody

comprises a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20; a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, or 51; a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NO:11; a heavy chain CDR2 comprising SEQ ID NO:46; a heavy chain CDR3 comprising SEQ ID NO:48; a light chain CDR1 comprising SEQ ID NO:52; a light chain CDR2 comprising SEQ ID NO:29; and a light chain CDR3 comprising SEQ ID NO:41. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising SEQ ID NO:13; a heavy chain CDR2 comprising SEQ ID NO:46; a heavy chain CDR3 comprising SEQ ID NO:48; a light chain CDR1 comprising SEQ ID NO:52; a light chain CDR2 comprising SEQ ID NO:29; and a light chain CDR3 comprising SEQ ID NO:41.

[00111] In some embodiments, an antibody that specifically binds ANGPTL8 comprises: (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, 15, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, 20, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, 51, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, 55, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; a light chain CDR2 comprising SEQ ID NOs:29, 30, 31, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions; and a light chain CDR3 comprising SEQ ID NOs:41, 42, 43, or a variant thereof comprising 1, 2, 3, or 4 amino acid substitutions. In some embodiments, the amino acid substitutions are conservative substitutions. In some embodiments, the substitutions are made as part of a humanization process. In some embodiments, the substitutions are made as part of a germline humanization process. In some embodiments, the substitutions are made as part of an affinity maturation process. In some embodiments, the substitutions are made as part of an optimization process.

[00112] In some embodiments, the antibody or antigen-binding fragment thereof comprising the CDRs described herein further comprises (a) a heavy chain FR1, a heavy chain FR2, a heavy chain FR3, and a heavy chain FR4; and/or (b) a light chain FR1, a light chain FR2, a light chain FR3, and a light chain FR4.

[00113] Sequence alignments of the heavy chain variable regions and the light chain variable regions of the exemplary antibodies described herein were prepared and are shown in Fig. 1. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID

NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y or V, and X₄ is Y or N (SEQ ID NO:59); a light chain CDR1 comprising X₁SSQSLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61).

[00114] In some embodiments, an antibody that specifically binds ANGPTL8 comprises a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:35, SEQ ID NO:44, or SEQ ID NO:56 and/or a light chain variable region having at least 80% sequence identity to SEQ ID NO:36, SEQ ID NO:45, or SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:35. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:44. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:56. In some embodiments, an anti-ANGPTL8 antibody comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:36. In some embodiments, an anti-ANGPTL8 antibody comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:45. In some embodiments, an anti-ANGPTL8 antibody comprises a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 97%, or at least about 99% sequence identity to SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region having at least about 95% sequence identity to SEQ ID NO:35, SEQ ID NO:44, or SEQ ID NO:56 and/or a light chain variable region having at least about 95% sequence identity to SEQ ID NO:36, SEQ ID NO:45, or SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region comprising SEQ ID NO:35, SEQ ID NO:44, or SEQ ID NO:56 and/or a light chain variable region comprising SEQ ID NO:36, SEQ ID NO:45, or SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region comprising SEQ ID NO:35, SEQ ID NO:44, or SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:36, SEQ ID NO:45, or SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region consisting essentially of SEQ ID NO:35, SEQ ID NO:44, or SEQ ID NO:56 and a light chain variable region consisting essentially of SEQ ID NO:36, SEQ ID NO:45, or

SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region consisting of SEQ ID NO:35, SEQ ID NO:44, or SEQ ID NO:56 and a light chain variable region consisting of SEQ ID NO:36, SEQ ID NO:45, or SEQ ID NO:57. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region comprising SEQ ID NO:35 and a light chain variable region comprising SEQ ID NO:36. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57.

[00115] In some embodiments, an antibody that specifically binds ANGPTL8 comprises a humanized version or humanized variant of any one of the antibodies described herein. In some embodiments, an antibody is a humanized antibody comprising (a) a heavy chain CDR1 from Table 1, a heavy chain CDR2 from Table 1, and a heavy chain CDR3 from Table 1; and/or (b) a light chain CDR1 from Table 1, a light chain CDR2 from Table 1, and a light chain CDR3 from Table 1. In some embodiments, an antibody is a humanized antibody comprising (a) a heavy chain CDR1 from Table 2, a heavy chain CDR2 from Table 2, and a heavy chain CDR3 from Table 2; and/or (b) a light chain CDR1 from Table 2, a light chain CDR2 from Table 2, and a light chain CDR3 from Table 2. In some embodiments, an antibody is a humanized antibody comprising (a) a heavy chain CDR1 from Table 3, a heavy chain CDR2 from Table 3, and a heavy chain CDR3 from Table 3; and/or (b) a light chain CDR1 from Table 3, a light chain CDR2 from Table 3, and a light chain CDR3 from Table 3.

[00116] In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region having at least about 80% sequence identity to SEQ ID NO:62 and a light chain variable region having at least about 80% sequence identity to SEQ ID NO:63. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity to SEQ ID NO:62 and a light chain variable region having at least about 85%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99% sequence identity to SEQ ID NO:63. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63. In some embodiments, an anti-ANGPTL8 antibody comprises a heavy chain comprising SEQ ID NO:64 and a light chain comprising SEQ ID NO:65.

[00117] Provided herein are antibodies that compete with one or more of the antibodies or antigen-binding fragments thereof described herein for binding to human ANGPTL8. In some embodiments, an antibody binds the same epitope as one of the antibodies described herein. In some embodiments, an antibody binds an epitope overlapping with an epitope bound by one of the antibodies described herein.

Antibodies and antigen-binding fragments that compete with or bind to the same epitope as the antibodies described herein are expected to show similar functional properties.

[00118] The antibodies described herein and antigen-binding fragments thereof include those comprising one, two, three, four, five, and/or six of the CDRs provided in Tables 1-3. The antibodies described herein and antigen-binding fragments include those comprising a heavy chain variable region and/or a light chain variable region provided in Tables 1-3. As a specific but non-limiting example, antibodies provided herein include those that compete with an antibody comprising: (a) one, two, three, four, five, or all six of the CDRs provided in Tables 1-3; (b) a heavy chain variable region and a light chain variable region selected from those provided in Tables 1-3; or (c) two light chains and two heavy chains comprising a heavy chain variable region and a light chain variable region as provided in Tables 1-3.

[00119] In some embodiments, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with an anti-ANGPTL8 antibody described herein. In some embodiments, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises: (a) comprises a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y or V, and X₄ is Y or N (SEQ ID NO:59); and/or (b) a light chain CDR1 comprising X₁SSQSLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61). In some embodiments, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:16, 17, 18, 19, or 20; a heavy chain CDR3 comprising SEQ ID NOs:21, 22, 23, or 24; a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 28; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:32, 33, or 34. In some embodiments, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20; a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69; a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43. In some embodiments, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain CDR1 comprising SEQ ID

NOs:11, 12, 13, 14, or 15; a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20; a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, or 51; a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55; a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31; and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43.

[00120] In some embodiments, an antibody or antigen-binding fragment thereof competes for binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises (a) a heavy chain variable region comprising SEQ ID NO:35, 44, 56, or 62; and (b) a light chain variable region comprising SEQ ID NO:36, 45, 57, or 63. In some embodiments, an antibody competes for specific binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:35 and a light chain variable region comprising SEQ ID NO:36. In some embodiments, an antibody competes for specific binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45. In some embodiments, an antibody competes for specific binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57. In some embodiments, an antibody competes for specific binding to ANGPTL8 with a reference antibody, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63.

[00121] In some embodiments, the ANGPTL8-binding agents described herein comprise antibodies (e.g., full-length antibodies or antigen-binding fragments thereof) in which at least one or more of the constant regions has been modified or deleted. In some embodiments, the antibodies may comprise modifications to one or more of the three heavy chain constant regions (CH1, CH2 or CH3) and/or to the light chain constant region (CL). In some embodiments, the heavy chain constant region of the modified antibodies comprises at least one human constant region. In some embodiments, the heavy chain constant region of the modified antibodies comprises more than one human constant region. In some embodiments, modifications to the constant region comprise additions, deletions, or substitutions of one or more amino acids in one or more regions. In some embodiments, one or more regions are partially or entirely deleted from the constant regions of the modified antibodies. In some embodiments, the entire CH2 domain has been removed from an antibody (ΔCH2 constructs). In some embodiments, a deleted constant region is replaced by a short amino acid spacer that provides some of the molecular flexibility typically imparted by the absent constant region. In some embodiments, a modified antibody comprises a CH3 domain directly fused to the hinge region of the antibody. In some embodiments, a modified antibody comprises a peptide spacer inserted between the hinge region and modified CH2 and/or CH3 domains.

[00122] It is known in the art that the constant region(s) of an antibody mediates several effector functions. For example, binding of the C1 component of complement to the Fc region of IgG or IgM antibodies (bound to antigen) activates the complement system. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and can be involved in autoimmune hypersensitivity. In addition, the Fc region of an antibody can bind a cell expressing a Fc receptor (FcR). There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell cytotoxicity or ADCC), release of inflammatory mediators, placental transfer, and control of immunoglobulin production.

[00123] In some embodiments, the modified antibodies provide for altered effector functions that, in turn, affect the biological profile of the administered antibody. For example, in some embodiments, the deletion or inactivation (through point mutations or other means) of a constant region may reduce Fc receptor binding of the circulating modified antibody. In some embodiments, the constant region modifications increase the serum half-life of the antibody. In some embodiments, the constant region modifications reduce the serum half-life of the antibody. In some embodiments, the constant region modifications decrease or remove ADCC and/or complement dependent cytotoxicity (CDC) of the antibody. For example, specific amino acid substitutions in a human IgG1 Fc region with corresponding IgG2 or IgG4 residues may reduce effector functions (e.g., ADCC and CDC) in the modified antibody. Thus, in some embodiments, an antibody does not have one or more effector functions. In some embodiments, the antibody has no ADCC activity and/or no CDC activity. In some embodiments, the antibody does not bind an Fc receptor and/or complement factors. In some embodiments, the antibody has no effector function(s). In some embodiments, the constant region is modified to eliminate disulfide linkages or oligosaccharide moieties. In some embodiments, the constant region is modified to add/substitute one or more amino acids to provide, for example, one or more cytotoxin or carbohydrate attachment sites.

[00124] Modifications to the constant region of antibodies described herein may be made using well known biochemical or molecular engineering techniques. In some embodiments, antibody variants can be prepared by introducing appropriate nucleotide changes into the encoding DNA, and/or by synthesis of the desired antibody or polypeptide. In this respect it may be possible to disrupt the activity or effector function provided by a specific sequence or region while substantially maintaining the structure, binding activity, and other desired characteristics of the modified antibody.

[00125] The present disclosure further embraces additional variants and equivalents that are substantially homologous to the recombinant, monoclonal, chimeric, humanized, and human antibodies, or antibody fragments thereof, described herein. In some embodiments, it may be desirable to improve the binding affinity of the antibody. In some embodiments, it may be desirable to modulate other biological properties of the antibody, including but not limited to, specificity, thermostability, expression level, effector function(s), glycosylation, immunogenicity, or solubility. Those skilled in the art will appreciate that amino acid changes may alter post-translational processes of an antibody, such as changing the number or position of glycosylation sites or altering membrane anchoring characteristics.

[00126] Variations may be a substitution, deletion, or insertion of one or more nucleotides encoding the antibody or polypeptide that results in a change in the amino acid sequence as compared with the native antibody or polypeptide sequence. Amino acid substitutions can be the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, such as the replacement of a leucine with a serine, *e.g.*, conservative amino acid replacements. Insertions or deletions may optionally be in the range of about 1 to 5 amino acids. In some embodiments, the substitution, deletion, or insertion includes less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the parent molecule. Variations in the amino acid sequence that are biologically useful and/or relevant may be determined by systematically making insertions, deletions, or substitutions in the sequence and testing the resulting variant proteins for activity as compared to the parental protein.

[00127] In some embodiments, variants may include addition of amino acid residues at the amino- and/or carboxyl-terminal end of the antibody or polypeptide. The length of additional amino acids residues may range from one residue to a hundred or more residues. In some embodiments, a variant comprises an N-terminal methionyl residue. In some embodiments, the variant comprises an additional polypeptide/protein, *i.e.*, a fusion protein. In some embodiments, a variant is engineered to be detectable and may comprise a detectable label and/or protein (*e.g.*, an enzyme).

[00128] In some embodiments, a cysteine residue not involved in maintaining the proper conformation of an antibody may be substituted or deleted to modulate the antibody's characteristics, *e.g.*, to improve oxidative stability and/or prevent aberrant disulfide crosslinking. Conversely, in some embodiments, one or more cysteine residues may be added to create disulfide bond(s) to improve stability.

[00129] In some embodiments, an antibody of the present disclosure is "deimmunized." The deimmunization of antibodies generally consists of introducing specific amino acid mutations (*e.g.*, substitutions, deletions, additions) to remove T-cell epitopes without significantly reducing the binding affinity or other desired activities of the antibody.

[00130] The variant antibodies or polypeptides described herein may be generated using methods known in the art, including but not limited to, site-directed mutagenesis, alanine scanning mutagenesis, and PCR mutagenesis.

[00131] In some embodiments, binding agents described herein are chemically modified. In some embodiments, the binding agents are antibodies that have been chemically modified by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, and/or linkage to a cellular ligand or other protein. Any of numerous chemical modifications may be carried out by known techniques.

[00132] The present disclosure encompasses binding agents built upon non-immunoglobulin backbones, wherein the agents bind to the same epitope or essentially the same epitope as an anti-ANGPTL8 antibody disclosed herein. In some embodiments, a non-immunoglobulin-based binding agent is an agent that competes with an anti-ANGPTL8 antibody described herein in a competitive binding assay. In some embodiments, alternative binding agents comprise a scaffold protein. Generally, scaffold proteins can be assigned to one of three groups based on the architecture of their backbone (1) scaffolds consisting of α -helices; (2) small scaffolds with few secondary structures or an irregular architecture of α -helices and β -sheets; and (3) scaffolds consisting of predominantly β -sheets. Scaffold proteins include, but are not limited to, anticalins, which are based upon the lipocalin scaffold; adnectins, which are based on the 10th domain of human fibronectin type 3; affibodies, which are based on the B-domain in the Ig-binding region of *Staphylococcus aureus* protein A; darpins, which are based on ankyrin repeat domain proteins; fynomers, which are based on the SH3 domain of the human Fyn protein kinase; affitins, which are based on Sac7d from *Sulfolobus acidocaldarius*; affilins, which are based on human γ -B-crystallin or human ubiquitin; avimers, which are based on the A-domains of membrane receptor proteins; knottins (cysteine knot miniproteins), which are based upon a stable 30-amino acid anti-parallel β -strand protein fold; and Kunitz domain inhibitor scaffolds, which are based upon a structure that contains three disulfide bonds and three loops. In some embodiments, an ANGPTL8-binding agent comprises an engineered scaffold protein comprising one or more CDRs as shown in Tables 1-3.

