

Anti-UNC5C antibodies and uses thereof

This application claims priority from GB 2319255.2 filed 15 December 2023, US 63/669394 filed 10 July 2024, and US 63/716754 filed 06 November 2024, the contents and elements of each of which are herein
5 incorporated by reference for all purposes.

Field of the Disclosure

The present invention relates to assays for detecting the level of soluble UNC5C in a biological fluid sample from a patient, and to antibodies and fragments thereof capable of binding to UNC5C, to the use of such
10 novel antibodies for detecting the level of soluble UNC5C in a biological fluid sample from a patient. Methods for detecting the level of soluble UNC5C in a biological fluid sample from a patient using anti-UNC5C antibodies in a clinical context are also described.

Background

15 Unc-5 netrin receptor C (UNC5C) belongs to the UNC5 family of cell membrane netrin receptors, which are part of the immunoglobulin superfamily (Leonardo, E., *et al.* (1997)). UNC5 family members have two extracellular Ig domains and two thrombospondin type 1 (TSP-1) domains. The intracellular c terminus consists of a zona occludens 5 (ZU5) domain, a DCC-binding domain and a death domain involved in apoptosis regulation (Ackerman SL, Knowles BB. 1998). Four subtypes of UNC5 have been identified,
20 namely UNC5 A-D (referred to as UNC5 H1-4 in other mammalian species), which are widely expressed in the central nervous system (CNS) (Rajasekharan, S., Kennedy, T.E. (2009)). Additional netrin receptors include Down's syndrome cell adhesion molecule (DSCAM), neogenin, Deleted in Colorectal Cancer (DCC) and CD146 (also known as Melanoma Cell Adhesion Molecule (MCAM or Mel-CAM). These receptors differ in the number of n-terminal Ig domains, inclusion of fibronectin type III domains and intracellular domains.
25 For an overview of cell membrane netrin receptors, see Dun et al., 2017.

UNC5C is a dependence receptor responsible for the regulation of neuronal apoptosis, and whether UNC5C promotes or inhibits apoptosis depends on its binding to netrin-1 (Lambi et al (2001); Poliak S, *et al.* 2015). UNC5 receptors have intracellular death domains. This domain can interact with death-associated protein kinase (DAPK1) to induce apoptosis in the absence of ligand binding, whilst the
30 receptors remain in monomeric form. The T835M mutation in the death domain of UNC5C further increases apoptosis. This increased signal allowed the determination of the involvement of death-associated protein kinase 1/protein kinase D/apoptosis signal-regulating kinase 1 (ASK1)/JNK/NADPH oxidase/caspases in this signalling cascade (Hashimoto et al 2016 Signal Transduction 291(23), 12282-12293)). UNC5-induced cell death is increased by caspase cleavage in its intracellular death domain which may lead to a feed-
35 forward loop once apoptosis is initiated. All Unc5 receptors are cleaved by caspase-3 *in vitro* (Lambi et al 2001). Cleavage of UNC5C by LGMN has also been proposed to increase UNC5C mediated neuronal apoptosis relevant to Alzheimer's disease (Chen et al 2021). However, in the presence of netrin-1, UNC5C forms a dimer and promotes neuronal survival, migration, and differentiation, *i.e.* it has anti-apoptotic role.

Multimerisation of UNC5 receptors is sufficient to prevent the apoptotic signal pathway activation (Mille et al 2009 Cell Death and Differentiation 16, 1344-1351) and netrin-1 directly inhibits apoptosis in neurones (Llambi et al 2001). UNC5C has also been shown to form heterodimers with DCC or DSCAM (Boyer and Gupton, 2018).

- 5 UNC5C has been implicated in Alzheimer's disease where truncated UNC5C resulting in enhanced neuronal apoptosis was found to be associated with accelerated AD pathology in mice (Chen et al., 2021). Further, several SNPs in UNC5C are linked to the development of Alzheimer's disease (AD). For example, the SNP T835M predisposes to the AD and increases neuronal apoptosis (Li Q, et al. 2018). UNC5C has also been implicated in Parkinson's Disease (PD) where cleavage of UNC5C was shown to
10 cause neuronal loss in a mouse model of PD (Chen et al. 2022).

Summary of the Disclosure

- The present inventors discovered novel antibodies to UNC5C. By studying the immune responses of individuals showing resilience to neurodegeneration despite increased risk of disease, the present inventors identified a convergent heavy chain variable (VH) domain sequence that was present in multiple resilient
15 individuals and therefore likely to have a protective function against neurodegeneration. This VH was paired with a light chain variable (VL) domain and target deconvolution for the resulting antibody revealed UNC5C as its target. Further testing of this antibody showed that this antibody competes with netrin 1, UNC5C's ligand, for binding to UNC5C. The present inventors further identified candidate antibodies derived from ATL_5262 by modification of the heavy chain sequence. These modifications were performed to further
20 improve properties, not limited to reduced immunogenicity, improved stability, improved binding potency, and improved pharmacokinetic properties. These antibodies were shown to be likely to improve or reverse neurodegeneration, for example in neurodegenerative diseases such as FTD, AD and ALS, by binding to UNC5C. The present inventors further identified additional novel anti-UNC5C antibodies with lower sequence homology compared to these therapeutic antibodies, and demonstrated their ability to detect
25 UNC5C in bodily fluid samples from subjects, such as e.g. subjects treated with a therapeutic that is expected to affect the level of soluble UNC5C in a treated subject. They further demonstrated that this can be used as an indicator of target engagement for such therapeutics. The present inventors further demonstrated that the level of soluble UNC5C in subjects are indicative of the presence of neurodegenerative diseases, and their severity. In other words, the level of soluble UNC5C in patient
30 samples (e.g. plasma) are higher in patients who are suffering from neurodegeneration than in healthy patients or patients suffering from other diseases. Therefore, the inventors demonstrated for the first time that levels of soluble UNC5C in patient samples can act as a reliable indicator of neurodegeneration. This can therefore be used for selection of patients for treatment of neurodegeneration, including but not limited to treatment with a therapeutic that is expected to affect the level of soluble UNC5C in a treated subject,
35 including but not limited to a UNC5C binding antibody.

The disclosure provides antibodies that specifically bind to UNC5C, and their use to detect soluble UNC5C in a sample obtained from a subject.

- In a first aspect, the disclosure provides an isolated antibody which specifically binds to UNC5C protein or
40 a fragment thereof, wherein the antibody comprises a heavy chain variable domain (VH) with the following

CDRs: CDRH1 comprising amino acid sequence SEQ ID NO: 142-149; CDRH2 comprising amino acid sequence SEQ ID NO: 150-158, and CDRH3 comprising amino acid sequence SEQ ID NO: 159-168, or a set of CDRs which contains one or two amino acid mutations, optionally substitutions, compared with the above set of CDRs. For example, the antibody may comprise a VH with the CDRs of any of the antibodies

5 ATL_6530 (SEQ ID NO:142, 150, 159); ATL_6531 (SEQ ID NO:143, 151, 160); ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO:145, 153, 162); ATL_6534 (SEQ ID NO:144, 152, 163); ATL_6535 (SEQ ID NO: 143, 154, 164); ATL_6536 (SEQ ID NO:146, 155, 165); ATL_6537 (SEQ ID NO:147, 156, 166); ATL_6538 (SEQ ID NO:148, 157, 167); ATL_6539 (SEQ ID NO:149, 158, 168).

10 In a second aspect, the disclosure provides the use of an antibody which specifically binds to UNC5C protein or a fragment thereof for detecting the level of soluble UNC5C in a biological fluid sample obtained from a subject. In embodiments, the use comprises contacting the biological fluid sample with the antibody. In a third aspect, there is provided a method for detecting the level of soluble UNC5C in a biological fluid sample obtained from a subject, wherein the method comprises contacting the biological fluid sample with an antibody or antibody fragment thereof which specifically binds to UNC5C protein or a fragment thereof.

15 In a fourth aspect, there is provided a method of determining the effect of a therapeutic compound or composition that affects the level of soluble UNC5C in a subject who has been administered the therapeutic compound or composition, wherein the method comprises determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject. In embodiments, determining the level of

20 soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or antibody fragment thereof. In embodiments, the method comprises: (a) determining a first level of soluble UNC5C in a first biological sample previously obtained from the subject, wherein the first biological fluid sample was obtained prior to administration of a therapeutic compound or

25 composition to the subject; (b) determining a second level of soluble UNC5C in a second biological fluid sample previously obtained from the subject, wherein the second biological fluid sample was obtained after administration of the therapeutic compound or composition to the subject; and (c) comparing the first level of soluble UNC5C and the second level of soluble UNC5C to determine the effect of the therapeutic compound or composition on the level of soluble UNC5C. In embodiments, determining the first level of

30 soluble UNC5C comprises contacting said first biological fluid sample previously obtained from the subject with a probe which specifically binds to UNC5C protein or a fragment thereof, and/or wherein determining the second level of soluble UNC5C comprises contacting said second biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. In embodiments, step (a) further comprises determining a first level of total protein in the first

35 biological fluid sample; step (b) further comprises determining a second level of total protein in the second biological fluid sample; and the comparison of step (c) comprises comparing (i) the first level of soluble UNC5C normalised to the first level of total protein and (ii) the second level of soluble UNC5C normalised to the second level of total protein. The first and second levels of total protein may be determined using a copper-based protein assay, a fluorescence-based protein assay, or a dye-based protein assay. In

40 embodiments, the first and second levels of total protein are determined using a bicinchoninic acid (BCA) assay.

In a fifth aspect, there is provided a method of detecting the presence and/or severity of a neurodegenerative disease in a subject and/or the likelihood that the subject will be resilient or sensitive to the neurodegenerative disease, the method comprising: (a) determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C, wherein a higher level of soluble UNC5C in the biological fluid sample relative to the reference level of soluble UNC5C is indicative of the presence and/or severity of the neurodegenerative disease and/or is indicative that the subject is likely to be sensitive / unlikely to be resilient to the neurodegenerative disease. The neurodegenerative disease may be: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease. The neurodegenerative disease may be selected from: amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and Parkinson's disease (PD). The neurodegenerative disease may be ALS.

In a sixth aspect, there is provided a method for selecting a subject for treatment with a therapeutic compound or composition that affects the level of soluble UNC5C, wherein the method comprises: (a) detecting the level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C in a reference biological fluid sample from a healthy control; and (c) selecting the subject for treatment with the therapeutic compound or composition that affects the level of soluble UNC5C when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C. The method may further comprise administering the treatment to the subject.

In a seventh aspect, there is provided a method for selecting a subject for participating in a clinical trial, wherein the method comprises: (a) detecting the level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C in a reference biological fluid sample from a healthy control; and (c) selecting the subject for participating in the clinical trial when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C. In embodiments, determining the level of soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. The clinical trial may be a clinical trial of a therapeutic compound or composition that affects the level of soluble UNC5C. The clinical trial may be a clinical trial of a therapeutic compound or composition for the treatment of a neurodegenerative disease. The neurodegenerative disease may be selected from: amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and Parkinson's disease (PD). The neurodegenerative disease may be ALS.

In embodiments of the fifth, sixth, or seventh aspects, determining the level of soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. In embodiments, step (a) comprises determining a level of total protein in the biological fluid sample and the comparison of step (b) comprises comparing (i) the level of soluble UNC5C normalised

to the level of total protein and (ii) the reference level of soluble UNC5C, wherein the reference level of soluble UNC5C corresponds to a level of soluble UNC5C normalised to a level of total protein. The reference level of soluble UNC5C may have been determined in a reference sample or set of samples. The reference level of soluble UNC5C may have been determined by detecting the level of soluble UNC5C in one or more reference biological fluid samples by contacting the biological fluid sample with an antibody or antibody fragment thereof which specifically binds to UNC5C protein or a fragment thereof, determining corresponding levels of total protein in the reference biological fluid sample(s), and calculating for each reference sample the level of soluble UNC5C normalised to the level of total protein in the sample. The levels of total protein in a biological fluid sample may be determined using a copper-based protein assay, a fluorescence-based protein assay, or a dye-based protein assay. In embodiments, the levels of total protein are determined using a bicinchoninic acid (BCA) assay.

In embodiments of the sixth aspect, the selection of step (c) comprises selecting the subject for treatment when the level of soluble UNC5C normalised to the level of total protein in the biological fluid sample is higher than the reference level of soluble UNC5C normalised to the level of total protein in the reference biological fluid sample(s). In embodiments, the method further comprises treating a selected subject with the treatment.

In an eighth aspect, there is provided a method of determining the effect of a therapeutic compound or composition for the treatment of a neurodegenerative disease in a subject who has been administered the therapeutic compound or composition, wherein the method comprises determining a level of soluble UNC5C in one or more biological fluid samples previously obtained from the subject. In embodiments, determining the level of soluble UNC5C in the biological fluid sample(s) previously obtained from the subject comprises contacting the biological fluid sample(s) with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or antibody fragment thereof. The therapeutic compound or composition may be a therapeutic compound or composition that affects the level of soluble UNC5C. The neurodegenerative disease may be: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease. The neurodegenerative disease may be selected from: amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and Parkinson's disease (PD). The neurodegenerative disease may be ALS. The method may comprise (a) determining a first level of soluble UNC5C in a first biological sample previously obtained from the subject, wherein the first biological fluid sample was obtained prior to administration of a therapeutic compound or composition to the subject; (b) determining a second level of soluble UNC5C in a second biological fluid sample previously obtained from the subject, wherein the second biological fluid sample was obtained after administration of the therapeutic compound or composition to the subject; and (c) comparing the first level of soluble UNC5C and the second level of soluble UNC5C to determine the effect of the therapeutic compound or composition on the level of soluble UNC5C. In embodiments, determining the first level of soluble UNC5C comprises contacting said first biological fluid sample previously obtained from the subject with a probe which specifically binds to UNC5C protein or a fragment thereof, and/or wherein determining the second level of soluble UNC5C comprises contacting said second biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. In embodiments, step (a) further comprises determining a

first level of total protein in the first biological fluid sample; step (b) further comprises determining a second level of total protein in the second biological fluid sample; and the comparison of step (c) comprises comparing (i) the first level of soluble UNC5C normalised to the first level of total protein and (ii) the second level of soluble UNC5C normalised to the second level of total protein. The first and second levels of total protein may be determined using a copper-based protein assay, a fluorescence-based protein assay, or a dye-based protein assay. In embodiments, the first and second levels of total protein are determined using a bicinchoninic acid (BCA) assay.

Embodiments of any aspects described herein may have any one or more of the following features.

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The antibody may comprise a heavy chain variable domain (VH) with the following CDRs:

CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147;

CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and

CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166;

15 The antibody may comprise a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166); In embodiments, the antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161).

20 The antibody may have a heavy chain variable domain (VH) with the following framework sequences:

HFWR1 of SEQ ID NO: 205, 209, 212, or 220;

HFWR2 of SEQ ID NO: 206, 210, or 221;

HFWR3 of SEQ ID NO: 207, 211, or 222; and

HFWR4 of SEQ ID NO: 208 or 223;

25 In embodiments, the antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6537 (SEQ ID NO: 220, 221, 222, 223). In embodiments, the antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO: 205, 206, 207, 208).

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The antibody may have a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533. The antibody may have a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.

35 The antibody may have a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH). The antibody may have a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%,
40 or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH). The antibody may have a heavy chain variable domain (VH) comprising a

sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 124 (ATL_6532 VH).

The antibody may comprise a light chain variable domain (VL) with the following CDRs:

CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174;

5 CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and

CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191.

The antibody may comprise a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191). The antibody may comprise a VL with the CDRs of antibody ATL_6532

10 (SEQ ID NO:171, 177, 186).

The antibody may have a light chain variable domain (VL) with the following framework sequences:

LFWR1 of SEQ ID NO: 230, 232, 233, or 235;

LFWR2 of SEQ ID NO: 240, 241, 238, or 244;

15 LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and

LFWR4 of SEQ ID NO: 256 or 258;

The antibody may comprise a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256). The antibody may comprise a VL with the FWRs of antibody

20 ATL_6532 (SEQ ID NO:232, 240, 249, 258).

The antibody may have a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136

25 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537). The antibody may have a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533). The antibody may have a light chain variable domain (VL) comprising a

sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).

30 sequence identity with SEQ ID NO: 134 (ATL_0006532).

The antibody may specifically bind the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein. In embodiments, the antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein.

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In embodiments, the antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282). In embodiments, wherein the antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL)

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set forth in SEQ ID NO: 134 (ATL_6532). Cross competition may be as assessed using ELISA or an MSD assay as described herein.

5 The antibody may bind human, mouse and/or cynomolgus monkey UNC5C protein. In embodiments, the antibody selectively binds to UNC5C over other one or more netrin receptors, optionally wherein the antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.

10 In embodiments, the subject is a mammal, optionally a mouse, monkey, or human. In embodiments, the subject is a human. In embodiments, the biological fluid sample is blood or cerebrospinal fluid (CSF). A blood sample may be selected from a whole blood, plasma or serum sample. In embodiments, the subject is a patient who has been treated with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF. In embodiments, the therapeutic compound is an anti-UNC5C antibody (therapeutic anti-UNC5C antibody). In embodiments, the therapeutic anti-UNC5C antibody binds the first immunoglobulin (Ig1) domain of UNC5C protein. In embodiments, the therapeutic anti-UNC5C antibody comprises a VH with the CDRs of antibody ATLX1282 (SEQ ID NO: 20, 22, 23) and
15 a VL with the CDRs of antibody ATLX1282 (SEQ ID NO: 24, 34, 40). In embodiments, the therapeutic anti-UNC5C antibody comprises the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282). In embodiments, the therapeutic anti-UNC5C antibody is ATLX1282.

20 In embodiments, the subject is a subject who has been diagnosed with a disease treatable with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF. In embodiments, the subject is a subject who has been diagnosed with a disease treatable with an anti-UNC5C antibody (also referred to herein as therapeutic anti-UNC5C antibody). In embodiments, the subject is a subject who has been diagnosed as having a neurodegenerative disease. In embodiments, the neurodegenerative disease is: (i) a prodromal or preclinical stage neurodegenerative
25 disease; or (ii) a symptomatic or clinical stage neurodegenerative disease. In embodiments, the subject is a patient who has been diagnosed with amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Parkinson's disease (PD), or REM Sleep Behaviour Disorder (RBD). In embodiments, the subject is a patient who has been diagnosed with amyotrophic lateral sclerosis (ALS).

30 The antibody may have a heavy chain variable domain (VH) with the following framework sequences: HFWR1 of SEQ ID NO: 199, 202, 205, 209, 212, 213, 216, 220, 224, 227; HFWR2 of SEQ ID NO: 200, 203, 206, 210, 214, 217, 221, 225, 228; HFWR3 of SEQ ID NO: 201, 204, 207, 211, 215, 218, 222, 226, 229; HFWR4 of SEQ ID NO: 62, 208, 219, 223. For example, the antibody may have a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6530 (SEQ ID NO: 199, 200, 201, 62); ATL_6531 (SEQ ID NO: 202, 203, 204, 62); ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533
35 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6535 (SEQ ID NO: 213, 214, 215, 208); ATL_6536 (SEQ ID NO: 216, 217, 218, 219); ATL_6537 (SEQ ID NO: 220, 221, 222, 223); ATL_6538 (SEQ ID NO: 224, 225, 226, 208); ATL_6539 (SEQ ID NO: 227, 228, 229, 208), such as a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL6533.