[00133] Generally speaking, antigen-antibody interactions are non-covalent and reversible, formed by a combination of hydrogen bonds, hydrophobic interactions, electrostatic and van der Waals forces. When describing the strength of an antigen-antibody complex, affinity and/or avidity are usually mentioned. The binding of an antibody to its antigen is a reversible process, and the affinity of the binding is typically reported as an equilibrium dissociation constant (K_D). K_D is the ratio of an antibody dissociation rate (k_{off}) (how quickly it dissociates from its antigen) to the antibody association rate (k_{on}) (how quickly it binds to its antigen). In some embodiments, K_D values are determined by measuring the k_{on} and k_{off} rates of a specific antibody/antigen interaction and then using a ratio of these values to calculate the K_D value.

K_D values may be used to evaluate and rank order the strength of individual antibody/antigen interactions. The lower the K_D of an antibody, the higher the affinity of the antibody for its target. Avidity gives a measure of the overall strength of an antibody-antigen complex. It is dependent on three major parameters: (i) affinity of the antibody for the epitope, (ii) valency of both the antibody and antigen, and (iii) structural arrangement of the parts that interact.

[00134] In some embodiments, an ANGPTL8-binding agent (e.g., an antibody) binds ANGPTL8 with a dissociation constant (K_D) of about 1 μ M or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, about 0.1 nM or less, 50 pM or less, 10 pM or less, or 1 pM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 20 nM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 10 nM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 1 nM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 0.5 nM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 0.1 nM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 50 pM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 25 pM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 10 pM or less. In some embodiments, an ANGPTL8-binding agent binds ANGPTL8 with a K_D of about 1 pM or less. In some embodiments, the dissociation constant of the binding agent (e.g., an antibody) to ANGPTL8 is the dissociation constant determined using an ANGPTL8 fusion protein comprising at least a portion or antigen-binding fragment of ANGPTL8 immobilized on a BIACORE chip. In some embodiments, the dissociation constant of the binding agent (e.g., an antibody) to ANGPTL8 is the dissociation constant determined using an ANGPTL8 peptide (e.g., SE1 peptide) immobilized on a BIACORE chip. In some embodiments, the dissociation constant of the binding agent (e.g., an antibody) to ANGPTL8 is the dissociation constant determined using the binding agent captured by an anti-human IgG antibody on a BIACORE chip and soluble ANGPTL8 or a fragment thereof.

[00135] In some embodiments, the ANGPTL8-binding agent (e.g., an antibody) binds ANGPTL8 with a half maximal effective concentration (EC50) of about 1 μ M or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, or about 0.1 nM or less. In some embodiments, an ANGPTL8-binding agent binds to human ANGPTL8 with an EC50 of about 1 μ M or less, about 100 nM or less, about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less, or about 0.1 nM or less. In some embodiments, an ANGPTL8-binding agent binds mouse ANGPTL8 and/or human ANGPTL8 with an EC50 of about 40 nM or less, about 20 nM or less, about 10 nM or less, about 1 nM or less or about 0.1 nM or less.

[00136] The binding agents (e.g., antibodies or antigen-binding fragments thereof) described herein can be produced by any suitable method known in the art. Such methods range from direct protein synthesis methods to constructing a DNA sequence encoding polypeptide sequences and expressing those sequences in a suitable host. In some embodiments, a DNA sequence is constructed using recombinant technology by isolating or synthesizing a DNA sequence encoding a wild-type protein of interest. Optionally, the sequence can be mutagenized by site-specific mutagenesis to provide functional variants thereof. In some embodiments, a DNA sequence encoding a polypeptide of interest is constructed by chemical synthesis using an oligonucleotide synthesizer. Oligonucleotides can be designed based on the amino acid sequence of the desired polypeptide and selecting those codons that are favored in the host cell in which the recombinant polypeptide of interest will be produced. Standard methods can be applied to synthesize a polynucleotide sequence encoding an isolated polypeptide of interest. For example, a complete amino acid sequence can be used to construct a back-translated gene. Further, a DNA oligomer containing a nucleotide sequence coding for the particular isolated polypeptide can be synthesized. For example, several small oligonucleotides coding for portions of the desired polypeptide can be synthesized and then ligated. The individual oligonucleotides typically contain 5' or 3' overhangs for complementary assembly.

[00137] Once assembled (by synthesis, site-directed mutagenesis, or another method), the polynucleotide sequences encoding a particular polypeptide of interest can be inserted into an expression vector and operatively linked to an expression control sequence appropriate for expression of the protein in a desired host. Proper assembly can be confirmed by nucleotide sequencing, restriction enzyme mapping, and/or expression of a biologically active polypeptide in a suitable host. As is well-known in the art, in order to obtain high expression levels of a transfected gene in a host, the gene must be operatively linked to transcriptional and translational expression control sequences that are functional in the chosen expression host.

[00138] In some embodiments, recombinant expression vectors are used to amplify and express DNA encoding antibodies, or fragments thereof, against human ANGPTL8. For example, recombinant expression vectors can be replicable DNA constructs which have synthetic or cDNA-derived DNA fragments encoding a polypeptide chain of an ANGPTL8-binding agent, such as an anti-ANGPTL8 antibody, or antigen-binding fragment thereof, operatively linked to suitable transcriptional and/or translational regulatory elements derived from mammalian, microbial, viral or insect genes. A transcriptional unit generally comprises an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, transcriptional promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription and translation initiation and termination sequences. Regulatory elements can include an

operator sequence to control transcription. The ability to replicate in a host, usually conferred by an origin of replication, and a selection gene to facilitate recognition of transformants can additionally be incorporated. DNA regions are “operatively linked” when they are functionally related to each other. For example, DNA for a signal peptide (secretory leader) is operatively linked to DNA for a polypeptide if it is expressed as a precursor which participates in the secretion of the polypeptide; a promoter is operatively linked to a coding sequence if it controls the transcription of the sequence; or a ribosome binding site is operatively linked to a coding sequence if it is positioned so as to permit translation. In some embodiments, structural elements intended for use in yeast expression systems include a leader sequence enabling extracellular secretion of translated protein by a host cell. In some embodiments, in situations where recombinant protein is expressed without a leader or transport sequence, a polypeptide may include an N-terminal methionine residue. This residue can optionally be subsequently cleaved from the expressed recombinant protein to provide a final product.

[00139] The choice of an expression control sequence and an expression vector generally depends upon the choice of host. A wide variety of expression host/vector combinations can be employed. Useful expression vectors for eukaryotic hosts include, for example, vectors comprising expression control sequences from SV40, bovine papilloma virus, adenovirus, and cytomegalovirus. Useful expression vectors for bacterial hosts include known bacterial plasmids, such as plasmids from *E. coli*, including pCR1, pBR322, pMB9 and their derivatives, and wider host range plasmids, such as M13 and other filamentous single-stranded DNA phages.

[00140] The ANGPTL8-binding agents (e.g., antibodies) of the present disclosure can be expressed from one or more vectors. For example, in some embodiments, a heavy chain polypeptide is expressed by one vector and a light chain polypeptide is expressed by a second vector. In some embodiments, a heavy chain polypeptide and a light chain polypeptide are expressed by one vector.

[00141] Suitable host cells for expression of an ANGPTL8-binding agent (e.g., an antibody) or an ANGPTL8 protein or fragment thereof to use as an antigen or immunogen include prokaryotes, yeast cells, insect cells, or higher eukaryotic cells under the control of appropriate promoters. Prokaryotes include gram-negative or gram-positive organisms, for example *E. coli* or *Bacillus*. Higher eukaryotic cells include established cell lines of mammalian origin as described herein. Cell-free translation systems may also be employed. Appropriate cloning and expression vectors for use with bacterial, fungal, yeast, and mammalian cellular hosts, as well as methods of protein production, including antibody production are well known in the art.

[00142] Various mammalian culture systems may be used to express recombinant polypeptides. Expression of recombinant proteins in mammalian cells may be desirable because these proteins are generally correctly folded, appropriately modified, and biologically functional. Examples of suitable

mammalian host cell lines include, but are not limited to, COS-7 (monkey kidney-derived), L-929 (murine fibroblast-derived), C127 (murine mammary tumor-derived), 3T3 (murine fibroblast-derived), CHO (Chinese hamster ovary-derived), HeLa (human cervical cancer-derived), BHK (hamster kidney fibroblast-derived), HEK-293 (human embryonic kidney-derived) cell lines and variants thereof.

Mammalian expression vectors can comprise non-transcribed elements such as an origin of replication, a suitable promoter and enhancer linked to the gene to be expressed, and other 5' or 3' flanking non-transcribed sequences, and 5' or 3' non-translated sequences, such as necessary ribosome binding sites, a polyadenylation site, splice donor and acceptor sites, and transcriptional termination sequences.

[00143] Expression of recombinant proteins in insect cell culture systems (e.g., baculovirus) also offers a robust method for producing correctly folded and biologically functional proteins. Baculovirus systems for production of heterologous proteins in insect cells are well-known to those of skill in the art.

[00144] Thus, the present disclosure provides cells comprising the ANGPTL8-binding agents described herein. In some embodiments, the cells produce the ANGPTL8-binding agents described herein. In some embodiments, the cells produce an antibody. In some embodiments, the cells produce an antibody that binds human ANGPTL8. In some embodiments, the cells produce an antibody that binds cyno ANGPTL8. In some embodiments, the cells produce an antibody that binds human ANGPTL8 and cyno ANGPTL8. In some embodiments, the cells produce an antibody designated 1E5. In some embodiments, the cells produce a humanized version of antibody 1E5, referred to as Hz1E5 or Hz1E5.A1. In some embodiments, the cells produce an antibody designated 1E9. In some embodiments, the cells produce an antibody designated 1A8. In some embodiments, the cell is a hybridoma cell. In some embodiments, the cell is a mammalian cell. In some embodiments, the cell is a prokaryotic cell. In some embodiments, the cell is an eukaryotic cell.

[00145] Proteins produced by a host cell can be purified according to any suitable method. Standard methods include chromatography (e.g., ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for protein purification. Affinity tags such as hexa-histidine, maltose binding domain, influenza coat sequence, and glutathione-S-transferase can be attached to the protein to allow easy purification by passage over an appropriate affinity column. Affinity chromatography used for purifying immunoglobulins can include Protein A, Protein G, and Protein L chromatography. Isolated proteins can be physically characterized using such techniques as proteolysis, size exclusion chromatography (SEC), mass spectrometry (MS), nuclear magnetic resonance (NMR), isoelectric focusing (IEF), high performance liquid chromatography (HPLC), and x-ray crystallography. The purity of isolated proteins can be determined using techniques known to those of skill in the art, including but not limited to, SDS-PAGE, SEC, capillary gel electrophoresis, IEF, and capillary isoelectric focusing (cIEF).

[00146] In some embodiments, supernatants from expression systems which secrete recombinant protein into culture media are first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. Following the concentration step, the concentrate can be applied to a suitable purification matrix. In some embodiments, an anion exchange resin is employed, for example, a matrix or substrate having pendant diethylaminoethyl (DEAE) groups. The matrices can be acrylamide, agarose, dextran, cellulose, or other types commonly employed in protein purification. In some embodiments, a cation exchange step is employed. Suitable cation exchangers include various insoluble matrices comprising sulfopropyl or carboxymethyl groups. In some embodiments, a hydroxyapatite media is employed, including but not limited to, ceramic hydroxyapatite (CHT). In some embodiments, one or more reverse-phase HPLC steps employing hydrophobic RP-HPLC media, e.g., silica gel having pendant methyl or other aliphatic groups, are employed to further purify a recombinant protein (e.g., an ANGPTL-binding agent). Some or all of the foregoing purification steps, in various combinations, can be employed to provide a homogeneous recombinant protein.

[00147] Anti-ANGPTL antibodies of the present disclosure may be analyzed for their physical/chemical properties and/or biological activities by various assays known in the art. In some embodiments, an anti-ANGPTL antibody is tested for its ability to bind ANGPTL8. Binding assays include, but are not limited to, BIACORE, ELISA, and FACS. In some embodiments, an anti-ANGPTL8 antibody is tested for its ability to inhibit, reduce, or block ANGPTL8 activity.

[00148] In some embodiments, monoclonal antibodies generated against ANGPTL8 are grouped based upon the epitope each individual antibody recognizes, a process known as “epitope binning.” Generally, in epitope binning antibodies are tested in a pairwise combinatorial manner and antibodies that compete with each other are grouped together into bins. For example, in a premix binning assay, a first antibody is immobilized on a surface and a premixed solution of the second antibody and antigen is flowed over the immobilized first antibody. In tandem, the target protein is immobilized on a surface and the two antibodies are flowed over the immobilized antigen and compete to bind. In each of these techniques, antibodies that block one another can be identified. A competitive blocking profile is created for each antibody relative to the others. The blocking results determine which bin each antibody is placed in. High-throughput methods of epitope binning are known in the art and allow for screening and characterization of large numbers of antibodies within a short period of time. Antibodies that bind similar epitopes often share a similar function. Conversely, antibodies that bind different epitopes may have different functional activities.

[00149] Epitope mapping is the process of identifying the binding site, or epitope on a target protein where an antibody (or other binding agent) binds. A variety of methods are known in the art for mapping epitopes on target proteins. These methods include mutagenesis, including but not limited to, shotgun

mutagenesis, site-directed mutagenesis, and alanine scanning; domain or fragment scanning, peptide scanning (e.g., pepscan); display methods (e.g., phage display, microbial display, and ribosome/mRNA display); methods involving proteolysis and mass spectroscopy; and structural determination (e.g., x-ray crystallography and NMR).

[00150] In some embodiments, purified anti-ANGPTL8 antibodies are characterized by assays including, but not limited to, N-terminal sequencing, amino acid analysis, high pressure liquid chromatography (HPLC), mass spectrometry, ion exchange chromatography, and papain digestion.

[00151] In some embodiments, assays are provided for identifying anti-ANGPTL8 antibodies or antigen-binding fragments thereof that affect ANGPTL8 activity. For example, overexpression of ANGPTL8 has been shown to increase triglyceride levels *in vivo* models. Thus, in some embodiments, candidate ANGPTL8-binding agents are screened for their ability to lower, reduce, or block ANGPTL8-induced increases in triglyceride levels. Clearance of plasma triglycerides is primarily mediated by the activity of lipoprotein lipase (LPL). Studies have suggested that ANGPTL8 is involved, either directly or indirectly, in regulation of LPL activity. Thus, in some embodiments, candidate ANGPTL8-binding agents (e.g., antibodies) are screened for their ability to affect LPL activity (e.g., enhance or increase LPL activity).

[00152] The present disclosure also provides conjugates comprising any one of the anti-ANGPTL8 antibodies described herein. In some embodiments, the antibody is attached to a second molecule. In some embodiments, the antibody is conjugated to a cytotoxic agent or moiety. In some embodiments, the antibody is conjugated to a cytotoxic agent to form an ADC (antibody-drug conjugate). In some embodiments, the cytotoxic moiety is a chemotherapeutic agent including, but not limited to, methotrexate, adriamycin/doxorubicin, melphalan, mitomycin C, chlorambucil, duocarmycin, daunorubicin, pyrrolobenzodiazepines (PBDs), or other intercalating agents. In some embodiments, the cytotoxic moiety is a microtubule inhibitor including, but not limited to, auristatins, maytansinoids (e.g., DMI and DM4), and tubulysins. In some embodiments, the cytotoxic moiety is an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof, including, but not limited to, diphtheria A chain, non-binding active fragments of diphtheria toxin, exotoxin A chain, ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), Momordica charantia inhibitor, curcin, crotin, Sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the trichothecenes. In some embodiments, an antibody is conjugated to one or more small molecule toxins, such as calicheamicins, maytansinoids, trichothenes, and CC1065. The derivatives of these toxins that retain cytotoxic activity can also be used.

[00153] In some embodiments, the antibody is conjugated to a radioisotope to produce a radioconjugate or a radioconjugated antibody. A variety of radionuclides are available for the production of radioconjugated antibodies including, but not limited to, ⁹⁰Y, ¹²⁵I, ¹³¹I, ¹²³I, ¹¹¹In, ¹³¹In, ¹⁰⁵Rh, ¹⁵³Sm, ⁶⁷Cu, ⁶⁷Ga, ¹⁶⁶Ho, ¹⁷⁷Lu, ¹⁸⁶Re, ¹⁸⁸Re and ²¹²Bi.

[00154] Conjugates comprising an antibody may be made using any suitable method known in the art. In some embodiments, conjugates are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene).

[00155] In some embodiments, antibodies are conjugated to detectable substances or molecules that allow the antibodies to be used for diagnosis and/or detection. The detectable substances may include but not limited to, enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, and acetylcholinesterase; prosthetic groups, such as streptavidin/biotin and avidin/biotin; fluorescent materials, such as, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride, and phycoerythrin; bioluminescent materials, such as luciferase, luciferin, and aequorin; chemiluminescent materials, such as luminol and acridinium; radioactive materials, such as ¹³¹I, ¹²⁵I, ¹²³I, and ¹²¹I, ¹⁴C, ³⁵S, ³H, ¹¹⁵In, ¹¹³In, ¹¹²In, and ¹¹¹In, ⁹⁹Tl, thallium ²⁰¹Tl, ⁶⁸Ga, ⁶⁷Ga, ¹⁰³Pd, ⁹⁹Mo, ¹³³Xe, ¹⁸F, ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, and ¹¹⁷Sn; positron emitting metals; and non-radioactive paramagnetic metal ions.

[00156] An antibody provided herein can also be conjugated to a second antibody to form an antibody heteroconjugate.

[00157] Antibodies that bind ANGPTL8 as described herein may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride, or polypropylene.

III. Polynucleotides

[00158] In some embodiments, the disclosure encompasses polynucleotides comprising polynucleotides that encode an ANGPTL8-binding agent described herein. The term “polynucleotides that encode a polypeptide” encompasses a polynucleotide which includes only coding sequences for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequences. The

polynucleotides of the disclosure can be in the form of RNA or in the form of DNA. DNA includes cDNA, genomic DNA, and synthetic DNA; and can be double-stranded or single-stranded, and if single stranded can be the coding strand or non-coding (anti-sense) strand.

[00159] In some embodiments, the polynucleotide comprises a polynucleotide (e.g., a nucleotide sequence) encoding a polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NOs:35, 36, 44, 45, 56, 57, 62, 63, 64, and 65. In some embodiments, the polynucleotide comprises a polynucleotide (e.g., a nucleotide sequence) encoding a polypeptide comprising more than one amino acid sequence selected from the group consisting of: SEQ ID NOs:35, 36, 44, 45, 56, 57, 62, 63, 64, and 65.

[00160] In some embodiments, a polynucleotide comprises a polynucleotide having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90% identical, at least about 95% identical, and in some embodiments, at least about 96%, 97%, 98%, or 99% identical to a polynucleotide encoding an amino acid sequence selected from the group consisting of: SEQ ID NOs:35, 36, 44, 45, 56, 57, 62, 63, 64, and 65. Also provided is a polynucleotide that comprises a polynucleotide that hybridizes to a polynucleotide encoding an amino acid sequence selected from the group consisting of: SEQ ID NOs:35, 36, 44, 45, 56, 57, 62, 63, 64, and 65. In some embodiments, the hybridization is under conditions of high stringency as is known to those skilled in the art.

[00161] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (e.g., an antibody) fused in the same reading frame to a polynucleotide which aids, for example, in expression and secretion of a polypeptide from a host cell (e.g., a leader sequence which functions as a secretory sequence for controlling transport of a polypeptide). The polypeptide can have the leader sequence cleaved by the host cell to form a “mature” form of the polypeptide.

[00162] In some embodiments, a polynucleotide comprises the coding sequence for a polypeptide (e.g., an antibody) fused in the same reading frame to a marker or tag sequence. For example, in some embodiments, a marker sequence is a hexa-histidine tag supplied by a vector which allows efficient purification of the polypeptide fused to the marker in the case of a bacterial host. In some embodiments, a marker sequence is a hemagglutinin (HA) tag derived from the influenza hemagglutinin protein when a mammalian host (e.g., COS-7 cells) is used. In some embodiments, the marker sequence is a FLAG-tag, a peptide of sequence DYKDDDDK (SEQ ID NO:66). In some embodiments, a marker may be used in conjunction with other affinity tags.

[00163] The present disclosure further relates to variants of the polynucleotides described herein, wherein the variant encodes, for example, fragments, analogs, and/or derivatives of a polypeptide. In some embodiments, the present disclosure provides a polynucleotide comprising a polynucleotide having a nucleotide sequence at least about 80% identical, at least about 85% identical, at least about 90%

identical, at least about 95% identical, and in some embodiments, at least about 96%, 97%, 98% or 99% identical to a polynucleotide encoding a polypeptide comprising an ANGPTL8-binding agent described herein.

[00164] As used herein, the phrase “a polynucleotide having a nucleotide sequence at least, for example, 95% “identical” to a reference nucleotide sequence” is intended to mean that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence can include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence can be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence can be inserted into the reference sequence. These mutations of the reference sequence can occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

[00165] The polynucleotide variants can contain alterations in the coding regions, non-coding regions, or both. In some embodiments, a polynucleotide variant contains alterations which produce silent substitutions, additions, or deletions, but does not alter the properties or activities of the encoded polypeptide. In some embodiments, a polynucleotide variant comprises silent substitutions that results in no change to the amino acid sequence of the polypeptide (due to the degeneracy of the genetic code). Polynucleotide variants can be produced for a variety of reasons, for example, to optimize codon expression for a particular host (i.e., change codons in the human mRNA to those preferred by a bacterial host such as *E. coli*). In some embodiments, a polynucleotide variant comprises at least one silent mutation in a non-coding or a coding region of the sequence.

[00166] In some embodiments, a polynucleotide variant is produced to modulate or alter expression (or expression levels) of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to increase expression of the encoded polypeptide. In some embodiments, a polynucleotide variant is produced to decrease expression of the encoded polypeptide. In some embodiments, a polynucleotide variant has increased expression of the encoded polypeptide as compared to a parental polynucleotide sequence. In some embodiments, a polynucleotide variant has decreased expression of the encoded polypeptide as compared to a parental polynucleotide sequence.

[00167] In some embodiments, the polynucleotides are isolated. In some embodiments, the polynucleotides are substantially pure.

[00168] Vectors and cells comprising the polynucleotides described herein are also provided. In some embodiments, an expression vector comprises a polynucleotide molecule. In some embodiments, a host

cell comprises an expression vector comprising the polynucleotide molecule. In some embodiments, a host cell comprises a polynucleotide molecule.

IV. Methods of use and pharmaceutical compositions

[00169] The ANGPTL8-binding agents (e.g., antibodies or antigen-binding fragments thereof) of the disclosure are useful in a variety of applications including, but not limited to, therapeutic treatment methods, such as treatment of diseases or disorders associated with elevated triglycerides. In some embodiments, an ANGPTL8-binding agent described herein is useful in methods for reducing or lowering triglyceride levels. In some embodiments, an ANGPTL8-binding agent described herein is useful in methods for reducing or lowering LDL-cholesterol levels. In some embodiments, an ANGPTL8-binding agent described herein is useful in methods for increasing HDL-cholesterol levels. In some embodiments, an ANGPTL8-binding agent described herein is useful in methods for lowering triglycerides levels and increasing HDL-cholesterol levels. In some embodiments, an ANGPTL8-binding agent described herein is useful in methods for reducing or lowering ANGPTL8 levels (e.g., ANGPTL8 levels in the blood). In some embodiments, an ANGPTL8-binding agent described herein is useful in methods for inhibiting ANGPTL8 activity.

[00170] In some embodiments, a method of lowering triglyceride levels in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of lowering triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of lowering triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8. In some embodiments, a method of lowering triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of lowering triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of lowering triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5.

[00171] In some embodiments, a method of treating a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of treating a disease or disorder associated with

elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of treating a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8. In some embodiments, a method of treating a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of treating a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of treating a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5.

[00172] In some embodiments, a method of treating a subject diagnosed with a disease or disorder associated with elevated triglyceride levels comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of treating a subject diagnosed with a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of treating a subject diagnosed with a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8. In some embodiments, a method of treating a subject diagnosed with a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of treating a subject diagnosed with a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of treating a subject diagnosed with a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5.

[00173] In some embodiments, a method of treating a subject having symptoms of a disease or disorder associated with elevated triglyceride levels comprises administering to the subject an ANGPTL8-binding

agent described herein. In some embodiments, a method of treating a subject having symptoms of a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of treating a subject having symptoms of a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8. In some embodiments, a method of treating a subject having symptoms of a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of treating a subject having symptoms of a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of treating a subject having symptoms of a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5.

[00174] In some embodiments, a method of preventing elevated triglyceride levels in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of preventing elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of preventing elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of preventing elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of preventing elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of preventing elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody is antibody Hz1E5.

[00175] In some embodiments, a method of preventing a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of preventing a disease or disorder associated with

elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of preventing a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of preventing a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of preventing a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of preventing a disease or disorder associated with elevated triglyceride levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody is antibody Hz1E5.

[00176] In some embodiments, a method of treating a subject at risk for elevated triglyceride levels comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of treating a subject at risk for elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of treating a subject at risk for elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of treating a subject at risk for elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of treating a subject at risk for elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of treating a subject at risk for elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody is antibody Hz1E5.

[00177] In some embodiments, a method of treating a subject at risk for a disease or disorder associated with elevated triglyceride levels comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of treating a subject at risk for a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically

effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of treating a subject at risk for a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of treating a subject at risk for a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of treating a subject at risk for a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of treating a subject at risk for a disease or disorder associated with elevated triglyceride levels comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody is antibody Hz1E5.

[00178] In some embodiments, a method of reducing ANGPTL8 levels in a subject wherein the ANGPTL8 levels are elevated, the method comprising administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of reducing ANGPTL8 levels in a subject wherein the ANGPTL8 levels are elevated, the method comprising administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of reducing ANGPTL8 levels in a subject wherein the ANGPTL8 levels are elevated, the method comprising administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of reducing ANGPTL8 levels in a subject wherein the ANGPTL8 levels are elevated, the method comprising administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of reducing ANGPTL8 levels in a subject wherein the ANGPTL8 levels are elevated, the method comprising administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of reducing ANGPTL8 levels in a subject wherein the ANGPTL8 levels are elevated, the method comprising administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody is antibody Hz1E5. In some embodiments, the method of reducing ANGPTL8 levels results in the lowering of triglycerides in the subject. In some embodiments, the method of reducing ANGPTL8 levels results in the prevention of elevated triglycerides in the subject.

[00179] In some embodiments, a method of inhibiting ANGPTL8 activity in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of inhibiting ANGPTL8 activity in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of inhibiting ANGPTL8 activity in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of inhibiting ANGPTL8 activity in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of inhibiting ANGPTL8 activity in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of inhibiting ANGPTL8 activity in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8, wherein the antibody is antibody Hz1E5. In some embodiments, the method of inhibiting ANGPTL8 activity results in the lowering of triglycerides in the subject. In some embodiments, the method of inhibiting ANGPTL8 activity results in the prevention of elevated triglycerides in the subject.

[00180] In some embodiments of the methods described herein, the disease or disorder associated with elevated triglyceride levels is metabolic syndrome, atherosclerosis, obesity, diabetes, cardiovascular disease, hyperlipidemia, hypertriglyceridemia, pancreatitis, renal disease, liver disease, or hypothyroidism.

[00181] In some embodiments of the methods described herein, the disease or disorder associated with elevated triglyceride levels is cardiovascular disease. Cardiovascular disease may include, but is not limited to, atherosclerosis, aneurysm, hypertension, angina, stroke, cerebrovascular diseases, congestive heart failure, coronary artery diseases, myocardial infarction, and peripheral vascular diseases. In some embodiments, the disease or disorder associated with elevated triglyceride levels is atherosclerosis.

[00182] In some embodiments, the disease or disorder associated with elevated triglyceride levels is dyslipidemia, atherogenic dyslipidemia, diabetic dyslipidemia, hypercholesterolemia, chylomicronemia, mixed dyslipidemia, lipodystrophy, or lipoatrophy.

[00183] In some embodiments, the disease or disorder associated with elevated triglyceride levels is hyperlipoproteinemia. There are several type of hyperlipoproteinemia, including primary and secondary hyperlipoproteinemia. Primary hyperlipoproteinemia, is often due to one or more familial or genetic conditions. Persistent hypertriglyceridemia, including hypertriglyceridemia that persists despite treatment of the underlying condition, may be associated with or caused by a primary form of hyperlipoproteinemia,

such as familial hypertriglyceridemia. Additional familial conditions associated with elevated triglyceride levels include familial combined lipidemia and familial dysbetalipoproteinemia (Type III hyperlipoproteinemia). Secondary hyperlipoproteinemia is associated with, and a compounding factor of, several conditions, such as obesity, diabetes, renal disease, liver disease, pancreatitis, hypothyroidism, and alcoholism.