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The antibody may have a heavy chain variable domain (VH) comprising a sequence selected that has at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 122 (ATL_0006530 VH); 123 (ATL_0006531 VH); 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 127 (ATL_0006535 VH); 128 (ATL_0006536 VH); 129 (ATL_0006537 VH); 130 (ATL_0006538 VH). For example, the antibody may have a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH).

10 The antibody may comprise a light chain variable domain (VL) with the following CDRs: CDRL1 comprising amino acid sequence SEQ ID NO: 169-176; CDRL2 comprising amino acid sequence SEQ ID NO: 177-183; CDRL3 comprising amino acid sequence SEQ ID NO: 184-193. For example, the antibody may comprise a VL with the CDRs of any of the antibodies ATL_6530 (SEQ ID NO:169, 177, 184); ATL_6531 (SEQ ID NO:170, 178, 185); ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6535 (SEQ ID NO:172, 179, 189); ATL_6536 (SEQ ID NO:173, 180, 190); ATL_6537 (SEQ ID NO:174, 181, 191); ATL_6538 (SEQ ID NO:175, 182, 192); ATL_6539 (SEQ ID NO:176, 183, 193).

The antibody may further have a light chain variable domain (VL) with the following framework sequences: 20 LFWR1 of SEQ ID NO: 230 to 237 and 68; LFWR2 of SEQ ID NO: 239 to 246; LFWR3 of SEQ ID NO: 247 to 255; and LFWR4 of SEQ ID NO: 256 to 258. For example, the antibody may comprise a VL with the FWRs of any of the antibodies ATL_6530 (SEQ ID NO:230, 238, 247, 256); ATL_6531 (SEQ ID NO:231, 239, 248, 257); ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6535 (SEQ ID NO:234, 242, 251, 257); ATL_6536 (SEQ ID NO:68, 243, 252, 257); ATL_6537 (SEQ ID NO:235, 244, 253, 256); ATL_6538 (SEQ ID NO:236, 245, 254, 256); ATL_6539 (SEQ ID NO:237, 246, 255, 256).

The light chain variable domain (VL) may comprise a sequence selected that has at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NO: 132-141 (ATL6530-6539 VL). For example, the antibody may have a light chain variable domain (VL) comprising a sequence that has at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); or SEQ ID NO: 135 (ATL_0006533).

35 The antibody may comprise an scFv antibody molecule, a nanobody, an antibody constant region, or a whole antibody.

The antibody may be a monoclonal antibody. The antibody may be a whole antibody. The antibody may be an immunoglobulin, such as an IgG, IgM, IgA IgD or IgE. The antibody may be an immunoglobulin G (IgG). The antibody may be an IgG1 or variant thereof. The antibody may be an IgG1 variant L234A/L235A (LALA). The antibody may be in any format known in the art. The antibody may be in any format known in the art to be suitable for use in an immunoassay.

Embodiments of the second to eighth aspects may have any one or more of the following optional features. In embodiments, the antibody or antibody fragment thereof which specifically binds to UNC5C protein or a fragment thereof is used as a capture antibody in an immunoassay. In embodiments, the immunoassay is a sandwich ELISA or a meso scale discovery (MSD) assay. In embodiments, the use or method comprises:

5 (a) detecting UNC5C bound by the capture antibody using a detection probe associated with a detectable label, and (b) detecting the detectable label to determine the level of soluble UNC5C in the biological fluid sample. In embodiments, the detection probe comprises an antibody, the detection antibody binds UNC5C, and: (i) the detection probe carries a detectable label; or (ii) the detection probe comprises a secondary antibody which binds the detection antibody, the detection antibody is detected using the secondary

10 antibody, and the secondary antibody carries a detectable label. In embodiments, the detection probe comprises a UNC5C ligand. In embodiments, the detection probe comprises a UNC5C ligand other than Netrin-1. In embodiments, the detection antibody is a non-human antibody which binds UNC5C. In embodiments, the detection antibody is a polyclonal antibody. In embodiments, the detection antibody is a goat polyclonal antibody. In embodiments, the secondary antibody is a non-human polyclonal antibody

15 which binds to antibodies of the species from which the detection antibody is derived. In embodiments, the detection antibody is a goat antibody, and the secondary antibody is an anti-goat polyclonal antibody. In embodiments, the secondary antibody is a mouse anti-goat polyclonal antibody or a donkey anti-goat polyclonal antibody. In embodiments the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label; for

20 example wherein the detectable label may be an electrochemiluminescent or enzymatically active label. In embodiments, the detectable label is a horseradish peroxidase (HRP) enzyme or a Sulfo-tag. In embodiments, the detectable label is an electrochemiluminescent label, optionally a Sulfo-tag. In some such embodiments, step (b) comprises: (i) applying an electrical current to the electrochemiluminescent label; and (ii) measuring the luminescence. In embodiments, the detectable label is a horseradish

25 peroxidase (HRP) enzyme. In some such embodiments, step (b) comprises: (i) contacting the horseradish peroxidase (HRP) enzyme with TMB (3, 3', 5, 5'-tetramethylbenzidine); and (ii) measuring the ultraviolet (UV) absorption at 450nm and/or 570nm.

In embodiments, a reference sample is associated with a healthy control subject. In embodiments, a healthy

30 control is a subject who has not been diagnosed with a neurodegenerative disease. In embodiments, the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), or Parkinson's disease (PD). For example, the healthy control may be a subject who has not been diagnosed with ALS.

35 Also described according to an ninth aspect is a kit comprising: (i) a capture antibody, wherein the capture antibody specifically binds to UNC5C protein or a fragment thereof; and (ii) a detection antibody, wherein the detection antibody binds UNC5C, optionally wherein the detection antibody carries a detectable label, wherein the capture antibody is different from the detection antibody. The kit may further comprise (iii) a

40 secondary antibody which binds the detection antibody, wherein the secondary antibody carries a detectable label.

In embodiments, the capture antibody comprises a heavy chain variable domain (VH) with the following CDRs:

CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147;

CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and

5 CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166;

In embodiments, the capture antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166). In embodiments, wherein the capture antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161). In embodiments, the capture

10 antibody has a heavy chain variable domain (VH) with the following framework sequences:

HFWR1 of SEQ ID NO: 205, 209, 212, or 220;

HFWR2 of SEQ ID NO: 206, 210, or 221;

HFWR3 of SEQ ID NO: 207, 211, or 222; and

HFWR4 of SEQ ID NO: 208 or 223.

15 In embodiments, the capture antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6537 (SEQ ID NO: 220, 221, 222, 223). In embodiments, the capture antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO: 205, 206, 207, 208). In embodiments, the

20 capture antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the capture antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.

In embodiments, the capture antibody has a heavy chain variable domain (VH) comprising a sequence selected that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH). In embodiments, the capture antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH). In embodiments, wherein the

25 capture antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 124 (ATL_6532 VH).

In embodiments, the capture antibody comprises a light chain variable domain (VL) with the following CDRs:

35 CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174;

CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and

CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191.

In embodiments, the capture antibody comprises a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 171, 177, 186); ATL_6533 (SEQ ID NO: 169, 177, 187); ATL_6534 (SEQ ID NO: 169, 177, 188); ATL_6537 (SEQ ID NO: 174, 181, 191). In embodiments, the capture antibody comprises a VL with

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the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186). In embodiments, the capture antibody has a light chain variable domain (VL) with the following framework sequences:

LFWR1 of SEQ ID NO: 230, 232, 233, or 235;

LFWR2 of SEQ ID NO: 240, 241, 238, or 244;

5 LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and

LFWR4 of SEQ ID NO: 256 or 258;

In embodiments, the capture antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256). In embodiments, the capture antibody
10 comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232, 240, 249, 258).

In embodiments, the capture antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ
15 ID NOs: 136 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537). In embodiments, the capture antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533). In embodiments, the capture antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at
20 least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).

In embodiments, the capture antibody specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein. In embodiments, the capture antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein. In embodiments,
25 the capture antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282). In embodiments, the capture antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO:
30 134 (ATL_6532). In embodiments, the capture antibody binds human, mouse and/or cynomolgus monkey UNC5C protein. In embodiments, the capture antibody selectively binds to UNC5C over other one or more netrin receptors. In embodiments, the capture antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.

35 In embodiments, the detection antibody is a non-human antibody which binds UNC5C. In embodiments, wherein the detection antibody is a polyclonal antibody. In embodiments, the detection antibody is a goat polyclonal antibody. In embodiments, the secondary antibody is a non-human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived. In embodiments, the detection antibody is a goat antibody, and the secondary antibody is an anti-goat polyclonal antibody. In
40 embodiments, the secondary antibody is a mouse anti-goat polyclonal antibody or a donkey anti-goat polyclonal antibody. In embodiments, the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label. In

embodiments, the detectable label is an electrochemiluminescent or enzymatically active label. In
embodiments, the detectable label is a horseradish peroxidase (HRP) enzyme or a Sulfo-tag. In
embodiments, the detectable label is a horseradish peroxidase (HRP) enzyme and the kit further comprises
TMB (3, 3', 5, 5'-tetramethylbenzidine). In embodiments, the capture antibody is immobilised to a solid
5 support. Thus, the kit may comprise a solid support onto which the capture antibody is immobilised. The kit
may further comprise means to detect levels of total protein in a biological fluid sample. The means to
detect levels of total protein in a biological fluid sample may comprise a copper-based protein assay kit, a
fluorescence-based protein assay kit, or a dye-based protein assay kit. In embodiments, the means to
detect levels of total protein comprise a bicinchoninic acid (BCA) assay kit. A copper-based protein assay
10 kit may comprise an alkaline copper salt solution. A copper-based protein assay kit may comprise a solution
comprising a colorimetric reagent that detects reduced cuprous ions (e.g. BCA or phosphomolybdic/
phosphotungstic acid) or unbound cupric ions.

Also described is an isolated VH domain of an antibody as described herein. Also described herein is an
15 isolated antibody VL domain of an antibody as described herein. Also described is an isolated nucleic acid
which comprises a nucleotide sequence encoding an antibody, including a VH or VL domain, as described
herein. Also described is a vector or set of vectors comprising such a nucleic acid. Also described is host
cell comprising the vector or set of vectors as described herein, or a host cell in vitro transformed with a
nucleic acid as described herein. Also described is a kit comprising an antibody described herein, and at
20 least one additional component.

Therapeutic antibodies described herein may have an epitope that at least partially overlaps the binding
site of Netrin-1. Antibodies described herein may bind human UNC5C with an EC50 of at most 9.81E-08
M, or at most 1E-08M, or at most 5.5E-09M as assessed by ELISA (such as binding of plated rhUNC5C).
25 The antibody may selectively bind to UNC5C over other one or more netrin receptors. The antibody may
selectively bind to UNC5C over one or more (or all of): neogenin, DCC, and DSCAM, UNC5A, UNC5B,
UNC5. Selective binding may refer to preferential binding (e.g. lower EC50) for one species (e.g. UNC5C)
over another (e.g. other netrin receptor), or to no detectable binding to comparative targets (e.g. other netrin
receptors). Selective binding may be verified by biolayer interferometry analysis as described herein. The
30 antibody may bind to monomeric UNC5C and/or dimeric UNC5C and/or homodimeric UNC5C. The
antibody may bind to monomeric UNC5C. The antibody may be an IgG1 or variant thereof, optionally
wherein the antibody is an IgG1 variant L234A/L235A (LALA).

Any antibody described herein may comprise an scFv antibody molecule, a nanobody, an antibody constant
region, or a whole antibody. The antibody may be a whole antibody.

35 The invention includes the combination of the aspects and preferred features described except where such
a combination is clearly impermissible or expressly avoided.

Summary of the Figures

Embodiments and experiments illustrating the principles of the invention will now be discussed with
40 reference to the accompanying figures in which:

Figure 1 shows schematically a process for unbiased antibody discovery.

Figure 2 shows results of ELISA assays for binding of antibodies identified in FTD resilient individuals to recombinant UNC5C. The pool of antibodies tested consisted of 18 antibodies identified from a FTD cohort, one antibody from a Huntington cohort, and an anti-PDL1 antibody, previously used as a positive control in a Retrogenix target deconvolution assay. The antibody, ATL_5262 binds to UNC5C. The blue bars indicate raw absorbance signals to recombinant UNC5C and the orange bars represent absorbance signals to an irrelevant protein, lysozyme. Figure shows absorbance at 450nm. The anti-Fc tag bars show the results for an anti-Fc-HRP treated well which controls that the UNC5C is correctly coated on the assay plate.

Figure 3 shows the presence of UNC5C antibodies in subjects who are resilient to neurodegeneration in different neurodegenerative disease cohorts. A. UNC5C antibodies were found in resilient centenarians (>100 year old subjects with no symptoms of neurodegeneration); resilient Alzheimer's disease (AD) patients (a resilient Alzheimer's disease subject showing slow cognitive progression), Frontotemporal dementia (FTD) patients (three subjects with resilience for FTD - carriers of high risk mutations and older age but no progression to FTD), Parkinson's disease (PD) patients (prodromal REM sleep behaviour disorder (RBD) but no Parkinson's diagnosis), and cognitively healthy controls (predominantly individuals over 60 years of age). Repertoires were searched for VH sequences containing the same V gene and J gene as ATL5262 and at most 2 amino acid mismatches across the junction region. Dots represent the number of matching sequences per repertoire and colour indicates the isotypes of sequences identified. The majority of matching sequences are class switched to IgG1 supporting a similar function for these antibodies across individuals. Unclassified PD subject SU_0001278 was initially included as a healthy control for this cohort, but subsequently removed since they have suspected REM sleep behaviour disorder, which is a prodromal marker for PD. Since they have no PD diagnosis they are 'potentially resilient'

Figure 4 shows the results of a Netrin 1 competition assay. ELISA plates were coated with rhUNC5C (recombinant human UNC5C) and preincubated with His-tagged recombinant Netrin 1 prior to adding ATL_5262 or control antibody ATL_5338. A. Netrin 1 was detected using an anti-His (horseradish peroxidase) HRP antibody. B. Antibody (ATL_5262 or control) was detected by anti-F(ab)'2-HRP detection. Assays were set up in duplicate whereby one replicate had Netrin 1 binding detected with anti-His-HRP (A) and the second replicate was used to determine ATL_0005262 antibody binding using the anti-Fab'2-HRP detection. B. Figure shows absorbance at 450nm.

Figure 5 shows the results of a concentration-dependent Netrin 1 competition assay. ELISA plates were coated with rhUNC5C and preincubated with a 12-point dilution series of His-tagged recombinant Netrin 1 (starting at 3nM) prior to adding ATL_5262. Netrin 1 was detected using anti-His HRP detection and is shown in the figure as "Netrin 1 signal (+ ATL_5262)". ATL_5262 was detected using anti-F(ab)'2-HRP detection and is shown in the figure as "ATL_0005262 signal (+ Netrin1)". Control wells contained Netrin 1 alone. Data represented as percentage of maximum absorbance signal.

Figure 6 shows the results of a Netrin 1 competition assay. ELISA plates were coated with rhUNC5C and preincubated with ATL_5262 or control antibody ATL_5338 prior to adding His-tagged recombinant Netrin 1. Netrin 1 was detected using anti-His HRP detection and is shown in the figure as "Netrin signal (+ ATL_0005262)" or "Netrin signal (+ ATL_0005338)". ATL_5262 was detected using anti-F(ab)'2-HRP detection. Figure shows absorbance at 450nm.

Figure 7 shows (A) a VH alignment of ATL_5262 and ATL_5262 homologues identified across neurodegeneration cohorts in resilient individuals. Figure shows IMGT numbering. (B) shows sequences of antibodies described herein (in particular, VH of ATL_0005262, SEQ ID NO: 1; VH of ATL_0006187, SEQ ID NO: 112; VH of ATL_0006191, SEQ ID NO: 113), with variable positions in the heavy chain highlighted. (B-1 to B-4) show the heavy chain sequences of the antibodies in (A) as well as ATL_6178 (VH of ATL_6178, SEQ ID NO: 93), aligned by IMGT position numbering. ATL_5262 and ATL_6178 have the same VL. Homologues were identified based on VH alone so only VH sequences are shown.

Figure 8 shows the results of an ELISA testing the binding of different VH variants of ATL_5262 to rhUNC5C. ATL_5338 was included as a negative control. Figure shows absorbance at 450nm.

Figure 9 shows the results of an ELISA testing the binding of different VH variants of ATL_5262 to rhUNC5C (A), rmUNC5C (B), and lysozyme (C). ATL_5338 was included as a negative control. ATL_6002 and ATL_6003 (VL pairing variants) were included for comparison. Figure shows absorbance at 450nm.

Figure 10 shows the results of an ELISA testing the binding of different VH variants of ATL_5262 to Fc tagged rhUNC5C (A), or His-tagged rhUNC5C (B). ATL_5338 was included as a negative control. ATL_6002 and ATL_6003 (VL pairing variants) were included for comparison. Figure shows absorbance at 450nm.

Figure 11 shows the results of an ELISA testing the binding of different VH variants of ATL_5262 to rhUNC5C (A), rmUNC5C (B), and lysozyme (C). ATL_5338 was included as a negative control. Figure shows absorbance at 450nm.

Figure 12 shows sequences of antibodies described herein, with variable positions in the heavy chain highlighted. A. Heavy chains, aligned by IMGT position numbering. VH of ATL_0005262, 5996, 5997, 5998, 5999, 6000, 6001, 6002, 6003, 6004, 6005: SEQ ID NO: 1; VH of ATL_0006033: SEQ ID NO: 2; VH of ATL_6034: SEQ ID NO: 3; VH of ATL_0006035: SEQ ID NO: 4; VH of ATL_0006036: SEQ ID NO: 5; VH of ATL_0006037: SEQ ID NO: 6; VH of ATL_0006038: SEQ ID NO: 7; VH of ATL_0006039: SEQ ID NO: 8; VH of ATL_0006177: SEQ ID NO: 91; VH of ATL_0006178: SEQ ID NO: 93. B. Light chains, aligned by IMGT position numbering. VL of ATL_0005262: SEQ ID NO: 9; VL of ATL_0005996: SEQ ID NO: 10; VL of ATL_0005997: SEQ ID NO: 11; VL of ATL_0005998: SEQ ID NO: 12; VL of ATL_0005999: SEQ ID NO: 13; VL of ATL_0006000: SEQ ID NO: 14; VL of ATL_0006001: SEQ ID NO: 15; VL of ATL_0006002: SEQ ID NO: 16; VL of ATL_0006003: SEQ ID NO: 17; VL of ATL_0006004: SEQ ID NO: 18; VL of ATL_0006005: SEQ ID NO: 19; VL of ATL_0006033, 6034, 6035, 6036, 6037, 6038, 6039, 6177: SEQ ID NO: 92; VL of ATL_0006178: SEQ ID NO: 94.