[00184] In some embodiments of the methods described herein, elevated triglyceride levels may be due to increased production and/or decreased elimination of very low-density lipoprotein (VLDL). In some embodiments, elevated triglyceride levels may be due to certain drug treatments, including but not limited to, beta blockers, birth control pills, estrogens, diuretics, steroids, tamoxifen, miconazole, spironolactone, isotretinoin (ACCUTANE), quinapril, or mirtazapine. In some embodiments of the methods described herein, elevated triglyceride levels may be due to genetic predisposition. In some embodiments of the methods described herein, elevated triglyceride levels may be due to diet and/or life style. In some embodiments, elevated triglyceride levels may be due to acute stress associated conditions. Acute stress associated states include but are not limited to, burns, trauma, myocardial infarction, and sepsis.

[00185] In some embodiments of the methods described herein, the elevated triglyceride levels are elevated blood or plasma triglyceride levels. It is generally accepted that a normal triglyceride level is less than 150 mg/dL (or less than 1.7 mmol/L). In some embodiments of the methods described herein, the elevated plasma triglyceride level is 150 mg/dL or higher. In some embodiments, the elevated plasma triglyceride level is about 150 to about 200 mg/dL. In some embodiments, the elevated plasma triglyceride level is about 200 to about 500 mg/dL. In some embodiments, the elevated plasma triglyceride level is about 500 or higher.

[00186] In some embodiments of the methods described herein, after administration of an anti-ANGPTL8 antibody the elevated triglyceride level is reduced by at least 5% as compared to triglyceride levels in the subject prior to the administration of the antibody. In some embodiments, the elevated triglyceride level is reduced by at least 10% as compared to triglyceride levels in the subject prior to the administration of the antibody. In some embodiments, the elevated triglyceride level is reduced by about 10-15% as compared to triglyceride levels in the subject prior to the administration of the antibody. In some embodiments, the elevated triglyceride level is reduced by about 10-20% as compared to triglyceride levels in the subject prior to the administration of the antibody. In some embodiments, the elevated triglyceride level is reduced by about 20-30% as compared to triglyceride levels in the subject prior to the administration of the antibody. In some embodiments, the elevated triglyceride level is reduced by more than 25% as compared to triglyceride levels in the subject prior to the administration of the antibody.

[00187] In some embodiments, a method of increasing HDL-cholesterol levels in a subject is provided. In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of increasing HDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5.

[00188] It is generally accepted that HDL-cholesterol levels of less than 40 mg/dL are considered a risk for heart disease, levels of 40-59 mg/dL are considered good, and levels of 60 mg/dL or higher are considered to be protective against heart disease. In some embodiments of the methods described herein, HDL-cholesterol levels are increased to 40 mg/dL or above.

[00189] In some embodiments of the disclosure, a method of lowering LDL-cholesterol levels in a subject is provided. In some embodiments, a method of lowering LDL-cholesterol levels in a subject comprises administering to the subject an ANGPTL8-binding agent described herein. In some embodiments, a method of lowering LDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an anti-ANGPTL8 antibody described herein. In some embodiments, a method of lowering LDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of lowering LDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of lowering LDL-cholesterol levels in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of lowering LDL-cholesterol levels in a subject comprises administering to the subject a

therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5.

[00190] It is generally accepted that LDL-cholesterol levels of less than 100 mg/dL are optimal, levels of 100-129 mg/dL are considered near optimal, levels of 130-159 mg/dL are considered borderline high, levels of 160-189 mg/dL are high, and levels of 190 mg/dL or above are very high. In some embodiments of the methods described herein, LDL-cholesterol levels are lowered to below about 190 mg/dL, to below about 160 mg/dL, to below about 130 mg/dL, or to below about 100 mg/dL.

[00191] In some embodiments of the methods described herein, the subject is overweight. In some embodiments, the subject is obese. In some embodiments, the subject has diabetes. In some embodiments, the subject has type 2 diabetes. In some embodiments, the subject has cardiovascular disease.

[00192] There has been at least one report that ANGPTL8 levels were elevated in patients with liver disease, such as non-alcoholic fatty liver disease (NAFLD) (see, e.g., Lee et al., 2016, *Nature Scientific Reports*, DOI: 10.1038/srep24013). Thus in some embodiments, a method of treating liver disease in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds human ANGPTL8. In some embodiments, a method of treating liver disease in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs provided in Tables 1-3. In some embodiments, a method of treating liver disease in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody comprises one, two, three, four, five, and/or six CDRs of antibody 1E5. In some embodiments, a method of treating liver disease in a subject comprises administering to the subject a therapeutically effective amount of an antibody that specifically binds ANGPTL8, wherein the antibody is antibody Hz1E5. In some embodiments, the liver disease is NAFLD. In some embodiments, the liver disease is non-alcoholic steatohepatitis (NASH).

[00193] In some embodiments of the methods described herein, a method comprises administering an ANGPTL8-binding agent described herein in combination with at least one additional therapeutic agent or therapeutic therapy. Treatment with two or more therapeutic agents often uses agents that work by different mechanisms of action, although this is not required. Combination therapy using agents with different mechanisms of action may result in additive or synergistic effects. Combination therapy may allow for a lower dose of each agent than is used in monotherapy, thereby reducing toxic side effects and/or increasing the therapeutic index of the agent(s). Combination therapy may decrease the likelihood that resistance to an agent will develop.

[00194] In some embodiments, the combination of an ANGPTL8-binding agent described herein and at least one additional therapeutic agent results in additive or synergistic results. In some embodiments, the combination therapy results in an increase in the therapeutic index of the ANGPTL8-binding agent. In some embodiments, the combination therapy results in an increase in the therapeutic index of the additional therapeutic agent(s). In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the ANGPTL8-binding agent. In some embodiments, the combination therapy results in a decrease in the toxicity and/or side effects of the additional therapeutic agent(s).

[00195] In some embodiments, an additional therapeutic agent can be administered prior to, concurrently with, and/or subsequently to, administration of the ANGPTL8-binding agent. In some embodiments, the at least one additional therapeutic agent comprises 1, 2, 3, or more additional therapeutic agents.

[00196] A combination treatment may comprise one additional therapeutic agent or two or more additional therapeutic agents. Treatment with an ANGPTL8-binding agent can occur prior to, concurrently with, or subsequent to administration of the additional therapeutic agents. Combined administration can include co-administration, either in a single pharmaceutical formulation or using separate formulations, or consecutive administration in either order but generally within a time period such that all active agents can exert their biological activities. Preparation and dosing schedules for additional therapeutic agents can be used according to manufacturers' instructions or as determined empirically by the skilled practitioner.

[00197] Additional therapeutic agents that may be administered in combination with the ANGPTL8-binding agents described herein include, but are not limited to, fibrates, statins, omega-3 fatty acids, and niacin. In some embodiments, an additional therapeutic agent is a fibrate. Fibrates are a class of amphipathic carboxylic acids and include, but are not limited to, aluminum clofibrate, bezafibrate, ciprofibrate, choline fenofibrae, clinofibrate, clofibrate (e.g., ATROMID-S), clofibrate, fenofibrate (e.g., FIBRICOR, LOFIBRA, TRICOR), gemfibrozil (e.g., LOPID), ronifibrate, simfibrate, and fenofibric acid. In some embodiments, an additional therapeutic agent is a statin. Statins are HMG-CoA reductase inhibitors and include, but are not limited to, atorvastatin (LIPITOR), fluvastatin (LESCOL), lovastatin (MEVACOR), pravastatin (PRAVACHOL), rosuvastatin (ZOCOR), and pitavastatin (LIVALO). In some embodiments, the additional therapeutic agent is niacin (vitamin B3). In some embodiments, the additional therapeutic agent is an omega-3 fatty acid.

[00198] In some embodiments, an additional therapeutic agent is an obesity drug. Obesity drugs include, but are not limited to, orlistat (XENICAL), phentermine/topiramate (QSYMIA), lorcaserin (BELVIQ), naltrexone/bupropion (CONTRAVE) and liraglutide (SAXENDA).

[00199] In some embodiments, an additional therapeutic agent is a diabetes drug. Anti-diabetic drugs include, but are not limited to, insulin; PPAR (peroxisome proliferator-activated receptor) γ -agonists, such as pioglitazone, troglitazone, ciglitazone, rivotril, rosiglitazone, and other 2,4-thiazolidinedione derivatives; DPP-4 inhibitors, such as sitagliptin (JANUVIA), vildagliptin, saxagliptin, linagliptin (TRADJENTA), dulaglitin, gemagliptin, and alogliptin (NESINA); GLP-1 analogs, such as exenatide, liraglutide, taspoglutide, albiglutide, and lixisenatide; biguanidine derivatives, such as metformin (GLUMETZA, GLUCOPHAGE), buformin, and phenformin; ATP-sensitive potassium channel modulators, such as metformin, repaglinide, and nateglinide; sulfonylurea derivatives, such as tolbutamide, chlorpropamide, tolazamide, acetohexamide, glipizide, gliclazide, glimepiride, gliclazide, glibornuride, glisoxepid, glibenclamide, glisentide, glisolamide, glyburide, and glycipyramide; α -glucosidase inhibitors, such as miglitol (GLYSET), acarbose (PRECOSE), and voglibose; and SGLT2 inhibitors, such as canagliflozin (INVOKANA), dapagliflozin (FARXIGA), and empagliflozin (JARDIANCE).

[00200] For the treatment of a disease, the appropriate dosage of an ANGPTL8-binding agent of the present disclosure depends on the disorder or disease to be treated, the severity and course of the disorder or disease, the responsiveness of the disorder or disease, whether the agent is administered for therapeutic or preventative purposes, previous therapy, the patient's clinical history, and so on. The ANGPTL8-binding agent can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved.

[00201] It will be appreciated that the combination of an ANGPTL8-binding agent described herein and at least one additional therapeutic agent may be administered in any order or concurrently. In some embodiments, the ANGPTL8-binding agent is administered to subjects that have previously undergone treatment with a therapeutic agent. In some embodiments, the ANGPTL8-binding agent and a second therapeutic agent is administered substantially simultaneously or concurrently. For example, a subject may be given an ANGPTL8-binding agent while undergoing a course of treatment with a second therapeutic agent (e.g., statins). In some embodiments, an ANGPTL8-binding agent is administered within 1 year of the treatment with a second therapeutic agent. In some embodiments, an ANGPTL8-binding agent is administered within 10, 8, 6, 4, or 2 months of any treatment with a second therapeutic agent. In some embodiments, an ANGPTL8-binding agent is administered within 4, 3, 2, or 1 weeks of any treatment with a second therapeutic agent. In some embodiments, an ANGPTL8-binding agent is administered within 5, 4, 3, 2, or 1 days of any treatment with a second therapeutic agent. It will further be appreciated that the two (or more) agents or treatments may be administered to the subject within a matter of hours or minutes (i.e., substantially simultaneously).

[00202] The dose of an ANGPTL8-binding agent described herein may vary depending on the nature and/or severity of the disease or disorder, as well as the condition of the subject. In some embodiments, dosage of the agent is from 0.01 μ g to 100 mg/kg of body weight, from 0.1 μ g to 100 mg/kg of body weight, from 1 μ g to 100 mg/kg of body weight, from 1 mg to 100 mg/kg of body weight, 1 mg to 80 mg/kg of body weight from 10 mg to 100 mg/kg of body weight, from 10 mg to 75 mg/kg of body weight, or from 10 mg to 50 mg/kg of body weight. In some embodiments, dosage of the agent is from about 0.1 mg to about 20 mg/kg of body weight. In some embodiments, dosage of the agent is about 0.5 mg/kg of body weight. In some embodiments, dosage of the agent is about 1 mg/kg of body weight. In some embodiments, dosage of the agent is about 1.5 mg/kg of body weight. In some embodiments, dosage of the agent is about 2 mg/kg of body weight. In some embodiments, dosage of the agent is about 2.5 mg/kg of body weight. In some embodiments, dosage of the agent is about 5 mg/kg of body weight. In some embodiments, dosage of the agent is about 7.5 mg/kg of body weight. In some embodiments, dosage of the agent is about 10 mg/kg of body weight. In some embodiments, dosage of the agent is about 12.5 mg/kg of body weight. In some embodiments, dosage of the agent is about 15 mg/kg of body weight. In some embodiments, the agent is dosed once or more daily, weekly, monthly, or yearly. In some embodiments, the agent is dosed once every week, once every two weeks, once every three weeks, or once every four weeks.

[00203] The present disclosure provides compositions comprising an ANGPTL8-binding agent described herein. The present disclosure also provides pharmaceutical compositions comprising an ANGPTL8-binding agent described herein and a pharmaceutically acceptable vehicle.

[00204] Formulations are prepared for storage and use by combining a purified antibody or agent of the present disclosure with a pharmaceutically acceptable vehicle (e.g., a carrier or excipient). Those of skill in the art generally consider pharmaceutically acceptable carriers, excipients, and/or stabilizers to be inactive ingredients of a formulation or pharmaceutical composition.

[00205] Suitable pharmaceutically acceptable vehicles include, but are not limited to, nontoxic buffers such as phosphate, citrate, and other organic acids; salts such as sodium chloride; antioxidants including ascorbic acid and methionine; preservatives such as octadecyldimethylbenzyl ammonium chloride, hexamethonium chloride, benzalkonium chloride, benzethonium chloride, phenol, butyl or benzyl alcohol, alkyl parabens, such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, 3-pentanol, and m-cresol; low molecular weight polypeptides (e.g., less than about 10 amino acid residues); proteins such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; carbohydrates such as monosaccharides, disaccharides, glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal

complexes such as Zn-protein complexes; and non-ionic surfactants such as TWEEN or polyethylene glycol (PEG). (*Remington: The Science and Practice of Pharmacy, 22nd Edition, 2012, Pharmaceutical Press, London.*). In some embodiments, the formulation is in the form of an aqueous solution. In some embodiments, the formulation is lyophilized or in an alternative dried form.

[00206] The therapeutic formulation can be in unit dosage form. Such formulations include tablets, pills, capsules, powders, granules, solutions or suspensions in water or non-aqueous media, or suppositories. In solid compositions such as tablets the principal active ingredient is mixed with a pharmaceutical carrier. Conventional tableting ingredients include corn starch, lactose, sucrose, sorbitol, talc, stearic acid, magnesium stearate, dicalcium phosphate or gums, and diluents (e.g., water). These can be used to form a solid preformulation composition containing a homogeneous mixture of a compound of the present disclosure, or a non-toxic pharmaceutically acceptable salt thereof. The solid preformulation composition is then subdivided into unit dosage forms of a type described above. The tablets, pills, etc. of the formulation or composition can be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner composition covered by an outer component. Furthermore, the two components can be separated by an enteric layer that serves to resist disintegration and permits the inner component to pass intact through the stomach or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials include a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[00207] The binding agents of the present disclosure may be formulated in any suitable form for delivery to a target cell/tissue. In some embodiments, an ANGPTL8-binding agent can be formulated as a liposome, microparticle, microcapsule, albumin microsphere, microemulsion, nano-particle, nanocapsule, or macroemulsion. In some embodiments, the pharmaceutical formulation includes an agent of the present disclosure complexed with liposomes. Methods to produce liposomes are known to those of skill in the art. For example, some liposomes can be generated by reverse phase evaporation with a lipid composition comprising phosphatidylcholine, cholesterol, and PEG-derivatized phosphatidylethanolamine (PEG-PE).

[00208] In some embodiments, an ANGPTL8-binding agent is formulated as a sustained-release preparation. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing an agent, where the matrices are in the form of shaped articles (e.g., films or microcapsules). Sustained-release matrices include but are not limited to polyesters, hydrogels such as poly(2-hydroxyethyl-methacrylate) or poly(vinyl alcohol), polylactides, copolymers of L-glutamic acid and 7 ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic

acid-glycolic acid copolymer and leuprolide acetate), sucrose acetate isobutyrate, and poly-D-(-)-3-hydroxybutyric acid.

[00209] The pharmaceutical compositions or formulations of the present disclosure can be administered in any number of ways for either local or systemic treatment. Administration can be topical by epidermal or transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders; pulmonary by inhalation or insufflation of powders or aerosols, including by nebulizer, intratracheal, and intranasal; oral; or parenteral including intravenous, intraarterial, intratumoral, subcutaneous, intraperitoneal, intramuscular (e.g., injection or infusion), or intracranial (e.g., intrathecal or intraventricular).

[00210] Various delivery systems are known and can be used to administer an ANGPTL8-binding agent described herein. In some embodiments, an ANGPTL8-binding agent or a composition described herein can be delivered in a controlled release or sustained release system. In some embodiments, a pump may be used to achieve controlled or sustained release. In some embodiments, polymeric materials can be used to achieve controlled or sustained release of the ANGPTL8-binding agent described herein. Examples of polymers used in sustained release formulations include, but are not limited to, poly 2-hydroxy ethyl methacrylate, polymethyl methacrylate, polyacrylic acid, polyethylene-co-vinyl acetate, polymethacrylic acid, polyglycolides (PLG), polyanhydrides, poly N-vinyl pyrrolidone, polyvinyl alcohol (PVA), polyacrylamide, polyethylene glycol (PEG), polylactides (PLA), polylactide-co-glycolides (PLGA), and polyorthoesters. Any polymer used in a sustained release formulation should be inert, free of leachable impurities, stable on storage, sterile, and biodegradable.

[00211] Additional delivery systems can be used to administer an ANGPTL8-binding agent described herein including, but not limited to, injectable drug delivery devices and osmotic pumps. Injectable drug delivery devices include, for example, hand-held devices (e.g., autoinjectors) or wearable devices. Different types of osmotic pump systems may include single compartment systems, dual compartment systems, and multiple compartment systems.

V. Assays and/or kits comprising ANGPTL8-binding agents

[00212] In some embodiments, the anti-ANGPTL8 antibodies and fragments thereof described herein are useful for detecting the presence of ANGPTL8 in a biological sample. Such anti-ANGPTL8 antibodies can include those that bind to human and/or cyno ANGPTL8, but do not inhibit ANGPTL8 activity. The term “detecting” as used herein encompasses quantitative or qualitative detection. In some embodiments, a biological sample comprises a cell, tissue, blood, or other bodily fluid.

[00213] In some embodiments, a method of detecting the presence of ANGPTL8 in a biological sample comprises contacting the biological sample with an anti-ANGPTL8 antibody under conditions permissive

for binding of the anti-ANGPTL8 antibody to ANGPTL8, and detecting whether a complex is formed between the anti-ANGPTL8 antibody and ANGPTL8. The methods may include assays known by those of skill in the art, such as Western blot analyses, radioimmunoassays, ELISAs (enzyme linked immunosorbent assays), “sandwich” immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

[00214] In some embodiments, the anti-ANGPTL8 antibody is tagged with a detectable label. The detectable label may be a fluorescent molecule, a chemiluminescent molecule, a bioluminescent molecule, an enzyme, or a radioisotope.

[00215] The present disclosure provides kits that comprise the ANGPTL8-binding agents described herein and that can be used to perform the methods described herein. In some embodiments, a kit comprises at least one purified ANGPTL8-binding agent in one or more containers. In some embodiments, the kits contain all of the components necessary and/or sufficient to perform a detection assay, including all controls, directions for performing assays, and any necessary software for analysis and presentation of results. One skilled in the art will readily recognize that the disclosed ANGPTL8-binding agents of the present disclosure can be readily incorporated into one of the established kit formats which are well known in the art.

[00216] Further provided are kits that comprise an ANGPTL8-binding agent as well as at least one additional therapeutic agent. In some embodiments, the second (or more) therapeutic agent is a fibrate, statin, niacin, and/or 3-omega fatty acid. In some embodiments, the second (or more) therapeutic agent is a second antibody.

EXAMPLES

Example 1

Overexpression of ANGPTL8 and ANGPTL8 variants in mice

[00217] It has previously been shown that overexpression of both mANGPTL8 and hANGPTL8 in mice significantly increased plasma triglycerides levels (see, e.g., International Publication No. WO 2016/054494). To further define the region(s) of human ANGPTL8 responsible for the increase in triglycerides, variants of human ANGPTL8 with deletions at the N-terminus were generated (Fig. 2A). Recombinant adeno-associated virus (rAAV) vectors expressing human ANGPTL8 or the deletion variants were produced by standard methods. One of skill in the art would know that the signal peptides are processed *in vivo* and human ANGPTL8 would correspond to SEQ ID NO:2. C57BL/6 mice (5-6 mice per a group) were administered 3×10^{11} IU rAAV by tail vein injection in a single dose. An AAV vector expressing green fluorescent protein (AAV-GFP) was used as a control. Twelve days post-injection, blood samples were obtained from tail veins and plasma triglyceride levels were measured.

Triglycerides were assayed using a L-Type Triglyceride M kit (Wako Diagnostics) following the manufacturer's instructions.

[00218] As shown in Fig. 2B, expression of wild-type hANGPTL8 and variant V3 increased plasma triglycerides levels by approximately three-fold. Variant V3 has a deletion of amino acids 22-31 of hANGPTL8 (SEQ ID NO:1). In contrast, expression of variants V2, V4 and V5 did not increase plasma triglyceride levels and appeared to actually reduce triglyceride levels below the control. Variant V2 has a deletion of amino acids 22-80 of hANGPTL8, variant V4 has a deletion of amino acids 22-45 of hANGPTL8, and variant V5 has a deletion of amino acids 22-52 of hANGPTL8. These results suggest that amino acids 32-46 are necessary for human ANGPTL8 to increase triglyceride levels.

Example 2

Generation of antibodies

[00219] Anti-ANGPTL8 antibodies were generated using several different immunogens. Mice were immunized with (i) DNA encoding human ANGPTL8, (ii) a peptide comprising amino acids 25-60 of human ANGPTL8 (hSE1 peptide; SEQ ID NO:6) or (iii) a purified fusion protein comprising human ANGPTL8 (SEQ ID NO:2) linked to human serum albumin (HSA) and a FLAG tag (Flag-HSA-hANGPTL8). Single cell suspensions of lymphocytes were obtained from the spleens and draining lymph nodes of immunized animals after the animals had been determined to have suitable antibody titers. Lymphocytes were fused with SP2/0 myeloma cells by electrofusion. Fused cells were plated into semi-solid media in the presence of HAT selection. After 7-10 days of culture, hybridoma clones were selected and screened as described herein in Example 3.

Example 3

Screening of antibodies

[00220] Hybridoma supernatants from the immunization and fusion campaigns described in Example 2 were screened by ELISA for binding to (i) human ANGPTL8, (ii) human SE1 peptide, (iii) a peptide comprising amino acids 22-46 of hANGPTL8 (SEQ ID NO:1), (iii) a peptide comprising amino acids 61-80 of hANGPTL8, (iv) a peptide comprising amino acids 80-138 of hANGPTL8, and (iv) a peptide comprising amino acids 139-198 of hANGPTL8. Hundreds of antibodies were identified as binders to human ANGPTL8 and/or fragments thereof.

[00221] A subpopulation of the antibodies identified by ELISA were tested *in vivo* for their ability to reduce plasma triglyceride levels in mice expressing hANGPTL8. C57BL/6J mice (6 mice per group) were administered one of the anti-hANGPTL8 antibodies or a control antibody (anti-KLH) by subcutaneous injection (Day -1). Antibodies were given as a single dose of 10 mg/kg. The next day (Day

0), the mice were injected with 1×10^{11} IU of rAAV expressing hANGPTL8 (AAV-hANGPTL8) or a control rAAV expressing green fluorescent protein (AAV-GFP). One day after administration of AAVhANGPTL8 or AAV-GFP, blood was drawn from the tail veins of individual mice and triglyceride levels were determined as described herein.

[00222] As shown in Fig. 3, of the antibodies tested, four different antibodies reduced triglyceride levels or inhibited increased triglyceride levels induced by the expression of hANGPTL8 by approximately three-fold as compared to no antibody treatment. Three of these antibodies, 1E5, 1A8, and 1E9, were determined to bind within the N-terminal region of ANGPTL8 (SE1 peptide; amino acids 25-60).

Example 4

Antibody Binding Affinity

[00223] The binding affinities of antibodies 1E5, 1A8, and 1E9 to human ANGPTL8 were measured using a BIACORE system (GE Healthcare LifeSciences). Briefly, anti-mouse Fc antibody (Sigma-Aldrich) was immobilized on all four flow cells of a CM5 chip using amine coupling reagents (GE Healthcare LifeSciences). Antibody from hybridoma supernatants were captured on flow cells 2, 3, and 4 using flow cell 1 as a reference. The SE1 peptide of human ANGPTL8 (hSE1; SEQ ID NO:6), a corresponding SE1 peptide of cynomolgus monkey ANGPTL8 (cSE1; SEQ ID NO:67), or human ANGPTL8 was injected at a flow rate of 50 μ L/min at 25°C. Kinetic data were collected over time and fit using the simultaneous global fit equation to yield affinity constants (K_D values) for each antibody.

[00224] Human ANGPTL8 (SEQ ID NO:2) was produced as a fusion with bacterial NusA protein (NusA-hANGPTL8). An *E. coli* BL21 transformant containing a NusA-hANGPTL8-expressing construct was induced with 1 mM IPTG at 37°C for 4 hours. The cells were harvested by centrifugation and the cell pellet was solubilized using BugBuster™ Protein Extraction Reagent (Novagen, Inc). NusA-hANGPTL8 was captured using a chelating Sepharose FF column and eluted using PBS containing 250 mM imidazole. The NusA-hANGPTL8 fusion protein was concentrated by ultrafiltration and further purified by column chromatography.

[00225] Assay results are shown in Table 4 and the BIACORE result for 1E5 binding to hANGPTL8 (NusA-hANGPTL8 fusion protein) is shown in Fig. 4.

Table 4

Anti-ANGPTL8 Antibody	hSE1	cSE1	NusA- hANGPTL8
Kd (nM)			
1E5	0.41	0.67	0.44
1A8	0.80	1.04	2.5
1E9	0.92	1.80	2.1

Example 5

Humanized Antibody

[00226] The heavy chain and light chain variable region sequences as well as CDR sequences of antibodies 1E5, 1E9, and 1A8 are shown in Tables 1-3. Antibody 1E5 was humanized by methods known by those skilled in the art and is referred to herein as Hz1E5. The heavy chain variable sequence of Hz1E5 is SEQ ID NO:62 and the light chain variable sequences of Hz1E5 is SEQ ID NO:63.

[00227] The binding affinity of Hz1E5 was determined using a BIACORE system as described herein and compared with the binding affinity of a chimeric 1E5 antibody containing the murine variable regions of 1E5 and a human Fc region. The humanized antibody Hz1E5 had a binding affinity to human SE1 peptide of 0.9 nM as compared to the chimeric antibody's binding affinity of 0.35 nM (Fig. 5A).

[00228] Humanized antibody Hz1E5 was tested *in vivo* for its ability to reduce plasma triglyceride levels in mice expressing hANGPTL8. C57BL/6J mice (6-8 mice per group) were administered parental antibody from hybridoma 1E5, chimeric antibody 1E5, humanized antibody Hz1E5, or a control antibody (anti-KLH) by subcutaneous injection (Day -1). Antibodies were administered as a single dose at 30 mg/kg. The next day (Day 0), the mice were injected with 1×10^{11} IU AAV-hANGPTL8 or control AAV-GFP. One day after injection with the AAVs, blood was obtained from the tail veins of individual mice and triglyceride levels were determined.

[00229] As shown in Fig. 5B, anti-hANGPTL8 antibody Hz1E5 lowered or inhibited the increase of plasma triglycerides induced by expression of hANGPTL8 to a similar extent as the parental antibody. These results suggest that humanization of antibody 1E5 had no effect on the activity of the antibody.

Example 6

Monkey studies

[00230] The ability of anti-ANGPTL8 antibody Hz1E5 to reduce plasma triglyceride levels *in vivo* was studied in cynomolgus monkeys. Hypertriglyceridemic male cynomolgus monkeys were selected and baseline values of serum triglycerides and serum HDL-cholesterol were measured prior to treatment (Day -3). On Day 0, one group of animals (n = 10) received a single dose of anti-hANGPTL8 antibody Hz1E5

(10 mg/kg) by subcutaneous injection. A group of control animals (n = 10) received a single dose of vehicle. Blood samples were taken on Days -2, .25 (6 hrs.), 1, 3, 5, 8, 15, 22, and 29 for determination of serum triglycerides and HDL-cholesterol. During the study, all animals were closely monitored for health status.

[00231] As shown in Fig. 6A, serum triglycerides in hypertriglyceridemic cynomolgus monkeys were significantly reduced as early as 6 hours (p < 0.05 vs vehicle) after a single treatment with anti-ANGPTL8 antibody Hz1E5 as compared to animals treated with control vehicle. The triglyceride level in treated animals gradually returned to pre-treatment levels by Day 29, but remained below levels observed in the control animals. In addition, the anti-ANGPTL8 antibody was effective in increasing HDL-cholesterol levels. HDL-cholesterol levels were significantly higher by Day 1 (p < 0.01 vs vehicle) in the animals treated with anti-ANGPTL8 antibody as compared to animals treated with control after a single dose of antibody (Fig. 6B). As with triglycerides levels, HDL-cholesterol levels gradually returned to pre-treatment levels by Day 29. These results demonstrated that in a non-human primate model for hypertriglyceridemia an exemplary anti-ANGPTL8 antibody is effective in reducing triglycerides levels and increasing HDL-cholesterol levels.