Figure 13 shows the results of a netrin-1 competition assay demonstrating that binding of ATLX-1282 and ATL_5262 to mouse brain tissue is blocked via Netrin-1. **(A)** Graph shows effect of Netrin-1 in competition with ATL_5262 in tissues with high UNC5C expression ("High expression region" =mouse brain tissue) or control tissues with no or low UNC5C expression ("Liver ctrl" =mouse liver tissue). **(B)** Fluorescent and Transmitted light tissue section images (20X), showing binding of ATL_5262 to mouse brain or control liver tissue under various blocking conditions (no netrin block; netrin-1 block; clusterin block (control)). **(C)** Effect of Netrin-1 and Netrin-4 blocking in competition with ATLX-1282 in naïve mouse brain sections. **(D)** Effect of Netrin-1 and Netrin-4 blocking in competition with ATLX-1282 in naïve (i.e., untreated) mouse brain sections, or liver control tissue ("Liver ctrl"). **(E)** Representative Fluorescent and Transmitted light tissue section images showing blocking effect of Netrin-4 and Netrin-1 of ATLX-1282 binding (resulting in reduced staining) in naïve mouse brain sections.

Figure 14 shows the results of a flow cytometry assay showing binding of ATL5262 and ATLX-1282 to UNC5C and the UNC5C T835M variant (AD variant) overexpressed in HEK-293 cells. **(A)** Quantification of UNC5C binding as measured by the % of IgG+ cells (detecting the antibody). **(B)** Quantification of UNC5C binding as measured by the Mean fluorescence intensity (MFI) of IgG.

Figure 15 shows the X-ray crystal structure of an ATL_5262 Fab fragment bound to human UNC5C Ig domains. **(A)** shows a cartoon view of the full Fab-UNC5C complex. **(B)-(F)** show close-up views of the specific interactions between the ATL_5262 and UNC5C as shown in **(A)**. **(G)** shows a protein alignment of human UNC5 family members B (SEQ ID NO: 260), A (SEQ ID NO: 261) and D (SEQ ID NO: 262) to region of human UNC5C Ig-like domain 1 that interacts with ATL_5262 (SEQ ID NO: 259).

Figure 16 shows sequences of antibodies described herein, with variable positions in the heavy chain highlighted. **A.** (A-1 to A-4) Heavy chains, aligned by IMGT position numbering. VH of ATL_0005262: SEQ ID NO: 1; VH of ATL_0006178: SEQ ID NO: 93; VH of ATL_0006530: SEQ ID NO: 122; VH of ATL_0006531: SEQ ID NO: 123; VH of ATL_0006532: SEQ ID NO:124; VH of ATL_0006533: SEQ ID NO:125; VH of ATL_0006534: SEQ ID NO:126; VH of ATL_0006535: SEQ ID NO:127; VH of ATL_0006536: SEQ ID NO:128; VH of ATL_0006537: SEQ ID NO:129; VH of ATL_0006538: SEQ ID NO:130; VH of ATL_0006539: SEQ ID NO:131. **B.** (B-1 to B-4) Light chains, aligned by IMGT position numbering. VL of ATL_0005262: SEQ ID NO: 9; VL of ATL_0006178: SEQ ID NO: 92; VL of ATL_0006530: SEQ ID NO: 132; VL of ATL_0006531: SEQ ID NO: 133; VL of ATL_0006532: SEQ ID NO:134; VL of ATL_0006533: SEQ ID NO:135; VL of ATL_0006534: SEQ ID NO:136; VL of ATL_0006535: SEQ ID NO:137; VL of ATL_0006536: SEQ ID NO:138; VL of ATL_0006537: SEQ ID NO:139; VL of ATL_0006538: SEQ ID NO:140; VL of ATL_0006539: SEQ ID NO:141.

Figure 17 shows ELISA capture and detection of UNC5C using ATL6532 and ATL6534. **A.** Capture of His tagged recombinant UNC5C by ATL6532 results in a much higher assay signal than capture by ATL6534. **B.** Considerably increasing coating concentration of ATL6534 results in an acceptable assay signal.

Figure 18 shows ELISA capture and detection of UNC5C using ATL6532 and ATL6533. **A.** Capture of his tagged UNC5C by ATL6532 and ATL6533 (both coated at 5µg/ml) and detection with an anti-His HRP results in a good titratable signal for both antibodies with ATL6532 providing greater sensitivity than ATL6533. **B.** No signal from irrelevant His tagged antigen, demonstrating the specificity of ATL6532 and ATL6533 for UNC5C.

Figure 19 shows sandwich format ELISA capture and detection of UNC5C using ATL6532 and ATL6533 as capture antibodies and goat polyclonal anti-UNC5C (Biotechnie #AF1005) as detection antibody. **A.** Titratable signal is observed with both capture antibodies, with greater signal and sensitivity observed with ATL6532 capture. **B.** No signal from the lysozyme antigen control, demonstrating the specificity of ATL6532 and ATL6533 for UNC5C.

Figure 20 shows results of antigen down format ELISA used to assess domain binding of ATL6532, ATL6533, ATL6534, and ATL6537. Truncated versions of human UNC5C were made that lack various parts of the extracellular domain. These proteins were used to identify which region of UNC5C the antibodies bind. The tested antigens were: full recombinant human UNC5C ("Full Human ECD"; SEQ ID NO: 263), recombinant human UNC5C with Ig1 domain only ("No Ig2, TSP1 or TSP2"; SEQ ID NO: 270), recombinant human UNC5C with Ig1 and Ig2 domain ("No TSP1 or TSP2; SEQ ID NO: 271), recombinant human UNC5C with Ig1, Ig2 and TSP-1 domain ("No TSP2"; SEQ ID NO: 272), and recombinant human UNC5C missing Ig1 domain only ("No Ig1"; SEQ ID NO: 273).

Figure 21 shows the assay in MSD format recognises mouse and cyno UNC5C as well as human, demonstrating the suitability of the assay for use in multiple species.

Figure 22 shows the assay in MSD format does not detect related family members UNC5A, UNC5B, UNC5D or DCC. SH-SY5Y cells express UNC5C and are used here to confirm the assay recognises endogenous UNC5C, not only recombinant.

Figure 23 shows the ability of the assay to detect UNC5C is not diminished by the presence of high concentrations of ATLX1282, thus demonstrating the suitability of this assay for measuring circulating UNC5C levels in the blood of patients dosed with ATLX1282.

Figure 24 shows the assay performs well in multiple relevant biological matrices, without significant matrix interference on assay recovery. **A.** Signal strength is comparable across PBS, 10% serum, 10% artificial CSF + 2% FBS, and 10% plasma conditions. **B.** Signal strength is comparable between PBS and 50% plasma conditions, with no signal for the isotype control ATL5338. **C.** A range of signal size is detected in 8 different plasma samples from healthy individuals and one serum sample.

Figure 25 shows results of an MSD assay to detect soluble UNC5C levels in plasma samples obtained from ALS patients as compared to the soluble UNC5C level in plasma samples obtained from healthy controls. Soluble UNC5C levels were normalised to total protein levels. The data show that soluble UNC5C levels are significantly higher in plasma samples from ALS patients than in healthy controls.

Figure 26 shows a correlation between sUNC5C levels and resiliency status. **A.** Results of an MSD assay to detect soluble UNC5C levels in plasma samples obtained from ALS patients stratified by resiliency status as compared to the soluble UNC5C level in plasma samples obtained from healthy controls. 'Progressor' encompasses rapid progressor and progressor, 'Resilient' encompasses both resilient and super resilient. Soluble UNC5C levels were normalised to total protein levels. **B.** Example ALSFRS-R slopes for each resilience category.

Figure 27 shows results of MSD assays to detect soluble UNC5C levels in serum samples obtained from healthy cynomolgus monkeys administered anti-UNC5C antibody ATLX1282. Following day 1 and day 22 dosing, serum samples were collected at 1, 6, 24, 48, 72 or 168 hours post dose. The results show that the MSD assay detects higher levels of sUNC5C in subjects following treatment with ATLX1282.

Figure 28 shows results of an MSD assay to detect soluble UNC5C levels in plasma samples obtained from NEFH-tTA × hTDP-43ΔNLS bigenic mice (rNLS mice; an ALS mouse model) administered anti-UNC5C antibody ATLX1282. Mice were dosed twice a week from age 5 weeks until age 12 weeks, and plasma samples obtained from terminal bleeds. The results show that the MSD assay detects higher levels of sUNC5C in subjects following treatment with ATLX1282.

Figure 29 shows results of an MSD assay to detect soluble UNC5C levels in plasma samples obtained from healthy controls, rheumatoid arthritis patients, ALS patients, REM Behaviour Disorder patients (prodromal Parkinson's Disease patients), Parkinson's Disease patients and Alzheimer's Disease patients. Soluble UNC5C levels were normalised to total protein levels. The results show that sUNC5C levels are significantly higher than healthy controls in all neurodegenerative condition samples (including prodromal PD samples), but not in the rheumatoid arthritis samples, demonstrating that soluble UNC5C is a neurodegeneration specific marker which can be used to detect prodromal/pre-clinical as well as clinical-stage neurodegenerative disease.

Detailed Description of the Invention

Aspects and embodiments of the present invention will now be discussed with reference to the accompanying figures. Further aspects and embodiments will be apparent to those skilled in the art.

Disclosed herein are antibodies and fragments thereof that are capable of specifically binding to UNC5C protein or a fragment thereof. As used herein, an antibody capable of "specific binding" or "specifically binding" a target is one able to bind through the association of the epitope recognition site with an epitope within the target. It is distinct from non-specific binding, for example Fc-mediated binding, ionic and/or hydrophobic interactions. In other words, an antibody which specifically binds a target recognises and binds to a specific protein structure within it rather than to proteins generally.

The present disclosure refers to antibodies described herein using references specified as "ATL_000xxx", "ATL_xxxx" or "xxx", where "xxx" is a four digits reference number specific to an antibody described herein. All of the above notations are used interchangeably to refer to the same antibody or a portion thereof

(e.g. a VH, VL or part thereof, of the antibody). For example, antibody ATL_0005262 is interchangeably referred to herein as ATL_5262 and 5262.

The term "antibody" as used herein encompasses whole antibodies as well as fragments thereof including e.g. an scFv antibody molecule, a nanobody, an antibody constant region, or an isolated VH region.

5 *Binding to UNC5C*

The antibodies described herein are capable of specifically binding UNC5C over other netrin receptors such as neogenin, DCC, DSCAM, UNC5A, UNC5B, and UNC5D. Binding of an antibody to a candidate protein can be verified for example by biolayer interferometry analysis as described herein.

10 Antibodies described herein may bind monomeric UNC5C and dimeric UNC5C. Antibodies described herein may bind homodimeric UNC5C.

Netrin-1 is one of several netrins, all members of the laminin superfamily. Netrins are secreted proteins that direct axon extension and cell migration during neural development and regulate cell adhesion, the maturation of cell morphology, cell survival and tumorigenesis (Rajasekharan, et al. (2009)). They are bifunctional proteins that act as attractants for some cell types and as repellents for others. The UNC-5
15 family of receptors mediate the repellent response to netrin. Netrin 1 consists of a laminin-like domain in the N-terminal (LN, also known as domain VI), followed by three epidermal growth factor (EGF) repeats (EGF1, EGF2 and EGF3, also known as domain V), and a C-terminal Netrin-like domain (NTR) that shares homology with domains found in complement and other proteins (Dun et al. 2017).

The antibodies of the disclosure specifically bind to UNC5C. Binding to UNC5C may be assessed by ELISA
20 assays as described herein. In embodiments, antibodies described herein specifically bind to UNC5C and mimics and/or competes with Netrin-1 for binding with UNC5C. Competition with Netrin-1 binding may be assessed by ELISA assays as described herein. Thus, antibodies described herein may compete for the same binding site on UNC5C as Netrin-1. Antibodies described herein may bind the same binding site on UNC5C as Netrin-1. An antibody mimicking netrin-1 may refer to the antibody interacting with UNC5C in a
25 way that mimics the effect of netrin-1 on UNC5C structure and/or activity. An antibody competing with netrin-1 for binding to UNC5C may refer to the binding of the antibody to UNC5C being dependent on the presence or absence of Netrin-1, and/or the binding of Netrin-1 to UNC5C being dependent on the presence or absence of the antibody. An antibody competing with netrin-1 for binding to UNC5C may refer to the binding of the antibody to UNC5C being inhibited by Netrin-1. For example, an antibody competing with
30 netrin-1 for binding to UNC5C may refer to the binding of the antibody to UNC5C being at least partially inhibited in the presence of Netrin-1. This may be assessed for example using an ELISA assay, such as described herein. For example, UNC5C may be pre-incubated with Netrin-1 prior to incubation with the antibody, and the amount of bound antibody may be compared to a control condition without Netrin-1 pre-incubation. A reduction in the amount of bound antibody may indicate that the antibody competes with
35 Netrin-1 for binding to UNC5C. In embodiments, a signal indicative of the presence of the antibody bound to UNC5C may be reduced by at least 50%, at least 60%, at least 70%, at least 80% or at least 90% in the presence of Netrin-1 (such as e.g. following pre-incubation with 200microg/l Netrin-1). In embodiments, binding of the antibody to UNC5C may be reduced by at least 50%, at least 60%, at least 70%, at least

80% or at least 90% in the presence of Netrin-1 (such as e.g. following pre-incubation with 200microg/l Netrin-1).

Antibodies as described herein may be such that binding of the antibody to UNC5C is inhibited by Netrin-1 in a concentration dependent manner. This can be assessed for example using an ELISA assay as described herein, or any other binding assay in combination with incubation with a plurality of different concentrations of Netrin-1 (e.g. a Netrin-1 dilution series). A reduction in binding of the antibody to UNC5C in the presence of Netrin-1 (e.g. when the antibody is incubated with UNC5C following pre-incubation with Netrin-1), which is dependent on the concentration of Netrin-1, may be considered to be indicative of Netrin-1 concentration-dependent inhibition of binding of the antibody to UNC5C. In embodiments, a signal indicative of the presence of the antibody bound to UNC5C may be reduced by at least 50% in the presence of at least 10^{-8} M of Netrin-1 (such as e.g. following pre-incubation with 10^{-8} M of Netrin-1). In embodiments, binding of the antibody to UNC5C may be reduced by at least 50% in the presence at least 10^{-8} M of Netrin-1 (such as e.g. following pre-incubation with 10^{-8} M of Netrin-1). In embodiments, a signal indicative of the presence of the antibody bound to UNC5C may be reduced by at least 80% in the presence of at least 10^{-7} M of Netrin-1 (such as e.g. following pre-incubation with 10^{-7} M of Netrin-1). In embodiments, binding of the antibody to UNC5C may be reduced by at least 80% in the presence at least 10^{-7} M of Netrin-1 (such as e.g. following pre-incubation with 10^{-7} M of Netrin-1). In embodiments, a signal indicative of the presence of the antibody bound to UNC5C may be reduced by at least 90% or at least 95% in the presence of at least 5×10^{-7} or 1×10^{-6} M of Netrin-1 (such as e.g. following pre-incubation with 5×10^{-7} or 1×10^{-6} M of Netrin-1). In embodiments, binding of the antibody to UNC5C may be reduced by at least 90% or at least 95% in the presence at least 5×10^{-7} or 1×10^{-6} M of Netrin-1 (such as e.g. following pre-incubation with 5×10^{-7} or 1×10^{-6} M of Netrin-1). Any pre-incubation may be for a suitable amount of time, such as e.g. around 30 minutes.

Antibodies described herein may have a binding site that at least partially overlaps with the netrin-1 binding site. Antibodies described herein may cause the dimerization of UNC5C.

The human gene encoding UNC5C (Gene ID: 8633) is present on 4q22.3 and consists of 20 exons. Reference non-human UNC5C amino acid and coding sequences are available in public databases.

The sequence of human UNC5C is available under Uniprot identifier O95185. It comprises 931 amino acids. Amino acids 62-159 form an Ig-like domain (also referred to herein as the first immunoglobulin domain/Ig1 domain; SEQ ID NO: 274), amino acids 161-256 form an Ig-like C2-type domain (also referred to herein as the second immunoglobulin domain/Ig2 domain; SEQ ID NO: 275), amino acids 260-314 and 316-368 form TSP type-1 1 (also referred to herein as the TSP1 domain; SEQ ID NO: 276) and TSP type-1 2 domains (also referred to herein as the TSP2 domain; SEQ ID NO: 277), amino acids 530-673 form a ZU5 domain and amino acids 850-929 form a death domain. The protein is cleaved at amino acids 415-416 by caspase-3. A reference human UNC5C amino acid sequence is provided as SEQ ID NO: 99.

The sequence of mouse UNC5C is available under Uniprot identifier O08747. It comprises 931 amino acids. Amino acids 62-159 form an Ig-like domain (also referred to herein as the first immunoglobulin domain or N terminal immunoglobulin domain), amino acids 161-256 form an Ig-like C2-type domain (also referred to

herein as the second immunoglobulin domain), amino acids 260-314 and 316-368 form TSP type-1 1 and TSP type-1 2 domains, amino acids 530-673 form a ZU5 domain and amino acids 850-929 form a death domain. The protein is cleaved at amino acids 415-416 by caspase-3. A reference mouse UNC5C amino acid sequence is provided as SEQ ID NO: 100.

5 However, as used herein, the term "UNC5C" encompasses truncations, derivatives, and variants of the sequence of UNC5C provided herein, and may refer to any protein with at least 80%, at least 90%, or at least 95% sequence identity. The antibodies described herein are capable of specifically binding a peptide or protein having or comprising the amino acid sequence of SEQ ID NO: 99 (including a full length UNC5C protein comprising said sequence, or a fragment of said protein comprising said sequence), or a fragment
10 thereof, such as e.g. a fragment comprising or consisting of amino acids 41-380 of human or murine (mouse) UNC5C (such as e.g. the amino acid sequence of SEQ ID NO: 101 or 102) or a corresponding homologous sequence. The antibodies described herein are capable of specifically binding a peptide or protein comprising the extracellular domain of UNC5C or a part thereof, such as e.g. a part comprising amino acids 41 to 380 of human or murine UNC5C, or a corresponding homologous sequence. Antibodies
15 described herein are capable of specifically binding human UNC5C and human mutant UNC5C T835M. Antibodies described herein may bind to mutant forms of human UNC5C that comprise one or more mutations that are not in the extracellular region of the protein. The antibody may bind to an epitope located in the extracellular region of UNC5C. For example, the antibody may bind to the N terminal immunoglobulin domain of UNC5C (also referred to as "first immunoglobulin domain"/"Ig1 domain"). Preferably, the antibody
20 does not bind the Ig1 domain of the UNC5C protein, i.e. preferably the antibody binds the Ig2, TSP1, and/or TSP2 domains of UNC5C, for example the Ig2 domain of UNC5C. The domain to which an antibody binds can be determined by testing binding of an antibody to truncated versions of UNC5C, for example using an ELISA-based domain binding assessment as disclosed herein.