[00232] Although the foregoing present disclosure has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the present disclosure. The embodiments of the present disclosure described herein are intended to be merely exemplary, and those skilled in the art will recognize numerous equivalents to the specific procedures described herein. All such equivalents are considered to be within the scope of the present disclosure and are covered by the embodiments.

[00233] All publications, patents, patent applications, internet sites, and accession numbers/database sequences including both polynucleotide and polypeptide sequences cited herein are hereby incorporated by reference in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

[00234] Following are the sequences disclosed in the application with the exception of the heavy chain and light chain CDR sequences defined in Tables 1-3.

Human ANGPTL8 amino acid sequence with predicted signal sequence underlined (SEQ ID NO:1)
MPVPALCLLWALAMVTRPASAAPMGGPELAQHEELLLLFHGTLQLGQALNGVYRTTEGRL
TKARNSLGLYGRTIELLGQEVSRGRDAAQELRASLLETQMEEDILQLQAEATAEVLGVA
QAQKVLRDSVQRLEVQRLRSAWLGPAYREFEVLKAHADKQSHILWALTGHVQRQRREMVAQ
QHRLRQIQERLHTAALPA

Human ANGPTL8 amino acid sequence without predicted signal sequence (SEQ ID NO:2)
 APMGGPELAQHEELTLLFHGTLQLGQALNGVYRTTEGRLTKARNSLGLYGRTIELLGQEV
 SRGRDAAQELRASLLETQMEEDILQLQAEATAEVILGEVAQAQKVLRDSVQRLEVQLRSAW
 LGPAYREFEVLKAHADKQSHILWALTGHVQRQRREMVAQQHRLRQIQLERLHTAALPA

Human ANGPTL8 amino acids 22-83 (SEQ ID NO:3)

APMGGPELAQHEELTLLFHGTLQLGQALNGVYRTTEGRLTKARNSLGLYGRTIELLGQEV
 SR

Human ANGPTL8 amino acids 84-138 (SEQ ID NO:4)

GRDAAQELRASLLETQMEEDILQLQAEATAEVILGEVAQAQKVLRDSVQRLEVQLR

Human ANGPTL8 amino acids 139-198 (SEQ ID NO:5)

SAWLGPAYREFEVLKAHADKQSHILWALTGHVQRQRREMVAQQHRLRQIQLERLHTAALPA

Human ANGPTL8 amino acids 25-60 (hSE1 peptide) (SEQ ID NO:6)

GGPELAQHEELTLLFHGTLQLG

Human ANGPTL8 amino acids 22-46 (Sumo peptide) (SEQ ID NO:7)

APMGGPELAQHEELTLLFHGTLQLG

Human ANGPTL8 amino acids 32-46 (SEQ ID NO:8)

HEELTLLFHGTLQLG

Cynomolgus monkey ANGPTL8 amino acid sequence with signal sequence (SEQ ID NO:9)
 MLVPALCLLWALAMVIQPASAAPVGSPELAEHEELTLLFHGTLQLGQALNGVYKTTEGRL
 TKARNSLGLYGRTVELLGQEVSRGRDAAQELRASLLETQMEEDILQLKAEAIAEVLEEVA
 QAQKVLQDSVRRLEVQLRSAWLGPAYQEFEVLKAHADKQSHILWALTGHVQRQRREMVAQ
 QHRLRQIQLERIHKAAALPA

Mouse ANGPTL8 amino acid sequence with signal sequence (SEQ ID NO:10)

MAVLALCCLLWTLASAVRPAPVAPLGGPEPAQYEELTLLFHGALQLGQALNGVYRATEARL
 TEAGHSLGLYDRALEFLGTEVRQGQDATQELRTSLSIQLQEDALHLRAEATARSLEVA
 RAQQALRDTVRRLQVQLRGAWLGQAHQEFTLKARADKQSHILWALTGHVQRQQREMAEQ
 QQWLRQIQLRLHTAALPA

1E5 Heavy chain variable region amino acid sequence (SEQ ID NO:35)

QVQLQQSGAELVKPGTSVRLSCKASGYFTDYTIHWVKLRSQGLEWIGWFYPGSDNIKY
 NAKFKDKATLTADKSSSTVYMDLGRLTSEDSAVYFCARHEAFSYYDVAWFAYWGQTLVT
 VSA

1E5 Light chain variable region amino acid sequence (SEQ ID NO:36)

DVVMQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYTVSNRF
 SGVPDRFSGSGSGDFTLNFSRVEAEDLGVYFCSQTTHFPYTGGGTKLEIK

1E9 Heavy chain variable region amino acid sequence (SEQ ID NO:44)

QVQLQQSGTELVKPGASVKLSCKASGYFTDYTIHWVKQRSQGLEWIGWFYPGSDNIKF
 NAKFRDKATLTADKSSSTVYMELSRLTSEDSAVYFCARHEAFYVYDVAWFANWGQGTIVT
 VST

1E9 Light chain variable region amino acid sequence (SEQ ID NO:45)

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWFLQKPGQSPNLLIYTVDNRF
SGVPDRFSGSGSGDFTLKISRVEAGDLGVYFCSQSTHFPYTFGGGTKLEIK

1A8 Heavy chain variable region amino acid sequence (SEQ ID NO:56)

QVQLQQSGAELVKPGASVKLSCKASGYFTDYTIHWVKQRSGQGLEWIGWFYPGSDNIKY
NEFKFDKATLTADKSSSIVYMELSRLTSEDSAVYFCARHEAYYVYDVAWFAYWGQGTIVT
VSA

1A8 Light chain variable region amino acid sequence (SEQ ID NO:57)

DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTFLHWFLQRPQSPKLLIYTVDNRF
SGVPDRFSGSGSGDFTLKISRVEAEDLGIVYFCSQSTHFPYTFGGGTLEIK

Heavy Chain CDR2 consensus sequence (SEQ ID NO:58)

WFYPGSDNIKX₁NX₂KFX₃D

X₁ = Y or F; X₂ = A or E; X₃ = K or R

Heavy chain CDR3 consensus sequence (SEQ ID NO:59)

HEAX₁X₂X₃YDVAWFAX₄

X₁ = F or Y; X₂ = S or Y; X₃ = Y or V; X₄ = Y or N

Light chain CDR1 consensus sequence (SEQ ID NO:60)

X₁SSQSLVHSNGNTX₂LH

X₁ = R or S X₂ = Y or F

Light chain CDR3 consensus sequence (SEQ ID NO:61)

SQX₁THFPYT

X₁ = T or S

H21E5.A1 Heavy chain variable region amino acid sequence (SEQ ID NO:62)

EVQLVQSGAEVKKPGSSVKVSCKASGYFTDYTIHWVRQAPGQGLEWMWFYPGSDNIKY
NAFKFDKATLTADKSTSTAYMELSSLRSEDTAVYYCARHEAFSYYDVAWFAYWGQGTIVT
VSS

H21E5.A1 Light chain variable region amino acid sequence (SEQ ID NO:63)

DVVMTQTPLSLPVTPGEPASISCRSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYTVDNRF
SGVPDRFSGSGSGDFTLKISRVEAEDVGIVYFCSQSTHFPYTFGQGTKEIK

H21E5.A1 Heavy chain amino acid sequence without signal sequence (SEQ ID NO:64)

EVQLVQSGAEVKKPGSSVKVSCKASGYFTDYTIHWVRQAPGQGLEWMWFYPGSDNIKY
NAFKFDKATLTADKSTSTAYMELSSLRSEDTAVYYCARHEAFSYYDVAWFAYWGQGTIVT
VSSASTKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEPVTVWSNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHPKSNTKVDKVEPKSCDKHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKVGFYPSDIAVEWESENQGPENNYKTPPVLDSDGSFFLYSKLVDK
SRWQQGNVFSCSVMHEALHNHTQKSLSLSPGK

H21E5.A1 Light chain amino acid sequence without signal sequence (SEQ ID NO:65)

DVVMTQTPLSLPVTPGEPASISCRSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYTVDNRF
SGVPDRFSGSGSGDFTLKISRVEAEDVGIVYFCSQSTHFPYTFGQGTKEIKRTVAAPSV
FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL

SSTLTL SKADYE KHKVY ACEVTHQGLSSPVTKS FNR GEC

FLAG tag (SEQ ID NO:66)
DYKDDDDK

Cyno ANGPTL8 amino acids 25-60 (cSEL peptide) (SEQ ID NO:67)
GSPELAEHEELTLLFHGTLQLGQALNGVYKTTEGRL

SEQUENCE LISTING

[00235] The present specification is being filed with a computer readable form (CRF) copy of the Sequence Listing. The CRF entitled 13370-057-228_ST25.txt, which was created on October 27, 2018 and is 37,767 bytes in size, is incorporated herein by reference in its entirety.

WHAT IS CLAIMED:

1. An antibody or antigen-binding fragment thereof that competes for binding to angiopoietin-like protein 8 (ANGPTL8) with a reference antibody, wherein the reference antibody comprises:
 - (a) a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y or V, and X₄ is Y or N (SEQ ID NO:59); and/or
 - (b) a light chain CDR1 comprising X₁SSQLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61).
2. An antibody or antigen-binding fragment thereof that binds an epitope on ANGPTL8 recognized by a reference antibody, wherein the reference antibody comprises:
 - (a) a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX₁NX₂KFX₃D, wherein X₁ is Y or F, X₂ is A or E and X₃ is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX₁X₂X₃YDVAWFAX₄, wherein X₁ is F or Y, X₂ is S or Y, X₃ is Y or V, and X₄ is Y or N (SEQ ID NO:59); and/or
 - (b) a light chain CDR1 comprising X₁SSQLVHSNGNTX₂LH, wherein X₁ is R or S and X₂ is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX₁THFPYT, wherein X₁ is T or S (SEQ ID NO:61).
3. The antibody or antigen-binding fragment thereof of claim 1 or claim 2, wherein the reference antibody comprises:
 - (a) a heavy chain CDR1 comprising SEQ ID NOS:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOS:16, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOS:21, 22, 23, or 24, and
 - (b) a light chain CDR1 comprising SEQ ID NOS:25, 26, 27, or 28, a light chain CDR2 comprising SEQ ID NOS:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOS:32, 33, or 34.

4. The antibody or antigen-binding fragment thereof of claim 1 or claim 2, wherein the reference antibody comprises:
 - (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69, and
 - (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43.
5. The antibody or antigen-binding fragment thereof of claim 1 or claim 2, wherein the reference antibody comprises:
 - (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, or 51, and
 - (b) a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43.
6. The antibody or antigen-binding fragment thereof of any one of claims 1 to 5, wherein the reference antibody further comprises (a) a heavy chain variable region FR1, a heavy chain variable region FR2, a heavy chain variable region FR3, and a heavy chain variable region FR4, and (b) a light chain variable region FR1, a light chain variable region FR2, a light chain variable region FR3, and a light chain variable region FR4.
7. The antibody or antigen-binding fragment thereof of any one of claims 1 to 6, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NOs:25, 44, 56, or 62; and a light chain variable region comprising SEQ ID NOs:26, 45, 57, or 63.
8. The antibody or antigen-binding fragment thereof of claim 7, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:25 and a light chain variable region comprising SEQ ID NO:26.

9. The antibody or antigen-binding fragment thereof of claim 7, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45.
10. The antibody or antigen-binding fragment thereof of claim 7, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57.
11. The antibody or antigen-binding fragment thereof of claim 7, wherein the reference antibody comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63.
12. The antibody or antigen-binding fragment thereof of any one of claims 1 to 11, wherein the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:3.
13. The antibody or antigen-binding fragment thereof of any one of claims 1 to 11, wherein the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:6.
14. The antibody or antigen-binding fragment thereof of any one of claims 1 to 11, wherein the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:7.
15. The antibody or antigen-binding fragment thereof of any one of claims 1 to 11, wherein the reference antibody binds an N-terminal region of ANGPTL8, wherein the N-terminal region comprises SEQ ID NO:8.
16. An antibody or antigen-binding fragment thereof that specifically binds an N-terminal region of human ANGPTL8, wherein the N-terminal region comprises amino acids 25-60 of SEQ ID NO:1 and wherein the antibody (i) lowers triglyceride levels, (ii) lowers LDL-cholesterol, and/or (iii) increases HDL-cholesterol in a subject.

17. An antibody or antigen-binding fragment thereof that specifically binds human ANGPTL8, which comprises:
 - (a) a heavy chain CDR1 comprising GYTFTDYTIH (SEQ ID NO:11); a heavy chain CDR2 comprising WFYPGSDNIKX1NX2KFX3D, wherein X1 is Y or F, X2 is A or E and X3 is K or R (SEQ ID NO:58); and a heavy chain CDR3 comprising HEAX1X2X3YDVAWFAX4, wherein X1 is F or Y, X2 is S or Y, X3 is Y or V, and X4 is Y or N (SEQ ID NO:59); and/or
 - (b) a light chain CDR1 comprising X1SSQSLVHSNGNTX2LH, wherein X1 is R or S and X2 is Y or F (SEQ ID NO:60); a light chain CDR2 comprising TVSNRFS (SEQ ID NO:29); and a light chain CDR3 comprising SQX1THFPYT, wherein X1 is T or S (SEQ ID NO:61).
18. The antibody or antigen-binding fragment thereof of claim 16 or claim 17, which comprises:
 - (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:16, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:21, 22, 23, or 24, and
 - (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 28, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:32, 33, or 34.
19. The antibody or antigen-binding fragment thereof of claim 16 or claim 17, which comprises:
 - (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:37, 17, 18, 19, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:38, 39, 68, or 69, and
 - (b) a light chain CDR1 comprising SEQ ID NOs:25, 26, 27, or 40, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43.
20. The antibody or antigen-binding fragment thereof of claim 16 or claim 17, which comprises:
 - (a) a heavy chain CDR1 comprising SEQ ID NOs:11, 12, 13, 14, or 15, a heavy chain CDR2 comprising SEQ ID NOs:46, 17, 18, 47, or 20, and a heavy chain CDR3 comprising SEQ ID NOs:48, 49, 50, or 51, and

- (b) a light chain CDR1 comprising SEQ ID NOs:52, 53, 54, or 55, a light chain CDR2 comprising SEQ ID NOs:29, 30, or 31, and a light chain CDR3 comprising SEQ ID NOs:41, 42, or 43.