Antibodies described herein bind UNC5C (e.g. mouse and/or human) with high affinity. High affinity binding
25 may be assessed by measuring the EC50 value, the concentration at which the antibody produces a half-maximal binding, for example via ELISA as described herein. For example, affinity of the antibody may be assessed by binding to plate-bound recombinant human UNC5C as described herein. High affinity as referred to herein means an EC50 value of at most 1E-07 M (i.e. 100 nM), at most 5E-07 M, at most 1 E-08 M, at most 2 E-08 M, at most 3 E-08 M, at most 4 E-08 M, at most 5 E-08 M, at most 6 E-08 M, at most
30 7 E-08 M, at most 8 E-08 M, at most 9 E-08 M, at most 1 E-09 M, at most 2 E-09 M, at most 3 E-09 M, at most 4 E-09 M, at most 5E-09 M, at most 6 E-09 M, at most 7 E-09 M; at most 8 E-09 M, at most 9 E-09 M, at most 1E-10 M, at most 2 E-10 M, at most 3 E-10 M, at most 4 E-10 M, at most 5E-10 M, at most 6 E-10 M, at most 7 E-10 M, at most 8 E-10 M, at most 9 E-10 M, at most 1E-11M, at most 2 E-11M, at most 3 E-11M, at most 4E-11M, at most 5 E-11M, at most 6E-11M, at most 7 E-11M, at most 8 E-11M, at most 9
35 E-11M, at most 4 E-12M, 5E-12M, at most 6 E-12M, at most 7 E-12M, at most 8 E-12M, at most 9 E-12M. In embodiments, the antibodies described herein bind UNC5C with an EC50 of at most 100 nM, preferably with an EC50 of at most 1 nM, as assessed via ELISA (e.g. as described herein).

In some embodiments, the antibody binds human UNC5C with an EC50 of at most 1.10E-08, at most 2.40E-08, at most 1.10E-09, at most 8.00E-09, at most 5.50E-10, at most 7.10E-10, at most 1.00E-11, at most 1.30E-11, at most 3.90E-11, at most 2.80E-12, or at most 9.20E-12.

5 In some embodiments, the antibody binds human UNC5C with an EC50 of about 1.10E-08, about 2.40E-08, about 1.10E-09, about 8.00E-09, about 5.50E-10, about 7.10E-10, about 1.00E-11, about 1.30E-11, about 3.90E-11, about 2.80E-12, or about 9.20E-12.

In some embodiments, the EC50 value is between 1E-07 M and 1E-13M, 5E-07 M and 1E-13M; 1E-08 M and 1E-13M; 5E-08 M and 1E-13M; 1E-09 M and 1E-13M; 5E-09 M and 1E-13M; 1E-10 M and 1E-13M; 5E-10 M and 1E-13M; 1E-11 M and 1E-13M; 5E-11 M and 1E-13M; 1E-12 M and 1E-13M; 5E-12 M and 1E-13M; between 1E-07 M and 1E-12M, 5E-07 M and 1E-12M; 1E-08 M and 1E-12M; 5E-08 M and 1E-12M; 1E-09 M and 1E-12M; 5E-09 M and 1E-12M; 1E-10 M and 1E-12M; 5E-10 M and 1E-12M; 1E-11 M and 1E-12M; 5E-11 M and 1E-12M; 1E-07 M and 1E-11M, 5E-07 M and 1E-11M; 1E-08 M and 1E-11M; 5E-08 M and 1E-11M; 1E-09 M and 1E-11M; 5E-09 M and 1E-11M; 1E-10 M and 1E-11M; 5E-10 M and 1E-11M; 1E-07 M and 1E-10M, 5E-07 M and 1E-10M; 1E-08 M and 1E-10M; 5E-08 M and 1E-10M; 1E-09 M and 1E-10M; 5E-09 M and 1E-10M; 1E-07 M and 1E-09M, 5E-07 M and 1E-09M; 1E-08 M and 1E-09M; 5E-08 M and 1E-09M; 1E-07 M and 1E-08M, 5E-07 M and 1E-08M. In embodiments, the EC50 value is between 1E-09 M and 1E-12M.

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In some embodiments, antibodies described herein are capable of specifically binding a UNC5C protein or protein fragments comprising or consisting of a UNC5C variant amino acid sequence. In some embodiments, the UNC5C variant protein or fragment comprises an amino acid sequence that has at least 70%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identity with SEQ ID NO: 99. In some embodiments, the antibodies disclosed herein are capable of specifically binding a UNC5C fragment comprising at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more of the UNC5C amino acid sequence, or a UNC5C variant sequence.

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25 In some embodiments, antibodies described herein, and antigen-binding fragments thereof, compete or cross-compete with other antibodies, or antigen binding fragments thereof, for example other antibodies described herein, such as ATL_6532. The term "compete", as used herein with regard to an antibody, means that a first antibody binds to an epitope of a protein (e.g., UNC5C) in a manner sufficiently similar to the binding of a second antibody, such that the result of binding of the first antibody with its epitope is detectably decreased in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. The alternative, where the binding of the second antibody to its epitope is also detectably decreased in the presence of the first antibody, can, but need not be the case. That is, a first antibody can inhibit the binding of a second antibody to its epitope without that second antibody inhibiting the binding of the first antibody to its respective epitope. However, where each detectably inhibits the binding of the other antibody with its epitope or ligand, whether to the same, greater, or lesser extent, the antibodies are said to "cross-compete" with each other for binding of their respective epitope(s). Both competing and cross-competing antibodies are within the scope of this disclosure. Regardless of the mechanism by which such competition or cross-competition occurs (e.g., steric hindrance, conformational change, or binding to a common epitope, or portion thereof), the skilled person would appreciate that such

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competing and/or cross-competing antibodies are encompassed and can be useful for the methods and/or uses provided herein, for example because competition and/or cross-competition with an antibody which does not bind the Ig1 domain of UNC5C (i.e. an antibody that binds elsewhere on the UNC5C protein, e.g. the Ig2, TSP1, and/or TSP2 domains of the UNC5C protein), such as ATL_6532, indicates that the detection of soluble UNC5C will not be inhibited by the presence of therapeutic anti-UNC5C antibodies that bind the Ig1 domain of UNC5C, such as ATLX-1282. Competition and/or cross competition may be assessed for example using an ELISA or MSD assay, such as described herein.

Conversely, in some embodiments, antibodies described herein, and antigen-binding fragments thereof, do not compete or cross-compete with other antibodies, or antigen binding fragments thereof, for example other antibodies described herein, such as ATLX-1282. That is, a first antibody may bind to an epitope of a protein (e.g., UNC5C) in a manner sufficiently different to the binding of a second antibody (e.g. the first antibody may bind a different epitope of the protein), such that the result of binding of the first antibody with its epitope is unchanged or substantially unchanged in the presence of the second antibody compared to the binding of the first antibody in the absence of the second antibody. That is, a first antibody can bind to its epitope without inhibiting the binding of the second antibody to its respective epitope. Such non-competing and/or non-cross-competing antibodies are encompassed and can be useful for the methods and/or uses provided herein. For example, anti-UNC5C antibodies (e.g. ATL_6532) that do not compete or cross-compete with therapeutic antibodies that bind the Ig1 domain of UNC5C, such as ATLX-1282, find particular utility in such methods and/or uses, as their detection of soluble UNC5C is not inhibited by the presence of therapeutic anti-UNC5C antibodies. Competition and/or cross competition may be assessed for example using an ELISA or MSD assay, such as described herein.

UNC5C may be human UNC5C, mouse UNC5C, or cynomolgus monkey UNC5C. Suitably, UNC5C may be human UNC5C. UNC5C may refer to human UNC5C unless context indicates otherwise. In other embodiments, for example when the individual to be treated is a non-human mammal, UNC5C may be non-human UNC5C.

Structural properties

Antibodies of the present disclosure may specifically target a single epitope on the UNC5C protein. The term epitope, also known as antigenic determinant, as used herein refers to any protein determinant capable of specific binding to an immunoglobulin or fragment thereof. Epitopic, or antigenic, determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics.

Antibodies described herein may bind to a discontinuous epitope of UNC5C. Antibodies described herein may bind to an epitope that comprises one or more of, or all of, the following residues of UNC5C provided as SEQ ID NO: 99: Thr89, Gln90, Gln102, Lys103, Val107, Asp108, Glu109, Arg110, Val111, Ile118, Arg120 or corresponding residues in a homologous sequence. In some embodiments, e.g. in instances where the antibody is a heavy-chain only antibody, the antibody binds to residues Thr89, Gln90, Gln102, Lys103, Val107, Glu109, Ile118, and Arg120.

In embodiments, one or more of (or all of) of VH residues 55, 63, 64, 65, 66, 74, 108, 110 in IMGT numbering interact with one or more residues in the epitope. In embodiments, one or more of (or all of) of VH residues 55 (Tyr), 63 (Gly), 64 (T, H or S, more specifically Thr), 65 (T or S, more specifically Thr), 66 (Asn), 74 (Ser), 108 (Arg), 110 (Met) in IMGT numbering interact with one or more residues in the epitope. All of these residues are conserved across the VH of antibodies 5262, 5996, 5997, 5998, 5999, 6000, 6001, 6002, 6003, 6004, 6005, 6033, 6034, 6035, 6036, 6037, 6038, 6039, 6177, 6178. All of these residues apart from 64 (Thr) and/or 65 (Thr) are conserved across the VH of antibodies 6187 (where position 64 is H and position 65 is T), 6191 (where position 64 is S and position 65 is S). This indicates that substitutions at these positions would be possible while maintaining binding to the epitope. Thus, in embodiments, one or more of (or all of) of VH residues 55 (Tyr), 63 (Gly), 64 (Thr or conservative substitution e.g. H or S), 65 (Thr or conservative substitution e.g. S), 66 (Asn), 74 (Ser), 108 (Arg), 110 (Met) in IMGT numbering interact with one or more residues in the epitope. In embodiments, one or more of (or all of) VL residues 38, 107, 108, 109, and 114 in IMGT numbering interact with one or more residues in the epitope. In embodiments, one or more of (or all of) VL residues 38 (Y, D, L or T, such as Tyr in specific embodiments), 107 (S, Y, F or W, such as Ser in specific embodiments), 108 (Y, D, T, or G, such as Tyr in specific embodiments), 109 (S, D, K or absent, such as Ser in specific embodiments), 114 (T, N, S, R, G, or A, such as Thr in specific embodiments) in IMGT numbering interact with one or more residues in the epitope. Position 38 is Tyr in antibodies 5262, 6178, 6530, 6531, 6533, 6534, 6537, 6539, D in antibody 6335, L in antibody 6336 and T in antibody 6538. Position 107 is S in antibodies 5262 and 6178 (same VL), Y in antibodies 6530, 6532, 6533, 6534, 6535 and 6536, F in 6531, and W in antibodies 6537, 6538 and 6539. Position 108 is Y in antibodies 5262 and 6178, D in antibodies 6530, 6532, 6533, 6534, 6535, 6537 and 6538, T in antibodies 6531 and 6536, and G in antibody 6539. Position 109 is S in antibodies 5262, 6178, 6530, 6533, 6534, 6535, 6536 and 6537, D in antibodies 6531 and 6538, K in antibody 6532 and absent in antibody 6539. Position 114 is T in antibodies 5262, 6178 and 6536, N in antibodies 6530, 6533 and 6538, S in antibodies 6531 and 6537, R in antibody 6532, G in antibody 6534, A in antibody 6535. Thus, in embodiments, the VH of an antibody as described herein has: at residue 55: Tyr, at residue 63: Gly, at residue 64: T, H or S, more specifically Thr, at residue 65: T or S, more specifically Thr, at residue 66: Asn, at residue 74: Ser, at residue 108: Arg, at residue 110: Met, where all positions are in IMGT numbering. Further, in embodiments, the VL of an antibody as described herein has: at residue 38: Y, D, L or T, such as Tyr in specific embodiments, at residue 107: S, Y, F or W, such as Ser in specific embodiments, at residue 108: Y, D, T, or G, such as Tyr in specific embodiments, at residue 109: S, D, K or absent (deletion), such as Ser in specific embodiments, at residue 114: T, N, S, R, G, or A, such as Thr in specific embodiments, where all positions are in IMGT numbering. Any of the amino acids at any of these positions can be combined with each other, and all resulting combinations are explicitly envisaged.

In some instances, (a) VH residue 64 (e.g. Thr) of antibodies described herein interacts with Thr 89 of the discontinuous epitope; and/or (b) VH residue 63 (e.g. Gly) of antibodies described herein interacts with Gln 90 of the discontinuous epitope; and/or (c) VH residue 65 (e.g. Thr) of antibodies described herein interacts with Gln 102 of the discontinuous epitope; and/or (d) VH residue 74 (e.g. Ser) of antibodies described herein interacts with Lys103 of the discontinuous epitope; and/or (e) VH residue 66 (Asn) of antibodies described herein interacts with Val 107 of the discontinuous epitope; and/or (f) VH residues 55 (e.g. Tyr),

66 (e.g. Asn), 108 (e.g. Arg), and/or 110 (e.g. Met) of antibodies described herein interact with Glu109 of the discontinuous epitope; and/or (g) VH residues 108 (e.g. Arg), and/or 110 (e.g. Met) of antibodies described herein interact with Ile118 of the discontinuous epitope; and/or (h) VH residue 55 (e.g. Tyr), 64 (e.g. Thr), and/or 65 (e.g. Thr) of antibodies described herein interacts with Arg120 of the discontinuous epitope. In some instances, (i) VL residue 114 (e.g. Thr) of antibodies described herein interacts with Val107 and/or Asp108, and/or Glu109 of the discontinuous epitope; and/or (j) VL residue 109 (e.g. Ser) of antibodies described herein interacts with Glu109 and/or Arg110 of the discontinuous epitope; and/or (k) VL residue 108 (e.g. Tyr) of antibodies described herein interacts with Arg110 and/or Val111 of the discontinuous epitope; wherein the UNC5C residues are those at the specified positions in SEQ ID NO: 99 or corresponding positions in a homologous sequence. The skilled person will be familiar with methods for mapping epitopes, including but not limited to X-ray co-crystallography as described herein; cryogenic electron microscopy as described, for example, in Renaud et al., 2018; mutagenesis strategies, such as alanine scanning mutagenesis as described, for example, in Cunningham et al., 1989.

An "antigen binding domain" describes the part of a molecule that binds to all or part of the target antigen. An antibody generally comprises six complementarity-determining regions (CDRs); three in the VH region: HCDR1, HCDR2 and HCDR3, and three in the VL region: LCDR1, LCDR2, and LCDR3. The six CDRs together define the paratope of the antigen binding domain, which is the part of the antigen binding domain which binds to the target antigen. The paratope of an antibody as described herein may include the following VH residues: 55 (e.g. Tyr), 63 (e.g. Gly), 64 (e.g. Thr, His or Ser), 65 (e.g. Thr or Ser), 66 (e.g. Asn), 74 (e.g. Ser), 108 (e.g. Arg), 110 (e.g. Met) in IMGT numbering. The paratope of an antibody as described herein may include the following VL residues: 38 (e.g. Tyr, D, L or T), 107 (e.g. Ser, Y, F, or W), 108 (e.g. Tyr, D, T or G), 109 (e.g. Ser, D, K or – (deletion)), 114 (e.g. Thr, N, S, R, G or A) in IMGT numbering. Thus, an antibody according to any embodiment of the present disclosure may comprise a VH with the following residues: at position 55: Tyr, at position 63: Gly, at position 64: Thr, at position 65: Thr, at position 66: Asn, at position 74: Ser, at position 108: Arg, at position 110: Met in IMGT numbering. Further, an antibody according to any embodiment of the present disclosure may comprise a VL with the following residues: at position 38: Tyr, at position 107: Ser, at position 108: Tyr, at position 109: Ser, at position 114: Thr in IMGT numbering. The present disclosure relates primarily to antibody molecules, whether whole antibody (e.g. IgG, such as IgG1) or antibody fragments (e.g. single-chain variable fragment (scFv), Antibody fragments (Fab) or bivalent antibody fragments (F(ab')₂), single-domain antibody (sdAb). Antibody antigen binding regions (also referred to as "antigen binding portions") are provided, as are antibody heavy chain variable (VH) and light chain variable (VL) domains. Within VH and VL domains are provided complementarity determining regions (CDRs), which may be provided within different framework regions (FRs), to form VH or VL domains, as the case may be. An antigen binding site may consist of an antibody VH domain and/or a VL domain. Thus, an antibody according to the present disclosure may be an scFv antibody molecule, a nanobody, or a whole antibody. The antibody may comprise an antibody constant region. The antibody constant region may be a human constant region, such as a human IgG1 constant region. The antibody may be a whole antibody. The antibody may be an IgG, such as an IgG1 or variant thereof. The antibody may be an IgG1 variant L234A/L235A (LALA).

Antibodies according to the present disclosure may be provided in isolated form. The term "antibody" encompasses a fragment or derivative thereof, or a synthetic antibody or synthetic antibody fragment.

The antigen-binding portion may be a part of an antibody (e.g. a Fab fragment) or a synthetic antibody fragment (e.g. an scFv). Suitable antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques ", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications ", J G R Hurrell (CRC Press, 1982). Chimeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 25 792-5 799).

An antibody or fragment thereof may be a monoclonal antibody (mAb). mAbs are homogenous populations of antibodies specifically targeting a single epitope on an antigen.

Fragments of antibodies, such as Fab and F(ab')₂ fragments, may also be provided, as can genetically engineered antibodies and antibody fragments. The VH and VL domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by "humanisation" of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent parent antibody (Morrison et al (1984) Proc. Natl. Acad. Sci. USA 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains as known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra et al (1988) Science 240, 1038); single-chain Fv (scFv) molecules where the VH and VL partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 20 242, 423; Huston et al (1988) Proc. Natl. Acad. Sci. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293- 299.

The term "scFv molecules" refers to molecules wherein the VH and VL partner domains are covalently linked, e.g. by a flexible oligopeptide. Fab, Fv, scFv and sdAb antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and F(ab')₂ fragments are "bivalent". The term "bivalent" means that the said antibodies and F(ab')₂ fragments have two antigen combining sites. In contrast, Fab, Fv, scFv and dAb fragments are monovalent, having only one antigen combining site.

A "fragment" of an antibody may comprise any number of residues of a "parental" antibody, whilst retaining target binding ability. A fragment may lack effector function, for example may be entirely unable to bind or show diminished binding to the Fc receptor, relative to the parent. A fragment is typically smaller than the parental antibody. A fragment may comprise 50%, 60%, 70%, 80%, 90%, 95% or more of the contiguous or non-contiguous amino acids of the parental antibody. A fragment may comprise 50, 100, 150, 200, 250, 300 or more contiguous or non-contiguous amino acids of the parental antibody. A fragment may comprise deletions in the Fc region, or of the Fc region. A fragment may retain the CDRs and/or the variable domains

of the parental antibody, unaltered. In some embodiments, a fragment is an Fab fragment or an F(ab')₂ fragment.

CDR sequences are described herein using the IMGT numbering (Lefranc, M.-P., Immunology Today, 18, 509 (1997)).