21. The antibody or antigen-binding fragment thereof of any one of claims 17 to 20, which further comprises: (a) a heavy chain FR1, a heavy chain FR2, a heavy chain FR3, and a heavy chain FR4; and/or (b) a light chain FR1, a light chain FR2, a light chain FR3, and a light chain FR4.

22. The antibody or antigen-binding fragment thereof of claim 16 or claim 17, which comprises:

- (a) a heavy chain variable region having at least 90% sequence identity to SEQ ID NOs:35, 44, 56, or 62; and/or
- (b) a light chain variable region having at least 90% sequence identity to SEQ ID NOs:36, 45, 57, or 63.

23. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:35 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:36.

24. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:44 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:45.

25. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:56 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:57.

26. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region having at least 95% sequence identity to SEQ ID NO:62 and a light chain variable region having at least 95% sequence identity to SEQ ID NO:63.

27. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region comprising SEQ ID NO:35, and a light chain variable region comprising SEQ ID NO:36.

28. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45.
29. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57.
30. The antibody or antigen-binding fragment thereof of claim 22, which comprises a heavy chain variable region comprising SEQ ID NO:62 and a light chain variable region comprising SEQ ID NO:63.
31. An antibody or antigen-binding fragment thereof that specifically binds ANGPTL8, which comprises the heavy chain CDR1, CDR2, and CDR3, and/or the light chain CDR1, CDR2, and CDR3 from:
 - (a) the antibody designated 1E5 that comprises a heavy chain variable region comprising SEQ ID NO:35 and a light chain variable region comprising SEQ ID NO:36;
 - (b) the antibody designated 1E9 that comprises a heavy chain variable region comprising SEQ ID NO:44 and a light chain variable region comprising SEQ ID NO:45; or
 - (c) the antibody designated 1A8 that comprises a heavy chain variable region comprising SEQ ID NO:56 and a light chain variable region comprising SEQ ID NO:57.
32. The antibody or antigen-binding fragment thereof of claim 31, which comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:35 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:36.
33. The antibody or antigen-binding fragment thereof of claim 31, which comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:44 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:45.
34. The antibody or antigen-binding fragment thereof of claim 31, which comprises the CDR1, CDR2, and CDR3 from a heavy chain variable region comprising SEQ ID NO:56 and the CDR1, CDR2, and CDR3 from a light chain variable region comprising SEQ ID NO:57.

35. An antibody or antigen-binding fragment thereof that specifically binds ANGPTL8, wherein the antibody comprises:
 - (a) a heavy chain variable region comprising a CDR1, a CDR2, and a CDR3 amino acid sequence depicted in Tables 1-3; and/or
 - (b) a light chain variable region comprising a CDR1, a CDR2, and a CDR3 amino acid sequence depicted in Tables 1-3.
36. An antibody that specifically binds ANGPTL8, which comprises a heavy chain of SEQ ID NO:64 and a light chain of SEQ ID NO:65.
37. The antibody or antigen-binding fragment thereof of any one of claims 1 to 36, wherein the antibody is a monoclonal antibody.
38. The antibody or antigen-binding fragment thereof of any one of claims 1 to 37, wherein the antibody is a humanized, human, or chimeric antibody.
39. The antibody or antigen-binding fragment thereof of any one of claims 1 to 38, which is a Fab, Fab', F(ab')₂, Fv, scFv, (scFv)₂, single chain antibody, dual variable region antibody, single variable region antibody, linear antibody, a V region, a bispecific antibody, or a multispecific antibody.
40. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which is conjugated to a detectable marker.
41. The antibody or antigen-binding fragment thereof of claim 40, wherein the detectable marker is selected from a radioisotope, a metal chelator, an enzyme, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.
42. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which is conjugated to a cytotoxic agent.
43. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which is an antagonist of ANGPTL8.

44. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which inhibits ANGPTL8 activity.
45. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which lowers triglyceride levels.
46. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which lowers LDL-cholesterol levels.
47. The antibody or antigen-binding fragment thereof of any one of claims 1 to 39, which increases HDL-cholesterol levels.
48. A pharmaceutical composition that comprises the antibody or antigen-binding fragment thereof of any one of claims 1 to 42, and a pharmaceutically acceptable carrier.
49. An isolated polynucleotide molecule that encodes the antibody of any one of claims 1 to 39.
50. A vector comprising the polynucleotide of claim 49.
51. An isolated cell comprising the polynucleotide of claim 49 or the vector of claim 50.
52. A host cell producing the antibody of any one of claims 1 to 39.
53. A hybridoma that produces the monoclonal antibody of claim 37.
54. A transgenic animal that produces the antibody of any one of claims 1 to 39.
55. A binding agent that binds to essentially the same epitope on ANGPTL8 as an antibody of any one of claims 1 to 39.
56. A binding agent that specifically binds ANGPTL8 which comprises a scaffold protein and comprises one or more CDRs as shown in Tables 1-3.

57. A binding agent which competes with the antibody of any one of claims 1 to 39 for binding to ANGPTL8.
58. A method of lowering triglyceride levels in a subject with elevated triglyceride levels, the method comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39.
59. A method of treating a disease or disorder associated with elevated triglyceride levels in a subject, the method comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39.
60. The method of claim 59, wherein the disease or disorder is metabolic syndrome, obesity, diabetes, cardiovascular disease, hyperlipidemia, hypertriglyceridemia, pancreatitis, renal disease, or hypothyroidism.
61. The method of any one of claims 58 to 60, wherein the elevated triglyceride levels are elevated plasma triglyceride levels.
62. The method of claim 61, wherein the elevated plasma triglyceride level is 150 mg/dL or higher.
63. The method of claim 61, wherein the elevated plasma triglyceride level is about 150 to about 200 mg/dL.
64. The method of claim 61, wherein the elevated plasma triglyceride level is about 200 to about 500 mg/dL.
65. The method of claim 61, wherein the elevated plasma triglyceride level is about 500 or higher.
66. The method of any one of claims 58 to 65, wherein the elevated triglyceride level is reduced by at least 5% as compared to triglyceride levels in the subject prior to the administration of antibody.
67. The method of any one of claims 58 to 65, wherein the elevated triglyceride level is reduced by at least 10% as compared to triglyceride levels in the subject prior to the administration of antibody.

68. The method of any one of claims 58 to 65, wherein the elevated triglyceride level is reduced by about 10-15% as compared to triglyceride levels in the subject prior to the administration of antibody.
69. The method of any one of claims 58 to 65, wherein the elevated triglyceride level is reduced by about 10-20% as compared to triglyceride levels in the subject prior to the administration of antibody.
70. The method of any one of claims 58 to 65, wherein the elevated triglyceride level is reduced by about 20-30% as compared to triglyceride levels in the subject prior to the administration of antibody.
71. The method of any one of claims 58 to 65, wherein the elevated triglyceride level is reduced by more than 25% as compared to triglyceride levels in the subject prior to the administration of antibody.
72. A method of increasing HDL-cholesterol levels in a subject, the method comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39.
73. A method of reducing LDL-cholesterol levels in a subject, the method comprising administering to the subject a therapeutically effective amount of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39.
74. The method of any one of claim 58 to 73, wherein the subject is overweight or obese.
75. The method of any one of claims 58 to 74, wherein the subject is administered one or more additional therapeutic agents.
76. The method of claim 75, wherein the therapeutic agent is selected from the group consisting of: a fibrate, a statin, omega-3 fatty acids, and niacin.
77. Use of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39 in the manufacture of a medicament for lowering triglyceride levels.

78. Use of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39 in the manufacture of a medicament for treatment of a disease or disorder associated with elevated triglyceride levels.
79. Use of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39 in the manufacture of a medicament for increasing HDL-cholesterol levels.
80. Use of the antibody or antigen-binding fragment thereof of any one of claims 1 to 39 in the manufacture of a medicament for lowering LDL-cholesterol levels.

FIG. 1B

Light chain variable region sequence alignments

	Kabat	1	10	20	24-27-----34	40	50-----56
AbM		1	10	20	24-----30-----34	40	50-----56
Chothia		1	10	20	26-----32	40	50--
Contact		1	10	20	30-----36	40	46-----55
IMGT		1			27-----38	41	56-65 69
AHon		1		23	42	58	72
1E5	DVVMQTPLSLPVLGQASISC		RSSQSLVHSNGNTYLH	WYIQLQKPGQSPKLLIY	TVSNRFS		
1E9	DVVMQTPLSLPVLGQASISC		RSSQSLVHSNGNTYLH	WFILQKPGQSPNLLIY	TVSNRFS		
1A8	DVVMQTPLSLPVLGQASISC		SSSQSLVHSNGNTFLH	WFILQRPQGQSPKLLIY	TVSNRFS		
Consensus			XSSQSLVHSNGNTXLH				
			(SEQ ID NO: 60)	(SEQ ID NO: 29)	(SEQ ID NO: 29)		
	Kabat	60	70	80	89-----97		
	AbM	60	70	80	89-----97		
	Chothia	60	70	80	91----96		
	Contact	60	70	80	89----96		
	IMGT	70	89	91	105-----117		
	AHon	73			107	138	
1E5	GVPDRFSGSGSGSDFTLNSRVEAEDLGVYFC		SQTTHEPYT	FGGGTKLEIK	(SEQ ID NO: 36)		
1E9	GVPDRFSGSGSGSGTDFTLKISRVEAGDLGVYFC		SQSTHEPYT	FGGGTKLEIK	(SEQ ID NO: 45)		
1A8	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC		SQSTHEPYT	FGGGTITLEIK	(SEQ ID NO: 57)		
Consensus			SQXTHFPYT				
			(SEQ ID NO: 61)				