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The disclosure further provides antibodies comprising a heavy chain variable domain (VH) with the CDRs of the VH of the antibodies ATL_6530; ATL_6531; ATL_6532; ATL_6533; ATL_6534; ATL_6535; ATL_6536; ATL_6537; ATL_6538; ATL_6539: CDRH1 comprising amino acid sequence SEQ ID NO: 142-149, CDRH2 comprising amino acid sequence SEQ ID NO: 150-158, and CDRH3 comprising amino acid sequence SEQ ID NO: 159-168. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6530, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 142, CDRH2 comprising amino acid sequence SEQ ID NO: 150, and CDRH3 comprising amino acid sequence SEQ ID NO: 159. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6531, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 143, CDRH2 comprising amino acid sequence SEQ ID NO: 151, and CDRH3 comprising amino acid sequence SEQ ID NO: 160. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6532, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 144, CDRH2 comprising amino acid sequence SEQ ID NO: 152, and CDRH3 comprising amino acid sequence SEQ ID NO: 161. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6533, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 145, CDRH2 comprising amino acid sequence SEQ ID NO: 153, and CDRH3 comprising amino acid sequence SEQ ID NO: 162. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6534, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 144, CDRH2 comprising amino acid sequence SEQ ID NO: 152, and CDRH3 comprising amino acid sequence SEQ ID NO: 163. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6535, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 143, CDRH2 comprising amino acid sequence SEQ ID NO: 154, and CDRH3 comprising amino acid sequence SEQ ID NO: 164. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6536, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 146, CDRH2 comprising amino acid sequence SEQ ID NO: 155, and CDRH3 comprising amino acid sequence SEQ ID NO: 165. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6537, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 147, CDRH2 comprising amino acid sequence SEQ ID NO: 156, and CDRH3 comprising amino acid sequence SEQ ID NO: 166.

In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6538, i.e. CDRH1 comprising

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amino acid sequence SEQ ID NO: 148, CDRH2 comprising amino acid sequence SEQ ID NO: 157, and CDRH3 comprising amino acid sequence SEQ ID NO: 167. In particular, antibodies according to the present disclosure may have a heavy chain variable domain (VH) with the CDRs of the heavy chain variable domain (VH) of antibody ATL_6539, i.e. CDRH1 comprising amino acid sequence SEQ ID NO: 149, CDRH2
5 comprising amino acid sequence SEQ ID NO: 158, and CDRH3 comprising amino acid sequence SEQ ID NO: 168. In an antibody according to the present disclosure at least one of the VH CDR 1-3 sequences may vary. For example, a variant may have VH CDRs that have up to 3 amino acid mutations over the above VH CDRs.

In an antibody according to the present disclosure at least one of the VH CDR 1-3 sequences may vary.

10 That is, the above set of CDRs may comprise one, two, or three mutations. Mutations as used herein refers to amino acid substitutions, insertions, or deletions. Mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. A variant may have one or two amino acid mutations, such as substitutions compared with the set of VH CDR1-3 described above. In embodiments, an antibody according to the disclosure comprises CDRs with sequences that have one or
15 two mutations, such as substitutions compared to the VH CDR sequences of any antibody described herein. For example, an antibody according to the disclosure may comprise VH CDRs with the sequences of any antibody above, except that one or two of the CDRHs comprise a substitution, where the total number of substitutions across the CDRHs does not exceed 2. In embodiments, a variant may have one, two or three, preferably at most one or two substitutions in each of one or more of the VH CDR1-3 described above.
20 CDRH1 regions of any antibodies or fragments described herein may have a length of 8 amino acids. CDRH2 regions of any antibodies or fragments described herein may have a length of 7 amino acids. CDRH3 regions of any antibodies or fragments described herein may have a length of 13 amino acids. In embodiments, a variant may have VH CDRs that have at least 70%, at least 80% or at least 90% sequence identity with any set of VH CDRs described herein. For example, a variant with a combined CDRH 1-3
25 length of 28 amino acids may have up to 8, up to 5, or up to 2 amino acid substitutions compared to a set of VH CDRs described herein. In embodiments, a variant may have one or two substitutions comprising or consisting of a substitution at position 58 in CDRH2. The substitution may be N58Y.

An antibody according to the present disclosure may have a VH with the following CDRHs: CDRH1 comprising amino acid sequence SEQ ID NO:144, CDRH2 comprising amino acid sequence SEQ ID
30 NO:152 and CDRH3 comprising amino acid sequence SEQ ID NO:161. These are the VH CDRs of ATL_6532. These are the CDRs of ATL_6533.

In an antibody according to the present disclosure at least one of the VL CDR 1-3 sequences may vary. A variant may have 1, 2, or 3 amino acid mutations, such as substitutions compared with the set of VL CDR1-3 described above. In embodiments, an antibody according to the disclosure comprises CDRs with
35 sequences that have between 1 and 3 mutations, such as substitutions compared to the VL CDR sequences of any antibody described herein. For example, an antibody according to the disclosure may comprise VL CDRs with the sequences of any antibody above, except that one to 3 of the CDRLs comprise a substitution, where the total number of substitutions across the CDRLs does not exceed 3. In embodiments, a variant may have one, two or three, preferably at most one or two substitutions in each of
40 one or more of the VL CDR1-3 described above. CDRL1 regions of any antibodies or fragments described

herein may have a length of between 6 and 12 amino acids, preferably 6 amino acids. CDRL2 regions of any antibodies or fragments described herein may have a length of 3 amino acids. CDRL3 regions of any antibodies or fragments described herein may have a length of 9 or 10 amino acids, preferably 9. In embodiments, a variant may have VL CDRs that have at least 70%, at least 80% or at least 90% sequence identity with any set of VL CDRs described herein.

Antibodies according to the present disclosure may have a VL comprising the framework sequences of any of ATL_6530, ATL_6531, ATL_6532, ATL_6533, ATL_6534, ATL_6535, ATL_6536, ATL_6537, ATL_6538, ATL_6539. The VH CDRs 1-3 and optionally VL CDRs 1-3 of any of the antibodies described above may also be particularly useful in conjunction with a number of different framework regions. Accordingly, light and/or heavy chains having CDRs 1-3 as described above may possess an alternative framework region. Suitable framework regions are known in the art and are described for example in M. Lefranc & G. Le Franc (2001) "The Immunoglobulin Facts Book", Academic Press.

An antibody of the disclosure may have the CDRH1, CDRH2 and CDRH3 of the VH domain within a germline framework. In some embodiments, the antibody of the disclosure has a heavy chain variable domain (VH) comprising CDRH1, CDRH2, and CDRH3 within a germline framework, provided that position 67 in standard IMGT numbering is Q.

The antibody according to the present disclosure may have a heavy chain variable domain (VH) comprising the framework sequences of any of antibodies ATL_6530, 6531, 6532, 6533, 6534, 6535, 656, 6537, 6538 or 6539, or framework sequences comprising one to five mutations, such as substitutions, compared to these framework sequences. Thus, antibodies according to the present disclosure may have a VH comprising

(a) HFWR1 of QVQLLETGGGLVQPGGSLRLSCAAS SEQ ID NO: 199; HFWR2 of MNWVRQAPGKGLEWVSS SEQ ID NO: 200; HFWR3 of

YYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYC SEQ ID NO: 201; HFWR4 of WGQGTTLTVSS SEQ ID NO: 62, (framework sequences of ATL_6530);

(b) HFWR1 of EVQLVESGGGVVQPGGSLRLSCAAS SEQ ID NO: 202; HFWR2 of MHWVRQAPGKGLEWVSL SEQ ID NO: 203; HFWR3 of

YYADSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYC SEQ ID NO: 204; HFWR4 of WGQGTTLTVSS SEQ ID NO: 62, (framework sequences of ATL_6531)

(c) HFWR1 of QVTLKESGAEVKKPGASVKVSKASSE SEQ ID NO: 205; HFWR2 of MHWVRQAPGQGLEWMGR SEQ ID NO: 206; HFWR3 of

NYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC SEQ ID NO: 207; HFWR4 of WGQGTMTVTVSS SEQ ID NO: 208, (framework sequences of ATL_6532)

(d) HFWR1 of QVQLQQSGGGLVKPGGSLRLSCAAS SEQ ID NO: 209; HFWR2 of MSWVRQAPGKGLEWVSY SEQ ID NO: 210; HFWR3 of

NYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYC SEQ ID NO: 211; HFWR4 of WGQGTMTVTVSS SEQ ID NO: 208, (framework sequences of ATL_6533)

(e) HFWR1 of QVQLVESGAEVKKPGASVKVSKAS SEQ ID NO: 212; HFWR2 of

MHWVRQAPGQGLEWMGR SEQ ID NO: 206; HFWR3 of

- NYAQKFQGRVTMTRDTSISTAYMELSRRLRSSDDTAVYYC SEQ ID NO: 207; HFWR4 of
WGQGTMTVTVSS SEQ ID NO: 208, (framework sequences of ATL_6534)
- (f) HFWR1 of EVQLLETGGGLVQPGRSLRLSCAAS SEQ ID NO: 13; HFWR2 of
MHWVRQAPGKGLEWVSG SEQ ID NO: 14; HFWR3 of
5 GYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYC SEQ ID NO: 15; HFWR4 of
WGQGTMTVTVSS SEQ ID NO: 208, (framework sequences of ATL_6535)
- (g) HFWR1 of EVQLLESAGGVVQLGRSLRLSCAAS SEQ ID NO: 216; HFWR2 of
MHWVRQAPGKGLEWVAI SEQ ID NO: 217; HFWR3 of
YYAGSVGGRFTISRDNASKNTLYLQMDSLRSSDDTAVYYC SEQ ID NO: 218; HFWR4 of
10 WGHGTMVTVSS SEQ ID NO: 219, (framework sequences of ATL_6536)
- (h) HFWR1 of QVQLVQSGAEVKKPGESLKISCKGS SEQ ID NO: 220; HFWR2 of
IGWVRQMPGKGLEWMGI SEQ ID NO: 221; HFWR3 of
RYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYC SEQ ID NO: 222; HFWR4 of
WGQGTMTVTVSS SEQ ID NO: 223, (framework sequences of ATL_6537)
- 15 (i) HFWR1 of QVQLQESGPGLVKPSGTLSTCAVS SEQ ID NO: 224; HFWR2 of
WSWVRQPPGKGLEWIGE SEQ ID NO: 225; HFWR3 of
NYNPSLKSRTISVDKSKNQFSLKLSSVTAADTAVYYC SEQ ID NO: 226; HFWR4 of
WGQGTMTVTVSS SEQ ID NO: 62, (framework sequences of ATL_6538)
- (j) HFWR1 of QVQLVESGGGLVQPGGSLRLSCSAS SEQ ID NO: 227; HFWR2 of
20 MSWVRQAPGKGLEWVST SEQ ID NO: 228; HFWR3 of
YYADSVKGRFTISRDNASKNTVYLQMNSLRAEDTAVYYC SEQ ID NO: 229; HFWR4 of
WGQGTMTVTVSS SEQ ID NO: 208, (framework sequences of ATL_6539)
- (k) or framework sequences comprising one to five mutations, such as substitutions, compared to
these framework sequences.
- 25 Mutations in the FWRH may include mutations at one or more, or all, of positions:
- In HFWR1: 1, optionally wherein the mutation is the substitution Q1E; 1, optionally wherein the
mutation is the substitution Q3T; 6, optionally wherein the mutation is the substitution E6Q; 7,
optionally wherein the mutation is the substitution S7T; 12, optionally wherein the mutation is the
substitution L12V; 13, optionally wherein the mutation is the substitution V13K; 15, optionally
30 wherein the mutation is the substitution P15L; 16, optionally wherein the mutation is the
substitution S16G; 19, optionally wherein the mutation is the substitution L19V; 21, optionally
wherein the mutation is the substitution L21V; 22, optionally wherein the mutation is the
substitution S22T;
 - In HFWR2: 48, optionally wherein the mutation is the substitution K48Q;
 - 35 - In HFWR3: 74, optionally wherein the mutation is the substitution S74G; 75, optionally wherein
the mutation is the substitution R75Q; 76, optionally wherein the mutation is the substitution
V76F; 78, optionally wherein the mutation is the substitution I78M; 79, optionally wherein the
mutation is the substitution S79T; 83, optionally wherein the mutation is the substitution S83A;
84, optionally wherein the mutation is the substitution K84I; 85, optionally wherein the mutation is
40 the substitution N85S; 89, optionally wherein the mutation is the substitution L89M; 93, optionally
wherein the mutation is the substitution S93R; 96, optionally wherein the mutation is the
substitution A96S;

- In HFWR4: 120, optionally wherein the mutation is the substitution Q120H.

In this specification, antibodies may have VH (and optionally VL) regions comprising an amino acid sequence that has a high percentage sequence identity to the VH and/or VL amino acid sequences described above.

- 5 For example, antibodies according to the present disclosure include antibodies that bind UNC5C and have a VH regions that comprises an amino acid sequence having at least 70%, more preferably one of at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the VH region amino acid sequence of any antibody described herein, such as ATL_6530-6539 (SEQ ID NOs: 122-131). Note that any sequence obtained by applying conservative one or more conservative amino acid substitutions to a sequence as described herein is also encompassed, provided that the overall sequence identity is within the ranges provided and that the resulting antibody still binds UNC5C.

- Antibodies described herein include antibodies that have a heavy chain variable domain (VH) comprising a sequence of the VH of any antibody described herein and a light chain variable domain (VL) comprising a sequence of the VL of any antibody described herein. In particular, antibodies described herein include antibodies that have: (i) a heavy chain variable domain (VH) comprising a sequence of the VH of ATL_6530, ATL_6531, ATL_6532, ATL_6533, ATL_6534, ATL_6535, ATL_6536, ATL_6537, ATL_6538, ATL_6539, and (ii) a light chain variable domain (VL) comprising a sequence of the VL of ATL_6530, ATL_6531, ATL_6532, ATL_6533, ATL_6534, ATL_6535, ATL_6536, ATL_6537, ATL_6538, ATL_6539.

- The antibodies of the disclosure are derived from human antibodies and may therefore be referred to as human antibodies. Antibodies of the disclosure were obtained from phage display libraries created from VH repertoires of patients who carried the original antibody ATL_0005262, and a VL repertoire from healthy donors. Thus, antibodies of the disclosure may differ from naturally occurring antibodies at least by their VH-VL pairing. Antibodies of the disclosure may comprise human VH and/or VL sequences. Antibodies of the disclosure may be formulated as human IgG1 antibodies or variants thereof. Antibodies of the disclosure may have a human framework sequence and/or a human Fc domain.

Overall percentage identity of a variable region or full length heavy/light chain sequence may be combined with specific CDR sequences from the same antibody.

- Percentage (%) sequence identity is defined as the percentage of amino acid residues in a candidate sequence that are identical with residues in a comparative sequence after aligning the sequences and introducing gaps if necessary, to achieve the maximum sequence identity, and not considering any conservative substitutions as part of the sequence identity. Sequence identity is preferably calculated over the entire length of the respective sequences. Where the aligned sequences are of different length, sequence identity of the shorter comparison sequence may be determined over the entire length of the longer given sequence or, where the comparison sequence is longer than the given sequence, sequence identity of the comparison sequence may be determined over the entire length of the shorter given sequence. Sequence identity may be defined with reference to the algorithm GAP (Wisconsin GCG package, Accelrys Inc, San Diego USA). GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally,

default parameters may be used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul et al. (1990) J. Mol. Biol. 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) PNAS USA 85: 2444-2448), SSEARCH (Smith and Waterman (1981) J. Mol Biol. 147: 195-197;), HMMER3 (Johnson LS et al BMC Bioinformatics. 2010 Aug 18; 11():431) or the TBLASTN program, of Altschul et al. (1990) supra, generally employing default parameters (see for example Pearson Curr Prot Bioinformatics (2013) Chapt 3 Uniy 3.1 doi:10.1002/0471250953.bi0301s42). In particular, the psi-Blast algorithm may be used (Altschul et al. Nucl. Acids Res. (1997) 25 3389-3402). Sequence identity and similarity may also be determined using Genomequest™ software (Gene-IT, Worcester MA USA). Sequence comparisons are preferably made over the full-length of the relevant sequences to be compared.

The antibodies of the present disclosure may comprise one or more substitutions within the framework of the VH and/or VL region. As used herein, "substitution" refers to the exchange of one amino acid for another at a specific position, relative to the same position in a baseline molecule. In some embodiments, the baseline molecules are exemplified antibodies herein, for example ATL_6532, ATL_6533, ATL_6534 or ATL_6537 (VH sequences of SEQ ID Nos: 124, 125, 126, 129; VL sequences of SEQ ID Nos: 134, 135, 136, 139). In some embodiments, the baseline molecule is ATL_6532 (VH sequence of SEQ ID No: 124; VL sequence of SEQ ID No: 134).

As used herein, a "biological fluid sample" refers to any fluid that originates from the subject's body, for example blood, urine, saliva, cerebrospinal fluid (CSF), and sweat. In embodiments, the biological fluid sample is blood or cerebrospinal fluid (CSF). In embodiments, the methods and uses described herein may be performed on whole blood, plasma, and/or serum derived from a blood sample obtained from a subject.

A subject may be any animal or human. In embodiments, the subject is mammalian, for example a mouse, rat, monkey or human, more preferably human. The subject may be male or female. The subject may be a patient, for example a patient diagnosed with amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Parkinson's disease (PD), or REM Sleep Behavior Disorder (RBD).

The subject may be a patient, for example a patient diagnosed with a condition treatable with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF. For example, the subject may be a patient who has been treated with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF. Conditions treatable in accordance with the present disclosure include any in which UNC5C plays a role, including neurodegenerative disorders, and in particular those characterised by increased neuronal apoptosis, decreased synaptic health and/or altered synaptic plasticity (e.g. reduced or abnormal synaptic plasticity). The antibodies of the present disclosure were originally identified through analysis of resilient frontotemporal dementia (FTD) patients. These antibodies were subsequently found in other resilient and control individuals in Alzheimer's disease (AD); Parkinson's disease (PD); and in resilient centenarians indicating relevance beyond FTD to other neurodegenerative diseases. Thus, also described herein are antibodies of the disclosure for use as a medicament. Also described are antibodies of the disclosure for use in the treatment or prevention of a neurodegenerative disease. Also described herein are antibodies of the disclosure for use in the manufacture of a medicament, such as a medicament for the treatment or

prevention of a neurodegenerative disease or disorder. Also described herein a method of treating a subject who has been diagnosed as having or being at risk of having a neurodegenerative disorder, the method comprising administering an antibody as described herein in a therapeutically effective amount to the subject.

- 5 The present disclosure refers to subjects that have been diagnosed as having or being likely to have a neurodegenerative disease or disorder, and to the diagnosis of subjects as likely to have a neurodegenerative disease or disorder. A neurodegenerative disease or disorder may be any disease or disorder associated with clinical symptoms of neurodegeneration, as well as any disease or disorder that is a prodromal or pre-clinical stage of a neurodegenerative disease or disorder. A neurodegenerative
- 10 disease or disorder can comprise one or more of the following: FTD, AD, HD, Parkinson's disease (PD), human immunodeficiency virus (HIV)-induced encephalitis, Chronic traumatic encephalopathy (CTE), vascular dementia, prion diseases, Lewy body disease, Spinal muscular atrophy (SMA); Motor Neuron Disease (MND), such as amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy (PSP), spinocerebellar ataxias (SCA) types 1, 2, 6, 7 and 17, Machado– Joseph disease (MJD/SCA3),
- 15 dentatorubral pallidoluysian atrophy (DRPLA), spinal bulbar muscular atrophy X-linked type 1 (SMA1/SBMA), Anderson-Fabry (X-linked Fabry Disease), and DNAJB6 Myopathies. In embodiments, the neurodegenerative disease is selected from FTD, AD, HD, MND, such as ALS, AD and PD. In embodiments, the neurodegenerative disease is a MND, such as ALS. In embodiments, the neurodegenerative disease is FTD, AD, HD, ALS, RBD (a prodromal stage of PD) or PD.
- 20 Described herein is a method of determining the effect of a therapeutic compound or composition that affects the level of soluble UNC5C in a subject who has been administered the therapeutic compound or composition, wherein the method comprises determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject. In embodiments, determining the level of soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid
- 25 sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or antibody fragment thereof. In embodiments, the method comprises: (a) determining a first level of soluble UNC5C in a first biological sample previously obtained from the subject, wherein the first biological fluid sample was obtained prior to administration of a therapeutic compound or composition to the subject; (b) determining a second level of soluble UNC5C in a second biological fluid sample previously
- 30 obtained from the subject, wherein the second biological fluid sample was obtained after administration of the therapeutic compound or composition to the subject; and (c) comparing the first level of soluble UNC5C and the second level of soluble UNC5C to determine the effect of the therapeutic compound or composition on the level of soluble UNC5C. In embodiments, determining the first level of soluble UNC5C comprises contacting said first biological fluid sample previously obtained from the subject with a probe which
- 35 specifically binds to UNC5C protein or a fragment thereof. In embodiments, determining the second level of soluble UNC5C comprises contacting said second biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. Also described herein is a method of determining the effect of a therapeutic compound or composition that affects the level of soluble UNC5C in a subject who has been administered the therapeutic compound or

composition, wherein the method comprises determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject, wherein the biological fluid sample was obtained after administration of the therapeutic compound or composition to the subject, and the method comprises comparing the level of soluble UNC5C in the sample to a reference value to determine the effect of the therapeutic compound or composition on the level of soluble UNC5C. The reference value may be a value that has been obtained by determining a first level of soluble UNC5C in a first biological sample previously obtained from the subject, wherein the first biological fluid sample was obtained prior to administration of a therapeutic compound or composition to the subject. The reference value may be a value that corresponds to an expected level of soluble UNC5C in a subject. The expected level of soluble UNC5C in a subject may be an expected level of soluble UNC5C in a subject that has been identified as having a neurodegenerative disease as described herein. In embodiments, the level or second level being higher than the first level or reference value may indicate target engagement of the therapeutic compound with UNC5C. In such embodiments, the therapeutic compound may be a therapeutic compound that stabilises soluble UNC5C (e.g. increase the half-life of UNC5C) when administered to a subject. In embodiments, step (i) further comprises determining a first level of total protein in the first biological fluid sample; step (ii) further comprises determining a second level of total protein in the second biological fluid sample; and the comparison of step (iii) comprises comparing the first level of soluble UNC5C normalised to the first level of total protein and the second level of soluble UNC5C normalised to the second level of total protein. In embodiments, the methods may comprise normalising a level of soluble UNC5C in a biological fluid sample relative to a level of total protein in the biological fluid sample. Such normalisation may be performed prior to any comparing step.

Described herein is a method of detecting the presence and/or severity of a neurodegenerative disease in a subject, or determining whether the subject is likely to be resilient or sensitive to a neurodegenerative disease, the method comprising: (a) determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C, wherein a higher level of soluble UNC5C in the biological fluid sample relative to the reference level of soluble UNC5C is indicative of the presence of the neurodegenerative disease and/or indicative of the severity of the neurodegenerative disease, and/or indicative of the subject being unlikely to be resilient (or likely to be sensitive) to the neurodegenerative disease. A subject being likely to be resilient to a neurodegenerative disease refers to a subject who has one or more risk factors for developing a neurodegenerative disease but who is unlikely to develop the neurodegenerative disease, likely to develop the neurodegenerative disease at a later age than a subject who is sensitive to the neurodegenerative disease or an average subject with the same risk factors, likely to develop a less severe form of the neurodegenerative disease than a subject who is sensitive to the neurodegenerative disease, and/or likely to have a slower progression of the neurodegenerative disease than a subject who is sensitive to the neurodegenerative disease. A subject being likely to be sensitive to a neurodegenerative disease refers to a subject who is likely to develop the neurodegenerative disease, likely to develop the neurodegenerative disease at an earlier age than a subject who is resilient to the neurodegenerative disease or at a similar or earlier age than an average subject with the same risk factors, likely to develop a more severe form of the neurodegenerative disease than a subject who is resilient to the neurodegenerative disease, and/or likely to

have a faster progression of the neurodegenerative disease than a subject who is resilient to the neurodegenerative disease. The neurodegenerative disease may be selected from: amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), and Parkinson's disease (PD). The neurodegenerative disease may be any disease that is a prodromal disease or disorder for a neurodegenerative disease with neurodegenerative symptoms, such as e.g. amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Huntington's Disease (HD), and Parkinson's disease (PD). The neurodegenerative disease may be ALS. In embodiments, the neurodegenerative disease is a prodromal or pre-clinical stage neurodegenerative disease, such as pre-clinical Alzheimer's disease (AD) or prodromal Parkinson's disease (PD), for example REM sleep Behavioural Disorder (RBD). Pre-clinical AD may be defined according to the NIA-AA AT(N) framework (Jack et al., 2018). REM sleep Behavioural Disorder (RBD) is considered a prodromal stage for Parkinson's Disease (Lee et al, 2023). In embodiments, the neurodegenerative disease is a symptomatic or clinical stage neurodegenerative disease. In embodiments, determining the level of soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. In embodiments, step (a) comprises determining a level of total protein in the biological fluid sample and the comparison of step (b) comprises comparing (i) the level of soluble UNC5C normalised to the level of total protein and (ii) the reference level of soluble UNC5C, wherein the reference level of soluble UNC5C corresponds to a level of soluble UNC5C normalised to a level of total protein. In embodiments, the presence and/or severity of the neurodegenerative disease is indicated by the level of soluble UNC5C in the biological fluid sample (optionally normalised to the level of total protein in the biological fluid sample) being at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100% higher than the reference level of soluble UNC5C (optionally normalised to the level of total protein in the reference biological fluid sample).

Described herein is a method of selecting a subject for treatment with a therapeutic compound or composition that affects the level of soluble UNC5C, or for participating in a clinical trial of a therapeutic compound or composition (e.g. a therapeutic compound or composition that affects the level of soluble UNC5C), wherein the method comprises: (a) detecting the level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C in a reference biological fluid sample from a healthy control; and (c) selecting the subject for treatment with the therapeutic compound or composition that affects the level of soluble UNC5C when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C. In embodiments, determining the level of soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof. The probe may be an antibody or fragment thereof. In embodiments, step (a) further comprises determining the level of total protein in the biological fluid sample; the comparison of step (b) comprises comparing (i) the level of soluble UNC5C normalised to the level of total protein and (ii) the reference level of soluble UNC5C normalised to the level of total protein in the reference biological fluid sample; and the selection of step (c) comprises selecting the subject for treatment

when the level of soluble UNC5C normalised to the level of total protein in the biological fluid sample is higher than the reference level of soluble UNC5C normalised to the level of total protein in the reference biological fluid sample. In embodiments, the selection of step (c) comprises selecting the subject for treatment or for participating in the clinical trial when the level of soluble UNC5C in the biological fluid sample (optionally normalised to the level of total protein in the biological fluid sample) is higher than the reference level of soluble UNC5C (optionally normalised to the level of total protein in the reference biological fluid sample) by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 100%. Any method of selecting a subject for treatment may further comprise administering to the subject a therapeutically effective amount of the treatment.

The skilled person will be familiar with methods for determining the level of total protein in a biological fluid sample, including but not limited to a bicinchoninic acid (BCA) assay such as Thermo Fisher/Pierce #23225 as used herein, Lowry assays, fluorescence-based protein assays, or a dye-based protein assays, for example Coomassie dye-based protein assays.

15 *Immunoassays*

In embodiments, the antibodies described herein are used as a capture antibody in an immunoassay. As used herein, the term "immunoassay" refers to a biochemical test that measures the presence or level (e.g. concentration) of a target analyte, e.g. a macromolecule (e.g. UNC5C) or a small molecule in a solution (e.g. in a biological sample obtained from a subject) through the use of an antibody. In embodiments, the immunoassay is a sandwich enzyme-linked immunosorbent assay (ELISA) or a meso scale discovery (MSD) assay.

As used herein, a sandwich ELISA refers to a type of immunoassay in which the analyte being measured is bound between two antibodies, each preferably detecting a different epitope of the antigen — the capture antibody and the detection antibody. The skilled person will be familiar with methods and protocols for performing a sandwich ELISA, for example as described herein. Typically, the capture antibody is immobilized on a plate, e.g. a multiwell microplate. The biological fluid sample is contacted with the capture antibody e.g. by contacting the immobilized capture antibody with the biological fluid sample to enable binding of the target analyte by the capture antibody. The plate is then washed to remove unbound analyte, before contacting the plate with the detection antibody. The detection antibody binds to the target analyte retained on the plate by the capture antibody. The plate is then washed again to remove unbound detection antibody. In embodiments, the detection antibody carries a detectable label (direct detection). In other embodiments, the detection antibody is detected using a secondary antibody which binds the detection antibody, wherein the secondary antibody carries a detectable label (indirect detection). Alternatively, the detection antibody may have bound to it biotin and binding of labelled streptavidin to the biotin is used to indirectly label the detection antibody. Detection of the detectable label is then used to determine the level of soluble UNC5C in the biological fluid sample. The skilled person will be familiar with various standard methods which may be used to detect the detectable label, depending on the specific detectable label used. For example, an example of an enzymatically active label is a horseradish peroxidase (HRP) enzyme, which may be detected by contacting the horseradish peroxidase (HRP) enzyme with TMB (3, 3', 5, 5'-

tetramethylbenzidine) and measuring the ultraviolet (UV) absorption at 450nm and/or 570nm, e.g. as described herein.

The skilled person will be familiar with methods and protocols for performing a meso scale discovery (MSD) assay, for example as described herein. In brief, the method comprises: immobilisation of the capture antibody on an MSD plate, contacting the biological fluid sample with the capture antibody e.g. by contacting the immobilized capture antibody with the biological fluid sample to enable binding of the target analyte by the capture antibody, washing away the unbound analyte, contacting the plate with the detection antibody, and washing the plate again to remove unbound detection antibody. In embodiments, the detection antibody carries a detectable label (direct detection). In other embodiments, the detection antibody is detected by contacting the detection antibody with a secondary antibody which binds the detection antibody, wherein the secondary antibody carries a detectable label (indirect detection ; followed by a wash step to remove unbound secondary antibody). Detection of the detectable label is then used to determine the level of soluble UNC5C in the biological fluid sample. In embodiments, the detectable label used in a meso scale discovery (MSD) assay is a SULFO-TAG (Meso Scale Discovery), which is an electrochemiluminescent label. Detection of a SULFO-TAG comprises addition of a read buffer, e.g. T (MSD #R92TC-3; a Tris-based buffer containing tripropylamine (TPA) as a co-reactant for light generation), and detection on a MSD plate reader, e.g. on an MSD QuickPlex SQ120MM.

Detection antibodies according to the present disclosure may be antibodies which bind UNC5C protein, for example which bind a different epitope of UNC5C protein to the capture antibody, which are detectably labelled or, at least, capable of detection. For example, the antibody may be labelled with a radioactive atom or a coloured molecule or a fluorescent molecule or a molecule which can be readily detected in any other way. Suitable detectable molecules include fluorescent proteins, luciferase, enzyme substrates, and radiolabels. The binding moiety (detection antibody or fragment thereof) may be directly labelled with a detectable label or it may be indirectly labelled. For example, the binding moiety may be an unlabelled detection antibody which can be detected by another antibody which is itself labelled (e.g. a secondary antibody as referred to herein). Alternatively, the detection antibody may have bound to it biotin and binding of labelled streptavidin to the biotin is used to indirectly label the detection antibody. In embodiments, the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label. For example, the detectable label may be an electrochemiluminescent or enzymatically active label. An example of an enzymatically active label is a horseradish peroxidase (HRP) enzyme. An example of an electrochemiluminescent label is a Sulfo-tag. As used herein, the term "electrochemiluminescent label" refers to a moiety which generates light when stimulated by electricity in the appropriate chemical environment. In embodiments, the detection antibody is a non-human antibody which binds UNC5C, for example a polyclonal antibody, for example a goat polyclonal antibody such as Biotechne #AF1005 used herein.

Secondary antibodies according to the present disclosure may be antibodies which bind a detection antibody, for example antibodies which bind antibodies of the species from which the detection antibody is derived. For example, a secondary antibody may be a polyclonal antibody which binds VH and/or VL chains of IgG protein of the species from which the detection antibody is derived. In embodiments, the secondary

antibody is a non-human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived, for example the detection antibody may be a goat antibody, and the secondary antibody may be an anti-goat polyclonal antibody, for example a mouse anti-goat polyclonal antibody labelled with HRP (e.g. Sigma #A9452) or a donkey anti-goat polyclonal sulfo-tagged antibody (e.g. MSD #R32AG-1), as described herein. Secondary antibodies according to the present disclosure may be detectably labelled or, at least, capable of detection. For example, the secondary antibody may be labelled with a radioactive atom or a coloured molecule or a fluorescent molecule or a molecule which can be readily detected in any other way. Suitable detectable molecules include fluorescent proteins, luciferase, enzyme substrates, and radiolabels. The binding moiety (secondary antibody or fragment thereof) may be directly labelled with a detectable label or it may be indirectly labelled. For example, the secondary antibody may have bound to it biotin and binding of labelled streptavidin to the biotin is used to indirectly label the secondary antibody. In embodiments, the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label. For example, the detectable label may be an electrochemiluminescent or enzymatically active label, for example a HRP label or SULFO-TAG label (Meso Scale Discovery).

The novel anti-UNC5C antibodies of the disclosure may be used as capture antibodies or as detection antibodies. In embodiments, the novel antibodies of the disclosure are used as capture antibodies.

Some methods of the present disclosure involve a sample containing cells. The sample may be a culture of cells grown *in vitro*. For example, the culture may comprise a suspension of cells or cells cultured in a culture plate or dish. Methods according to the present disclosure may be performed, or products may be present, *in vitro*, *ex vivo*, or *in vivo*. The term "*in vitro*" is intended to encompass experiments with materials, biological substances, cells and/or tissues in laboratory conditions or in culture whereas the term "*in vivo*" is intended to encompass experiments and procedures with intact multi-cellular organisms. "*Ex vivo*" refers to something present or taking place outside an organism, e.g. outside the human or animal body, which may be on tissue (e.g. whole organs) or cells taken from the organism.

According to some aspects of the present disclosure a kit of parts is provided comprising an antibody according to the present invention. In some embodiments, the kit comprises an antibody according to the present invention and one or more of: reagents for use in immunochemistry e.g. a detection antibody and/or a secondary antibody; the antibodies immobilised to a solid support; means for labelling the antibodies.

The features disclosed in the foregoing description, or in the following claims, or in the accompanying drawings, expressed in their specific forms or in terms of a means for performing the disclosed function, or a method or process for obtaining the disclosed results, as appropriate, may, separately, or in any combination of such features, be utilised for realising the invention in diverse forms thereof. While the invention has been described in conjunction with the exemplary embodiments described above, many equivalent modifications and variations will be apparent to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the invention set forth above are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the spirit and scope of the invention. For the avoidance of any doubt, any theoretical explanations provided herein are provided for the purposes of improving the understanding of a reader. The inventors do not wish

to be bound by any of these theoretical explanations. Any section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. Throughout this specification, including the claims which follow, unless the context requires otherwise, the word "comprise" and "include", and variations such as "comprises", "comprising", and "including" will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps. It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Ranges may be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by the use of the antecedent "about," it will be understood that the particular value forms another embodiment. The term "about" in relation to a numerical value is optional and means for example +/- 10%.

All references cited herein are incorporated herein by reference in their entirety. For standard molecular biology techniques, see Sambrook, J., Russel, D.W. *Molecular Cloning, A Laboratory Manual*. 3 ed. 2001, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Embodiments of the disclosure are described in the following numbered clauses.

1. Use of an antibody which specifically binds to UNC5C protein or a fragment thereof for detecting the level of soluble UNC5C in a biological fluid sample obtained from a subject.

2. The use according to embodiment 1, wherein the antibody comprises a heavy chain variable domain (VH) with the following CDRs: CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147; CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166; optionally wherein the antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166); optionally wherein the antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161).

3. The use according to embodiment 1 or embodiment 2, wherein the antibody has a heavy chain variable domain (VH) with the following framework sequences: HFWR1 of SEQ ID NO: 205, 209, 212, or 220; HFWR2 of SEQ ID NO: 206, 210, or 221; HFWR3 of SEQ ID NO: 207, 211, or 222; and HFWR4 of SEQ ID NO: 208 or 223; optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6537 (SEQ ID NO: 220, 221, 222, 223); optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO: 205, 206, 207, 208).

4. The use according to any preceding embodiment, wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.

5. The use according to any preceding embodiment, wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH); optionally
- 5 wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH); optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ
- 10 ID NO: 124 (ATL_6532 VH).
6. The use according to any preceding embodiment, wherein the antibody comprises a light chain variable domain (VL) with the following CDRs: CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174; CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191; optionally wherein the antibody comprises a VL
- 15 with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191); optionally wherein the antibody comprises a VL with the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186).
7. The use according to any preceding embodiment, wherein the antibody has a light chain variable
- 20 domain (VL) with the following framework sequences: LFWR1 of SEQ ID NO: 230, 232, 233, or 235; LFWR2 of SEQ ID NO: 240, 241, 238, or 244; LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and LFWR4 of SEQ ID NO: 256 or 258; optionally wherein the antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256); optionally
- 25 wherein the antibody comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232, 240, 249, 258).
8. The use according to any preceding embodiment, wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134
- 30 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537); optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); optionally wherein the antibody has a light chain variable domain (VL) comprising a
- 35 sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).
9. The use according to any preceding embodiment, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein.