FIG. 2A

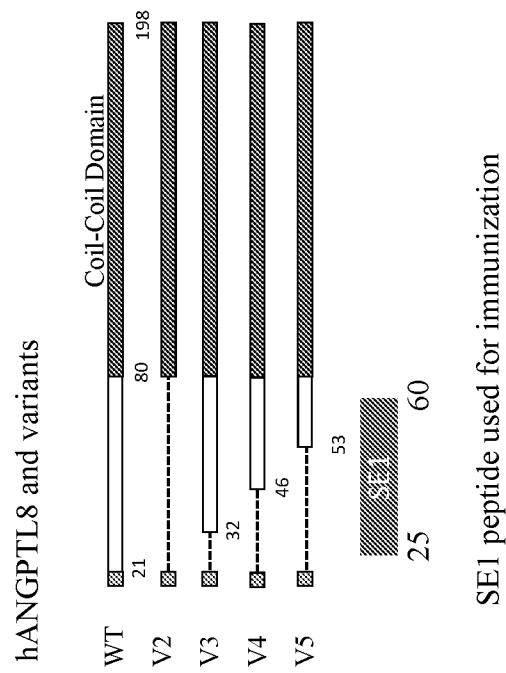


FIG. 2B

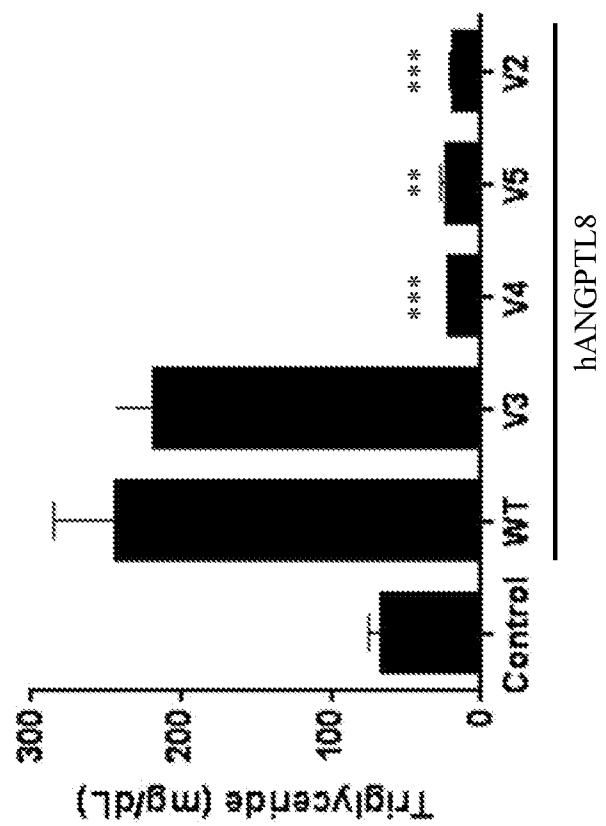


FIG. 3

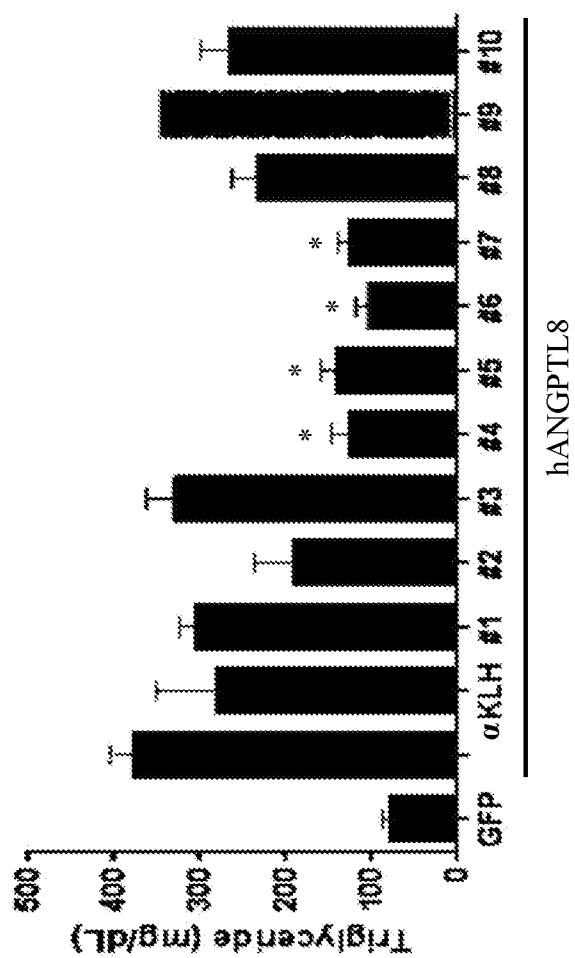


FIG. 4

NusA-hANGPTL8



FIG. 5A
hANGPTL8 SE1 peptide

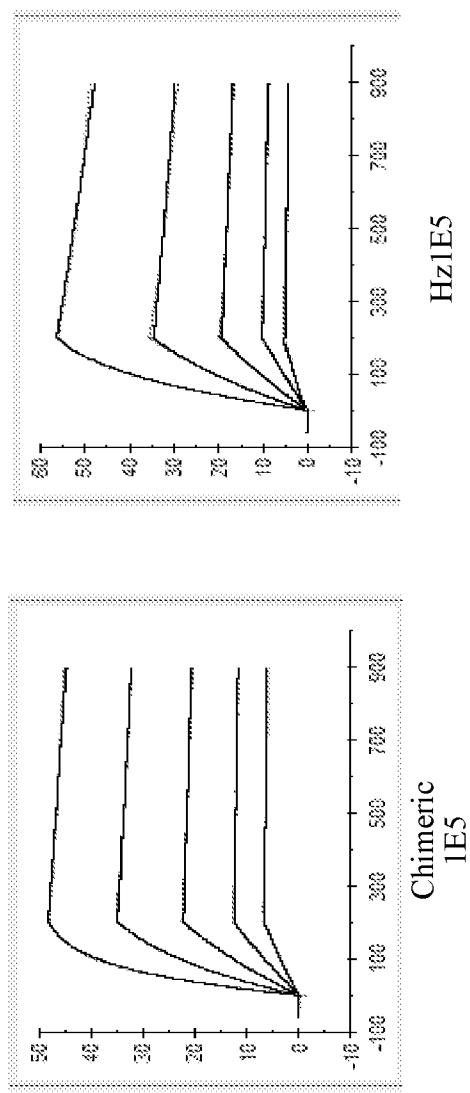


FIG. 5B

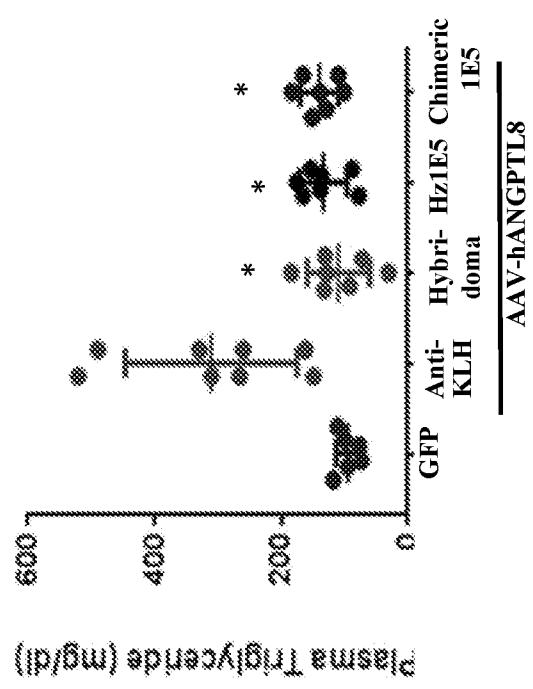


FIG. 6A

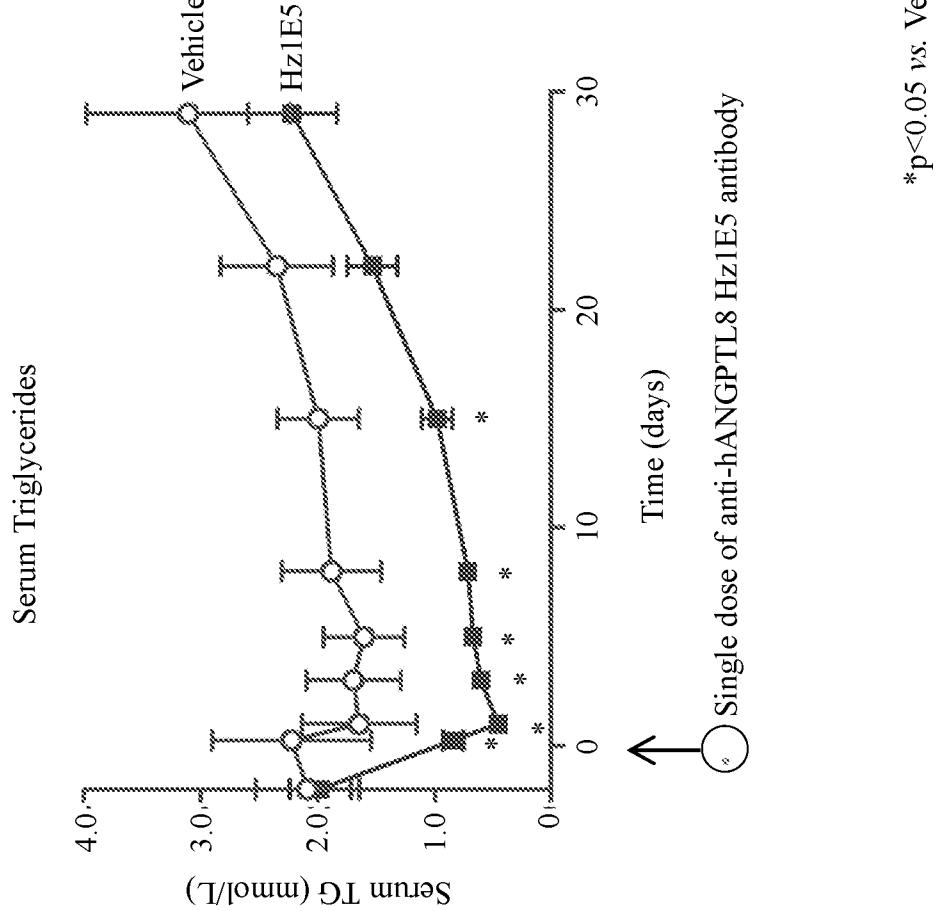
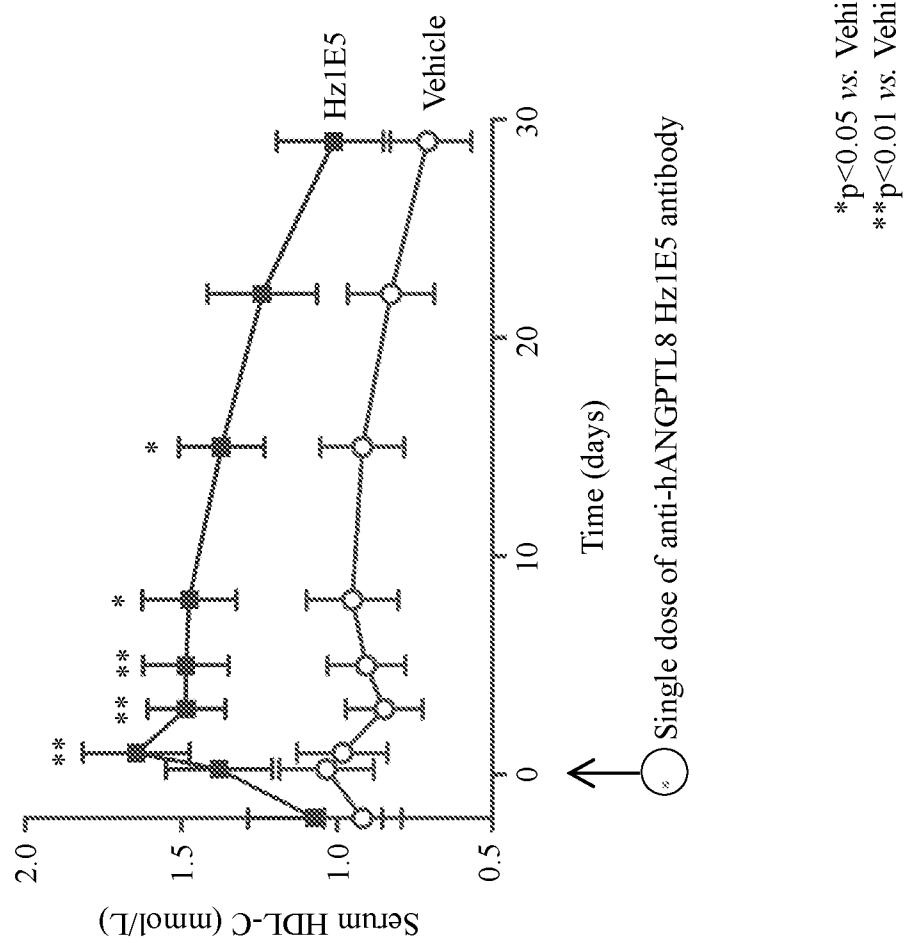


FIG. 6B

Serum HDL-cholesterol



WO 2019/094533

INTERNATIONAL SEARCH REPORT

PCT/US2018/059734

International application No.

PCT/US 18/59734

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 6-15, 21, 37-55, 57-80 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

- see extra sheet for Box No. III Observations where unity of invention is lacking -

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-2, 16, 17, 22, 35, 56 limited to SEQ ID NOS: 11, 16, 21, 35

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/59734

A. CLASSIFICATION OF SUBJECT MATTER
 IPC(8) - C07K 16/26, A61K 39/395 (2019.01)
 CPC - C07K 16/26, A61K 39/39575, C07K 16/22, C07K 2317/34, C07K 2317/76, C07K 2317/92

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017/0037124 A1 (REGENERON PHARMACEUTICALS, INC.) 09 February 2017 (09.02.2017) para [0008], [0010], [0012], [0020] SEQ ID NO: 337	16
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A		1-2, 17, 22, 35, 56

A	WO 2011/084714 A2 (BIOGEN IDEC MA Inc.) 14 July 2011 (14.07.2011) abstract, SEQ ID NO: 59	1-2, 17, 22, 35, 56
A	US 2016/0264662 A1 (THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICE et al.) 15 September 2016 (15.09.2016) para [0167]	1-2, 17, 22, 35, 56

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance
 "E" earlier application or patent but published on or after the international filing date
 "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
 "O" document referring to an oral disclosure, use, exhibition or other means
 "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
 "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
 "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
 "&" document member of the same patent family

Date of the actual completion of the international search

04 March 2019

Date of mailing of the international search report

22 MAR 2019

Name and mailing address of the ISA/US

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 Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 18/59734

Continuation of:

Box No. III. Observations where unity of invention is lacking

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I+: Claims 1-5, 16-20, 22-36, 56, drawn to an antibody or antigen-binding fragment thereof that binds to angiopoietin-like protein 8 (ANGPTL8). The anti-ANGPTL8 antibody will be searched to encompass:
HCDR1 comprising GYTFTDYTIH (SEQ ID NO: 11);
HCDR2 comprising WFYPGSDNIK-X1-N-X2-KF-X3-D, wherein X1 is Y, X2 is A, X3 is K (SEQ ID NO: 58) (corresponding to WFYPGSDNIKYNNAKFKD (SEQ ID NO: 16));
HCDR3 comprising HEA-X1-X2-X3-YDVAWFAY-X4, wherein X1 is F, X2 is S, X3 is Y, and X4 is Y (SEQ ID NO: 59) (corresponding to HEAFSYYDVAWFAY (SEQ ID NO: 21));
Heavy chain comprising SEQ ID NO: 35.

It is believed that claims 1-2, 16, 17, 22, 35, 56, encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass SEQ ID NOs: 11, 16, 21, 35. Additional anti-ANGPTL8 antibody will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected anti-ANGPTL8 antibody. Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched.

An exemplary election would be an anti-ANGPTL8 antibody encompasses:
LCDR1 comprising X1-SSQSLVHSNGNT-X2-LH, wherein X1 is R, X2 is Y (SEQ ID NO: 60) (corresponding to RSSQSLVHSNGNTYLH (SEQ ID NO: 25));
LCDR2 comprising TVSNRFS (SEQ ID NO: 29);
LCDR3 comprising SQ-X1-THFPYT, wherein X1 is T (corresponding to SQTTHFPYT (SEQ ID NO: 32).);
Light chain comprising SEQ ID NO: 36 (Claims 1-3, 16-18, 22, 23, 31, 32, 35, 56, limited to SEQ ID NOs: 25, 29, 32, 36).

The inventions listed as Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

No technical features are shared between the amino acid sequences of anti-ANGPTL8 antibodies of Groups I+ and, accordingly, these groups lack unity a priori.

Additionally, even if Groups I+ were considered to share the technical features of including: an anti-ANGPTL8 antibody, these shared technical features are previously disclosed by US 2017/0037124 A1 to Regeneron Pharmaceuticals, Inc. (hereinafter 'Regeneron').

Regeneron teaches (instant claim 1) an antibody or antigen-binding fragment thereof that competes for binding to angiopoietin-like protein 8 (ANGPTL8) with a reference antibody (para [0010], a monoclonal antibody or antigen-binding fragment thereof that specifically binds to ANGPTL8.; [0020], j) cross-competes with a reference antibody.), wherein the reference antibody comprises: a heavy chain CDR1, CDR2 and CDR3; and/or a light chain CDR1, CDR2, and CDR3 (para [0020], wherein the reference antibody comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR) amino acid sequence selected from the group consisting of any of the HCVR and LCVR amino acid sequences of Table 1.).

Regeneron teaches (instant claim 2) an antibody or antigen-binding fragment thereof that binds an epitope on ANGPTL8 recognized by a reference antibody (para [0010], a monoclonal antibody or antigen-binding fragment thereof that specifically binds to ANGPTL8.; [0012], b) binds specifically to a linear epitope in the N-terminal region of human ANGPTL8.; [0020], j) cross-competes with a reference antibody.).

Regeneron teaches (instant claim 16) an antibody or antigen-binding fragment thereof that specifically binds an N-terminal region of human ANGPTL8, wherein the N-terminal region comprises amino acids 25-60 of SEQ ID NO: 1 (para [0012], b) binds specifically to a linear epitope in the N-terminal region of human ANGPTL8 as defined by SEQ ID NO: 337, amino acid residues 4-39 is 100% match to amino acid residues 25-60 of claimed SEQ ID NO: 1.); and wherein the antibody (i) lowers triglyceride levels (para [0008], One aspect of the invention provides human antibodies and antigen-binding fragments thereof that bind to/interact with ANGPTL8, whereby such binding and/or interaction results in the lowering of triglyceride levels in a mammal.).

Regeneron teaches (instant claims 17, 31, 35) an antibody or antigen-binding fragment thereof that specifically binds human ANGPTL8 (para [0010], a monoclonal antibody or antigen-binding fragment thereof that specifically binds to ANGPTL8.).

The inventions of Group I+ further shares the feature of the technical feature of a binding agent that comprises a scaffold protein and one or more CDRs (instant claim 56), which is disclosed by US 2016/0264662 A1 to The United States Of America, As Represented By The Secretary, Department Of Health And Human Service et al. (hereinafter 'HHS') (para [0167], one or more of the heavy and/or light chain complementarity determining regions (CDRs) from a disclosed antibody is expressed on the surface of another protein, such as a scaffold protein.).

As said technical features were known in the art at the time of the invention, these cannot be considered special technical features that would otherwise unify the groups.

Groups I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.