10. The use according to embodiment 9, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein.
11. The use according to any preceding embodiment, wherein the antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).
12. The use according to any preceding embodiment, wherein the antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO: 134 (ATL_6532).
13. The use according to any preceding embodiment, wherein the antibody binds human, mouse and/or cynomolgus monkey UNC5C protein.
14. The use according to any preceding embodiment, wherein the antibody selectively binds to UNC5C over other one or more netrin receptors, optionally wherein the antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.
15. The use according to any preceding embodiment, wherein the subject is a mammal, optionally wherein the subject is a mouse, monkey, or human, optionally wherein the subject is a human.
16. The use according to any preceding embodiment, wherein the biological fluid sample is blood or cerebrospinal fluid (CSF), optionally wherein a blood sample is selected from a whole blood, plasma or serum sample.
17. The use according to embodiment 16, wherein the subject is a patient who has been treated with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF, optionally wherein the therapeutic compound is an anti-UNC5C antibody and/or wherein the therapeutic compound or composition increases the half-life of soluble UNC5C.
18. The use according to any preceding embodiment, wherein the subject is a patient who has been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease, optionally wherein the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), REM sleep Behavioural Disorder (RBD) or Parkinson's disease (PD).
19. The use according to any preceding embodiment, wherein the use comprises contacting the biological fluid sample with the antibody.
20. The use according to embodiment 19, wherein the antibody is used as a capture antibody in an immunoassay, optionally wherein the immunoassay is a sandwich ELISA or a meso scale discovery (MSD) assay.
21. The use according to embodiment 20, further comprising: (a) detecting UNC5C bound by the capture antibody using a detection probe, wherein the detection probe binds UNC5C, optionally a detection antibody, UNC5C binding protein or ligand, wherein: (i) the detection probe carries a detectable label; or

(ii) the detection probe is detected using a secondary antibody which binds the detection probe, wherein the secondary antibody carries a detectable label; and (b) detecting the detectable label to determine the level of soluble UNC5C in the biological fluid sample.

22. The use according to embodiment 21, wherein the detection antibody is a non-human antibody which
5 binds UNC5C; optionally wherein the detection antibody is a polyclonal antibody; optionally wherein the detection antibody is a goat polyclonal antibody.

23. The use according to embodiment 21 or embodiment 22, wherein the secondary antibody is a non-human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived; optionally wherein the detection antibody is a goat antibody, and the secondary antibody is an
10 anti-goat polyclonal antibody; optionally wherein the secondary antibody is a mouse anti-goat polyclonal antibody or a donkey anti-goat polyclonal antibody.

24. The use according to any one of embodiments 21 to 23, wherein the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label; optionally wherein the detectable label is an electrochemiluminescent or
15 enzymatically active label; optionally wherein the detectable label is a horseradish peroxidase (HRP) enzyme or a Sulfo-tag.

25. A method for detecting the level of soluble UNC5C in a biological fluid sample obtained from a subject, wherein the method comprises contacting the biological fluid sample with an antibody or antibody fragment thereof which specifically binds to UNC5C protein or a fragment thereof.

26. The method according to embodiment 25, wherein the antibody comprises a heavy chain variable domain (VH) with the following CDRs: CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147; CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166; optionally wherein the antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID
25 NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166); optionally wherein the antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161).

27. The method according to embodiment 25 or embodiment 26, wherein the antibody has a heavy chain variable domain (VH) with the following framework sequences: HFWR1 of SEQ ID NO: 205, 209, 212, or 220; HFWR2 of SEQ ID NO: 206, 210, or 221; HFWR3 of SEQ ID NO: 207, 211, or 222; and HFWR4 of SEQ ID NO: 208 or 223; optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6537 (SEQ ID
30 NO: 220, 221, 222, 223); optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO: 205, 206, 207, 208).

28. The method according to any one of embodiments 25 to 27, wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.

29. The method according to any one of embodiments 25 to 28, wherein the antibody has a heavy chain variable domain (VH) comprising a sequence selected that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH);
5 optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH); optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity
10 with SEQ ID NO: 124 (ATL_6532 VH).

30. The method according to any one of embodiments 25 to 29, wherein the antibody comprises a light chain variable domain (VL) with the following CDRs: CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174; CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191; optionally wherein the antibody
15 comprises a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191); optionally wherein the antibody comprises a VL with the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186).

31. The method according to any one of embodiments 25 to 30, wherein the antibody has a light chain variable domain (VL) with the following framework sequences: LFWR1 of SEQ ID NO: 230, 232, 233, or 235; LFWR2 of SEQ ID NO: 240, 241, 238, or 244; LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and LFWR4 of SEQ ID NO: 256 or 258; optionally wherein the antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256); optionally
25 wherein the antibody comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232, 240, 249, 258).

32. The method according to any one of embodiments 25 to 31, wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537); optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); optionally wherein the antibody has a light chain variable domain (VL) comprising a
30 sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).

33. The method according to any one of embodiments 25 to 32, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein.

34. The method according to embodiment 33, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein.
35. The method according to any one of embodiments 25 to 34, wherein the antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).
36. The method according to any one of embodiments 25 to 35, wherein the antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO: 134 (ATL_6532).
37. The method according to any one of embodiments 25 to 36, wherein the antibody binds human, mouse and/or cynomolgus monkey UNC5C protein.
38. The method according to any one of embodiments 25 to 37, wherein the antibody selectively binds to UNC5C over other one or more netrin receptors, optionally wherein the antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.
39. The method according to any one of embodiments 25 to 38, wherein the subject is a mammal, optionally wherein the subject is a mouse, monkey, or human, optionally wherein the subject is a human.
40. The method according to any one of embodiments 25 to 39, wherein the biological fluid sample is blood or cerebrospinal fluid (CSF), optionally wherein a blood sample is selected from a whole blood, plasma or serum sample.
41. The method according to embodiment 40, wherein the subject is a patient who has been treated with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF, optionally wherein the therapeutic compound is an anti-UNC5C antibody and/or wherein the therapeutic compound or composition increases the half-life of soluble UNC5C.
42. The method according to any one of embodiments 25 to 41, wherein the subject is a patient who has been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease, optionally wherein the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), REM sleep Behavioural Disorder (RBD) or Parkinson's disease (PD).
43. The method according to any one of embodiments 25 to 42, wherein the antibody is used as a capture antibody in an immunoassay, optionally wherein the immunoassay is a sandwich ELISA or a meso scale discovery (MSD) assay.
44. The method according to embodiment 43, further comprising: (a) contacting UNC5C bound by the capture antibody with a detection probe, wherein the detection probe binds UNC5C, wherein: (i) the detection probe carries a detectable label; or (ii) the detection probe is detected using a secondary antibody which binds the detection probe, wherein the secondary antibody carries a detectable label; and (b) detecting the detectable label to determine the level of soluble UNC5C in the biological fluid sample;

optionally wherein the detection probe comprises an anti-UNC5C antibody, UNC5C binding protein or UNC5C ligand.

45. The method according to embodiment 44, wherein the detection antibody is a non-human antibody which binds UNC5C; optionally wherein the detection antibody is a polyclonal antibody; optionally wherein
5 the detection antibody is a goat polyclonal antibody.

46. The method according to embodiment 44 or embodiment 45, wherein the secondary antibody is a non-human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived; optionally wherein the detection antibody is a goat antibody, and the secondary antibody is an anti-goat polyclonal antibody; optionally wherein the secondary antibody is a mouse anti-
10 goat polyclonal antibody or a donkey anti-goat polyclonal antibody.

47. The method according to any one of embodiments 44 to 46, wherein the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label; optionally wherein the detectable label is an electrochemiluminescent or enzymatically active label; optionally wherein the detectable label is a horseradish peroxidase (HRP)
15 enzyme or a Sulfo-tag.

48. The method according to embodiment 47, wherein the detectable label is an electrochemiluminescent label, optionally a Sulfo-tag, wherein step (b) comprises: (i) applying an electrical current to the electrochemiluminescent label; and (ii) measuring the luminescence.

49. The method according to embodiment 47, wherein the detectable label is a horseradish peroxidase (HRP) enzyme, wherein step (b) comprises: (i) contacting the horseradish peroxidase (HRP) enzyme with TMB (3, 3', 5, 5'-tetramethylbenzidine); and (ii) measuring the ultraviolet (UV) absorption at 450nm and/or 570nm.
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50. A method of determining the effect of a therapeutic compound or composition that affects the level of soluble UNC5C in a subject who has been administered the therapeutic compound or composition,
25 wherein the method comprises contacting one or more biological fluid samples previously obtained from the subject with an antibody which specifically binds to UNC5C protein or a fragment thereof.

51. The method according to embodiment 50, wherein the antibody comprises a heavy chain variable domain (VH) with the following CDRs: CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147; CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166; optionally wherein the antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166); optionally wherein the antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161).
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52. The method according to embodiment 50 or embodiment 51, wherein the antibody has a heavy chain variable domain (VH) with the following framework sequences: HFWR1 of SEQ ID NO: 205, 209, 212, or 220; HFWR2 of SEQ ID NO: 206, 210, or 221; HFWR3 of SEQ ID NO: 207, 211, or 222; and HFWR4 of SEQ ID NO: 208 or 223; optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533
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(SEQ ID NO:209, 210, 211, 208); ATL_6534 (SEQ ID NO:212, 206, 207,208); ATL_6537 (SEQ ID NO:220, 221, 222, 223); optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO:205, 206, 207, 208).

53. The method according to any one of embodiments 50 to 52, wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.

54. The method according to any one of embodiments 50 to 53, wherein the antibody has a heavy chain variable domain (VH) comprising a sequence selected that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH); optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH); optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 124 (ATL_6532 VH).

55. The method according to any one of embodiments 50 to 54, wherein the antibody comprises a light chain variable domain (VL) with the following CDRs: CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174; CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191; optionally wherein the antibody comprises a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191); optionally wherein the antibody comprises a VL with the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186).

56. The method according to any one of embodiments 50 to 55, wherein the antibody has a light chain variable domain (VL) with the following framework sequences: LFWR1 of SEQ ID NO: 230, 232, 233, or 235; LFWR2 of SEQ ID NO: 240, 241, 238, or 244; LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and LFWR4 of SEQ ID NO: 256 or 258; optionally wherein the antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256); optionally wherein the antibody comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232, 240, 249, 258).

57. The method according to any one of embodiments 50 to 56, wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537); optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135

(ATL_0006533); optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).

58. The method according to any one of embodiments 50 to 57, wherein the antibody or antibody
5 fragment thereof specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein.

59. The method according to embodiment 58, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein.

60. The method according to any one of embodiments 50 to 59, wherein the antibody does not compete
10 or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).

61. The method according to any one of embodiments 50 to 60, wherein the antibody competes or cross
15 competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO: 134 (ATL_6532).

62. The method according to any one of embodiments 50 to 61, wherein the antibody binds human, mouse and/or cynomolgus monkey UNC5C protein.

63. The method according to any one of embodiments 50 to 62, wherein the antibody selectively binds to
20 UNC5C over other one or more netrin receptors, optionally wherein the antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.

64. The method according to any one of embodiments 50 to 63, wherein the subject is a mammal, optionally wherein the subject is a mouse, monkey, or human, optionally wherein the subject is a human.

65. The method according to any one of embodiments 50 to 64, wherein the biological fluid sample is
25 blood or cerebrospinal fluid (CSF), optionally wherein a blood sample is selected from a whole blood, plasma or serum sample.

66. The method according to any one of embodiments 50 to 65, wherein the therapeutic compound is an anti-UNC5C antibody and/or wherein the therapeutic compound increases the half-life of soluble UNC5C.

67. The method according to embodiment 66, wherein the therapeutic anti-UNC5C antibody binds the
30 first immunoglobulin (Ig1) domain of UNC5C protein.

68. The method according to embodiment 66 or 67, wherein the therapeutic anti-UNC5C antibody comprises a VH with the CDRs of antibody ATLX1282 (SEQ ID NO: 20, 22, 23) and a VL with the CDRs of antibody ATLX1282 (SEQ ID NO: 24, 34, 40).

69. The method according to any one of embodiments 66 to 68, wherein the therapeutic anti-UNC5C
35 antibody comprises the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).

70. The method according to any one of embodiments 66 to 69, wherein the therapeutic anti-UNC5C antibody is ATLX1282.

71. The method according to any one of embodiments 50 to 70, wherein the subject is a patient who has been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is:

- 5 (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease, optionally wherein the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), REM sleep Behavioural Disorder (RBD) or Parkinson's disease (PD).

- 10 72. The method according to any one of embodiments 50 to 71, wherein the antibody or antibody fragment thereof is used as a capture antibody in an immunoassay, optionally wherein the immunoassay is a sandwich ELISA or a meso scale discovery (MSD) assay.

- 15 73. The method according to embodiment 72, further comprising: (a) contacting UNC5C bound by the capture antibody with a detection probe, wherein the detection probe binds UNC5C, wherein: (i) the detection probe carries a detectable label; or (ii) the detection probe is detected using a secondary antibody which binds the detection probe, wherein the secondary antibody carries a detectable label; and (b) detecting the detectable label to determine the level of soluble UNC5C in the biological fluid sample; optionally wherein the detection probe comprises an anti-UNC5C antibody, UNC5C binding protein or UNC5C ligand.

- 20 74. The method according to embodiment 73, wherein the detection antibody is a non-human antibody which binds UNC5C; optionally wherein the detection antibody is a polyclonal antibody; optionally wherein the detection antibody is a goat polyclonal antibody.

- 25 75. The method according to embodiment 73 or embodiment 74, wherein the secondary antibody is a non-human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived; optionally wherein the detection antibody is a goat antibody, and the secondary antibody is an anti-goat polyclonal antibody; optionally wherein the secondary antibody is a mouse anti-goat polyclonal antibody or a donkey anti-goat polyclonal antibody.

- 30 76. The method according to any one of embodiments 73 to 75, wherein the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label; optionally wherein the detectable label is an electrochemiluminescent or enzymatically active label; optionally wherein the detectable label is a horseradish peroxidase (HRP) enzyme or a Sulfo-tag.

77. The method according to embodiment 76, wherein the detectable label is an electrochemiluminescent label, optionally a Sulfo-tag, wherein step (b) comprises: (i) applying an electrical current to the electrochemiluminescent label; and (ii) measuring the luminescence.

- 35 78. The method according to embodiment 76, wherein the detectable label is a horseradish peroxidase (HRP) enzyme, wherein step (b) comprises: (i) contacting the horseradish peroxidase (HRP) enzyme with TMB (3, 3', 5, 5'-tetramethylbenzidine); and (ii) measuring the ultraviolet (UV) absorption at 450nm and/or 570nm.

79. A kit comprising: (i) a capture antibody, wherein the capture antibody specifically binds to UNC5C protein or a fragment thereof; and (ii) a detection probe, wherein the detection probe binds UNC5C, optionally wherein the detection antibody carries a detectable label, wherein the detection probe comprises an anti-UNC5C antibody, UNC5C binding protein or UNC5C ligand, and/or wherein the
5 capture antibody is different from the detection antibody.
80. The kit according to embodiment 79, further comprising: (iii) a secondary antibody which binds the detection antibody, wherein the secondary antibody carries a detectable label.
81. The kit according to embodiment 79 or embodiment 80, wherein the capture antibody comprises a heavy chain variable domain (VH) with the following CDRs: CDRH1 comprising amino acid sequence
10 SEQ ID NO: 144, 145, or 147; CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166; optionally wherein the capture antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166); optionally wherein the capture antibody comprises a VH with the CDRs of
15 antibody ATL_6532 (SEQ ID NO: 144, 152, 161).
82. The kit according to any one of embodiments 79 to 81, wherein the capture antibody has a heavy chain variable domain (VH) with the following framework sequences: HFWR1 of SEQ ID NO: 205, 209, 212, or 220; HFWR2 of SEQ ID NO: 206, 210, or 221; HFWR3 of SEQ ID NO: 207, 211, or 222; and HFWR4 of SEQ ID NO: 208 or 223; optionally wherein the capture antibody has a heavy chain variable
20 domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6537 (SEQ ID NO: 220, 221, 222, 223); optionally wherein the capture antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO: 205, 206, 207, 208).
- 25 83. The kit according to any one of embodiments 79 to 82, wherein the capture antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the capture antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.
84. The kit according to any one of embodiments 79 to 83, wherein the capture antibody has a heavy
30 chain variable domain (VH) comprising a sequence selected that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH); optionally wherein the capture antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%
35 sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH); optionally wherein the capture antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 124 (ATL_6532 VH).
85. The kit according to any one of embodiments 79 to 84, wherein the capture antibody comprises a light
40 chain variable domain (VL) with the following CDRs: CDRL1 comprising amino acid sequence SEQ ID

- NO: 169, 171, or 174; CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191; optionally wherein the capture antibody comprises a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191); optionally wherein the capture antibody comprises a VL with the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186).
86. The kit according to any one of embodiments 79 to 85, wherein the capture antibody has a light chain variable domain (VL) with the following framework sequences: LFWR1 of SEQ ID NO: 230, 232, 233, or 235; LFWR2 of SEQ ID NO: 240, 241, 238, or 244; LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and LFWR4 of SEQ ID NO: 256 or 258; optionally wherein the capture antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256); optionally wherein the capture antibody comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232, 240, 249, 258).
87. The kit according to any one of embodiments 79 to 86, wherein the capture antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537); optionally wherein the capture antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); optionally wherein the capture antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).
88. The kit according to any one of embodiments 79 to 87, wherein the capture antibody specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein.
89. The kit according to embodiment 88, wherein the capture antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein.
90. The kit according to any one of embodiments 79 to 89, wherein the capture antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).
91. The kit according to any one of embodiments 79 to 90, wherein the capture antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO: 134 (ATL_6532).
92. The kit according to any one of embodiments 79 to 91, wherein the capture antibody binds human, mouse and/or cynomolgus monkey UNC5C protein.

93. The kit according to any one of embodiments 79 to 92, wherein the capture antibody selectively binds to UNC5C over other one or more netrin receptors, optionally wherein the capture antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.

94. The kit according to any one of embodiments 79 to 93, wherein the detection antibody is a non-
5 human antibody which binds UNC5C;

optionally wherein the detection antibody is a polyclonal antibody;

optionally wherein the detection antibody is a goat polyclonal antibody.

95. The kit according to any one of embodiments 80 to 94, wherein the secondary antibody is a non-
human polyclonal antibody which binds to antibodies of the species from which the detection antibody is
10 derived; optionally wherein the detection antibody is a goat antibody, and the secondary antibody is an
anti-goat polyclonal antibody; optionally wherein the secondary antibody is a mouse anti-goat polyclonal
antibody or a donkey anti-goat polyclonal antibody.

96. The kit according to any one of embodiments 79 to 95, wherein the detectable label is selected from
the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or
15 enzymatically active label; optionally wherein the detectable label is an electrochemiluminescent or
enzymatically active label; optionally wherein the detectable label is a horseradish peroxidase (HRP)
enzyme or a Sulfo-tag.

97. The kit according to embodiment 96, wherein the detectable label is a horseradish peroxidase (HRP)
enzyme and the kit further comprises TMB (3, 3', 5, 5'-tetramethylbenzidine).

20 98. The kit according to any one of embodiments 79 to 95, wherein the capture antibody is immobilised to
a solid support.

99. The method according to any of embodiments 50 to 78, the method comprising: (a) determining a first
level of soluble UNC5C by contacting a first biological fluid sample previously obtained from the subject
with an antibody or antibody fragment thereof which specifically binds to UNC5C protein or a fragment
25 thereof, wherein the first biological fluid sample was obtained prior to administration of a therapeutic
compound or composition to the subject; (b) determining a second level of soluble UNC5C in a second
biological fluid sample previously obtained from the subject by contacting said second biological fluid
sample with an antibody or antibody fragment thereof which specifically binds to UNC5C protein or a
fragment thereof, wherein the second biological fluid sample was obtained after administration of the
30 therapeutic compound or composition to the subject; and (c) comparing the first level of soluble UNC5C
and the second level of soluble UNC5C to determine the effect of the therapeutic compound or
composition on the level of soluble UNC5C.

100. The method according to embodiment 99, wherein: step (a) further comprises determining a first
level of total protein in the first biological fluid sample; step (b) further comprises determining a second
35 level of total protein in the second biological fluid sample; and the comparison of step (c) comprises
comparing (i) the first level of soluble UNC5C normalised to the first level of total protein and (ii) the
second level of soluble UNC5C normalised to the second level of total protein.

101. The method according to embodiment 100, wherein the first and second levels of total protein are determined using a copper-based protein assay, a fluorescence-based protein assay, or a dye-based protein assay, optionally wherein the first and second levels of total protein are determined using a bicinchoninic acid (BCA) assay.

5 102. A method for selecting a subject for treatment with a therapeutic compound or composition that affects the level of soluble UNC5C, or for participating in a clinical trial of a therapeutic compound or composition that affects the level of soluble UNC5C, the method comprising: (a) detecting the level of soluble UNC5C in a biological fluid sample previously obtained from the subject by contacting the biological fluid sample with an antibody or antibody fragment thereof which specifically binds to UNC5C
10 protein or a fragment thereof; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C in a reference biological fluid sample from a healthy control; and (c) selecting the subject for treatment or for participating in the clinical trial when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C.

103. The method according to embodiment 102, wherein: step (a) further comprises determining the level
15 of total protein in the biological fluid sample; the comparison of step (b) comprises comparing (i) the level of soluble UNC5C normalised to the level of total protein and (ii) the reference level of soluble UNC5C normalised to the level of total protein in the reference biological fluid sample; and the selection of step (c) comprises selecting the subject for treatment or for participating in the clinical trial when the level of soluble UNC5C normalised to the level of total protein in the biological fluid sample is higher than the
20 reference level of soluble UNC5C normalised to the level of total protein in the reference biological fluid sample.

104. The method according to embodiment 103, wherein the level of total protein is determined using a copper-based protein assay, a fluorescence-based protein assay, or a dye-based protein assay, optionally wherein the level of total protein is determined using a bicinchoninic acid (BCA) assay.

25 105. The method according to any one of embodiments 102 to 104, wherein the antibody comprises a heavy chain variable domain (VH) with the following CDRs: CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147; CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166; optionally wherein the antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161);
30 ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166); optionally wherein the antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161).

106. The method according to any one of embodiments 102 to 105, wherein the antibody has a heavy chain variable domain (VH) with the following framework sequences: HFWR1 of SEQ ID NO: 205, 209,
35 212, or 220; HFWR2 of SEQ ID NO: 206, 210, or 221; HFWR3 of SEQ ID NO: 207, 211, or 222; and HFWR4 of SEQ ID NO: 208 or 223; optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO: 205, 206, 207, 208); ATL_6533 (SEQ ID NO: 209, 210, 211, 208); ATL_6534 (SEQ ID NO: 212, 206, 207, 208); ATL_6537 (SEQ ID NO: 220, 221, 222, 223); optionally wherein the antibody has a heavy chain variable domain (VH)
40 with the framework sequences of antibody ATL_6532 (SEQ ID NO: 205, 206, 207, 208).

107. The method according to any one of embodiments 102 to 106, wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.
- 5 108. The method according to any one of embodiments 102 to 107, wherein the antibody has a heavy chain variable domain (VH) comprising a sequence selected that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH); optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that
- 10 has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH); optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 124 (ATL_6532 VH).
- 15 109. The method according to any one of embodiments 102 to 108, wherein the antibody comprises a light chain variable domain (VL) with the following CDRs: CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174; CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191; optionally wherein the antibody comprises a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533
- 20 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191); optionally wherein the antibody comprises a VL with the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186).
110. The method according to any one of embodiments 102 to 109, wherein the antibody has a light chain variable domain (VL) with the following framework sequences: LFWR1 of SEQ ID NO: 230, 232,
- 25 233, or 235; LFWR2 of SEQ ID NO: 240, 241, 238, or 244; LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and LFWR4 of SEQ ID NO: 256 or 258; optionally wherein the antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256); optionally wherein the antibody comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232,
- 30 240, 249, 258).
111. The method according to any one of embodiments 102 to 110, wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136 (ATL_0006534); SEQ ID NO:
- 35 139 (ATL_0006537); optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95%
- 40 sequence identity with SEQ ID NO: 134 (ATL_0006532).

112. The method according to any one of embodiments 102 to 111, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein.
113. The method according to embodiment 112, wherein the antibody specifically binds the second
5 immunoglobulin (Ig2) domain of UNC5C protein.
114. The method according to any one of embodiments 102 to 113, wherein the antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).
- 10 115. The method according to any one of embodiments 102 to 114, wherein the antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO: 134 (ATL_6532).
116. The method according to any one of embodiments 102 to 115, wherein the antibody binds human,
15 mouse and/or cynomolgus monkey UNC5C protein.
117. The method according to any one of embodiments 102 to 116, wherein the antibody selectively binds to UNC5C over other one or more netrin receptors, optionally wherein the antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.
118. The method according to any one of embodiments 102 to 117, wherein the subject is a mammal,
20 optionally wherein the subject is a mouse, monkey, or human, optionally wherein the subject is a human.
119. The method according to any one of embodiments 102 to 118, wherein the biological fluid sample is blood or cerebrospinal fluid (CSF), optionally wherein a blood sample is selected from a whole blood, plasma or serum sample.
120. The method according to any one of embodiments 102 to 119, wherein the therapeutic compound or
25 composition that affects the concentration of soluble UNC5C is an anti-UNC5C antibody and/or wherein the therapeutic compound or composition increases the half-life of soluble UNC5C, optionally wherein the therapeutic anti-UNC5C antibody binds the first immunoglobulin (Ig1) domain of UNC5C protein.
121. The method according to embodiment 120, wherein the therapeutic anti-UNC5C antibody comprises a VH with the CDRs of antibody ATLX1282 (SEQ ID NO: 20, 22, 23) and a VL with the CDRs of antibody
30 ATLX1282 (SEQ ID NO: 24, 34, 40).
122. The method according to embodiment 121, wherein the therapeutic anti-UNC5C antibody comprises the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282), optionally wherein the therapeutic anti-UNC5C antibody is ATLX1282.
- 35 123. The method according to any one of embodiments 102 to 122, wherein the subject is a patient who has been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease, optionally wherein the neurodegenerative disease is selected from

amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), REM sleep Behavioural Disorder (RBD) or Parkinson's disease (PD).

124. The method according to any one of embodiments 102 to 123, wherein the antibody is used as a capture antibody in an immunoassay, optionally wherein the immunoassay is a sandwich ELISA or a meso scale discovery (MSD) assay.

125. The method according to embodiment 124, further comprising: (a) contacting UNC5C bound by the capture antibody with a detection probe, wherein the detection probe binds UNC5C, wherein: (i) the detection probe carries a detectable label; or (ii) the detection probe is detected using a secondary antibody which binds the detection probe, wherein the secondary antibody carries a detectable label; and (b) detecting the detectable label to determine the level of soluble UNC5C in the biological fluid sample; optionally wherein the detection probe comprises an anti-UNC5C antibody, UNC5C binding protein or UNC5C ligand.

126. The method according to embodiment 125, wherein the detection antibody is a non-human antibody which binds UNC5C; optionally wherein the detection antibody is a polyclonal antibody; optionally wherein the detection antibody is a goat polyclonal antibody.

127. The method according to embodiment 125 or embodiment 126, wherein the secondary antibody is a non-human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived; optionally wherein the detection antibody is a goat antibody, and the secondary antibody is an anti-goat polyclonal antibody; optionally wherein the secondary antibody is a mouse anti-goat polyclonal antibody or a donkey anti-goat polyclonal antibody.

128. The method according to any one of embodiments 125 to 127, wherein the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label; optionally wherein the detectable label is an electrochemiluminescent or enzymatically active label; optionally wherein the detectable label is a horseradish peroxidase (HRP) enzyme or a Sulfo-tag.

129. The method according to embodiment 128, wherein the detectable label is an electrochemiluminescent label, optionally a Sulfo-tag, wherein step (b) comprises: (i) applying an electrical current to the electrochemiluminescent label; and (ii) measuring the luminescence.

130. The method according to embodiment 128, wherein the detectable label is a horseradish peroxidase (HRP) enzyme, wherein step (b) comprises: (i) contacting the horseradish peroxidase (HRP) enzyme with TMB (3, 3', 5, 5'-tetramethylbenzidine); and (ii) measuring the ultraviolet (UV) absorption at 450nm and/or 570nm.

131. The method according to any one of embodiments 102 to 130, wherein the healthy control is a subject who has not been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease, optionally wherein the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), REM sleep Behavioural Disorder (RBD) or Parkinson's disease (PD).

132. A method of selecting a subject for treatment with a therapeutic compound or composition that affects the level of soluble UNC5C, the method comprising: (a) detecting the level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C; and (c) selecting the subject for treatment with the therapeutic compound or composition when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C.

133. The method of embodiment 132, wherein the reference level corresponds to the level of soluble UNC5C in a healthy control sample.

134. The method of embodiment 132 or embodiment 133, wherein the method has the features of any of embodiments 102 to 131.

135. A method of selecting a subject for participating in a clinical trial of a therapeutic compound or composition that affects the level of soluble UNC5C, the method comprising: (a) detecting the level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C; and (c) selecting the subject for participating in the clinical trial when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C.

136. The method of embodiment 135, wherein the reference level corresponds to the level of soluble UNC5C in a healthy control sample.

137. The method of embodiment 135 or embodiment 136, wherein the method has the features of any of embodiments 102 to 131.

138. A method of detecting the presence and/or severity of a neurodegenerative disease in a subject, the method comprising: (a) determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C, wherein a higher level of soluble UNC5C in the biological fluid sample relative to the reference level of soluble UNC5C is indicative of the presence of the neurodegenerative disease and/or indicative of a higher severity of the neurodegenerative disease than a severity associated with the reference level.

139. A method of determining whether a subject is likely to be resilient or sensitive to a neurodegenerative disease, the method comprising: (a) determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject; (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C, wherein a higher level of soluble UNC5C in the biological fluid sample relative to the reference level of soluble UNC5C is indicative of the subject being unlikely to be resilient (or likely to be sensitive) to the neurodegenerative disease.

140. A method according to embodiment 138 or embodiment 139, wherein the method has the features of any of embodiments 1 to 49.

Sequences

Example sequence of antibodies described herein are provided in Figure 16, 7B, 12 (aligned by IMGT position numbering) and in the table below.

SEQ ID NO	Description	Sequence
1	ATL_5262 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWTWIRQPPGKRL EWIGYIHNSGTTNQNP SLKSRVTISIDTSKNHFSLKLSSVTAADTA VYYCARGRGMATLPFDYWGQGLTVTVSS
2	ATL_6033 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKG LEWIGYIHNSGTTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGLTVTVSS
3	ATL_6034 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWTWIRQPPGKG LEWIGYIHNSGTTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGLTVTVSS
4	ATL_6035 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKR LEWIGYIHNSGTTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGLTVTVSS
5	ATL_6036 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKG LEWIGYIHNSGTTNQNP SLKSRVTISVDTSKNQFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGLTVTVSS
6	ATL_6037 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKG LEWIGYIHNSGTTNYPNPSLKSRVTISIDTSKNQFSLKLSSVTAADTA VYYCARGRGMATLPFDYWGQGLTVTVSS
7	ATL_6038 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKG LEWIGYIHNSGTTNYPNPSLKSRVTISVDTSKNHFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGLTVTVSS
8	ATL_6039 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWTWIRQPPGKRL EWIGYIHNSGTTNQNP SLKSRVTISIDTSKNHFSLKLSSVTAADTA VYYCARGRGMATLPFDYWGQGLTVTVSS
9	ATL_5262 VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQS YSTPLTFGGGGTKVEIK
10	ATL_5996 VL	DIQMTQSPSSLSASVGDRVTITCQASQDITNYLNWYQQKPGKAPK LLIYDASNLETGVPSRFSGSGSGTDFTFTISLQPEDATYYCQQY DNLPTFTFGGQGTKVEIR
11	ATL_5997 VL	EIVLTQSPGTLSLSPGERATLSCRASQSVSSTYLAWYQQKPGQA PRLIYAASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQ QYGSSPWTFGGQGTKVEIK
12	ATL_5998 VL	DIVMTQSPDSLAVSLGERATINCKSSQSVLYSSKNKNYLAWYQQK PGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQAEDVAV YYCQQYYSTPWTFGGQGTKVEIK
13	ATL_5999 VL	DIQMTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQKPGKAP KLLIYKASSLESGVPSRFSGSGSGTEFTLTISLQPDDEFATYYCQQ YSSYSPTFGGQGTKVEIK

14	ATL_6000 VL	QSALTQPASVSGSPGQSITISCTGTSSDVGGYNYVSWYQQHPGK APKLMYDVSNRPSGVSNRFGSGSKSGNTASLTISGLQTEDEAEYY CSSYTSSSTFVFGPGTKVTVL
15	ATL_6001 VL	DIQMTQSPSSLSASVGDRVITTCRASQSSISNYLNWYQQKPGKAPN LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQS YSTPPTFGPGTKVDIK
16	ATL_6002 VL	DIQMTQSPSSLSASVGDRVITTCRASQSSISRYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQS YTTPLTFGQGTKVEIK
17	ATL_6003 VL	DIQMTQSPSSLSASVGDRVITTCRASQTISNYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQS YSTPLTFGGGKVEIK
18	ATL_6004 VL	EIVLTQSPGTLSSLSPGERVTLSCRASQNIISNYLAWYQQKPGQAP RLLIYGASSRATGIPDRFSGSGSGTDFTLTISLQPEDFAVYCHQY GSIPLTFGGGKVEIK
19	ATL_6005 VL	QSALTQPPSASGSPGQSVTISCTGTSSDVGGYNYVSWFQQHPG KAPKLIIEVTKRPSGVPDRFSGSKSGNAASLTVSGLQAEDEADY YCSSYAGSNNLLFGGGTKLTVL
20	CDRH1 (all ATL_5262 variants)	GGSISSYF
21	CDRH2 (all ATL_5262 variants except ATL_6178)	IHNSGTT
22	CDRH2 ATL_6178	IHYSGTT
23	CDRH3 (all ATL_5262 variants)	ARGRGMATLPFDY
24	CDRL1 (ATL_5262; 6033; 6034; 6035; 6036; 6037; 6038; 6039; 6177; 6178)	QSISSY
25	CDRL1 (ATL_5996)	QDITNY
26	CDRL1 (ATL_5997)	QSVSSTY
27	CDRL1 (ATL_5998)	QSVLYSSKNKNY
28	CDRL1 (ATL_5999)	QSISSW
29	CDRL1 (ATL_6000; ATL_6005)	SSDVGGYNY
30	CDRL1 (ATL_6001)	QSSISNY
31	CDRL1 (ATL_6002)	QSSISRY
32	CDRL1 (ATL_6003)	QTISNY

33	CDRL1 (ATL_6004)	QNIISNY
34	CDRL2 (ATL_5262; 5997; 5998; 6001; 6002; 6003; 6033; 6034; 6035; 6036; 6037; 6038; 6039; 6177; 6178)	AAS
35	CDRL2 (ATL_5996)	DAS
36	CDRL2 (ATL_5999)	KAS
37	CDRL2 (ATL_6000)	DVS
38	CDRL2 (ATL_6004)	GAS
39	CDRL2 (ATL_6005)	EVT
40	CDRL3 (ATL_5262; 6003;6033; 6034; 6035; 6036; 6037; 6038;6039; 6177; 6178)	QQSYSTPLT
41	CDR3L (ATL_5996)	QQYDNLPLYT
42	CDR3L (ATL_5997)	QQYGSSPWT
43	CDR3L (ATL_5998)	QQYYSTPWT
44	CDR3L (ATL_5999)	QQYSSYSPT
45	CDR3L (ATL_6000)	SSYTSSSTFV
46	CDR3L (ATL_6001)	QQSYSTPPT
47	CDR3L (ATL_6002)	QQSYTTPLT
48	CDR3L (ATL_6004)	HQYGSIPLT
49	CDR3L (ATL_6005)	SSYAGSNNLL
50	HFWR1 (all ATL_5262 variants and homologues 6187 and 6191)	QVQLQESGPGLVKPSSETLSLTCTVS
51	HFWR2 (ATL_5262; 5996; 5997; 5998; 5999; 6000; 6001; 6002; 6003; 6004; 6005; 6039)	WTWIRQPPGKRLEWIGY
52	HFWR2 (ATL_6033; 6036; 6037; 6038; 6177; 6178; 6187)	WSWIRQPPGKGLEWIGY
53	HFWR2 (ATL_6034	WTWIRQPPGKGLEWIGY
54	HFWR2 (ATL_6035)	WSWIRQPPGKRLEWIGY
55	HFWR3 (ATL_5262; 5996; 5997; 5998; 5999; 6000; 6001; 6002; 6003; 6004; 6005;)	NQNPSLKSRVTISIDTSKNHFSKLSSVTAADTAVYYC

56	HFWR3 (ATL_6033; 6034; 6035)	NYNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC
57	HFWR3 (ATL_6036)	NQNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYC
58	HFWR3 (ATL_6037)	NYNPSLKSRVTISIDTSKNQFSLKLSSVTAADTAVYYC
59	HFWR3 (ATL_6038)	NYNPSLKSRVTISVDTSKNHFSKLSSVTAADTAVYYC
60	HFWR3 (ATL_6039)	NQNPSLKSRVTISIDKSKNHFSKLSSVTAADTAVYYC
61	HFWR3 (ATL_6177; 6178)	NQNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYC
62	HFWR4 (all ATL_5262 variants, and homologue ATL_0006191, phage display antibodies 6530, 6531, 6538)	WGQGTLVTVSS
63	LFWR1 (ATL_5262; 6001; 6002; 6003; 6033; 6034; 6035; 6036; 6037; 6038; 6039; 6177; 6178)	DIQMTQSPSSLSASVGDRVITICRAS
64	LFWR1 (ATL_5996)	DIQMTQSPSSLSASVGDRVITICQAS
65	LFWR1 (ATL_5997)	EIVLTQSPGTLSPGERATLSCRAS
66	LFWR1 (ATL_5998)	DIVMTQSPDSLAVSLGERATINCKSS
67	LFWR1 (ATL_5999)	DIQMTQSPSTLSASVGDRVITICRAS
68	LFWR1 (ATL_6000, 6536)	QSALTQPASVSGSPGQSITISCTGT
69	LFWR1 (ATL_6004)	EIVLTQSPGTLSPGERVTLSCRAS
70	LFWR1 (ATL_6005)	QSALTQPPSASGSPGQSVTISCTGT
71	LFWR2 (ATL_5262; 5996; 6002; 6003; 6033; 6034; 6035; 6036; 6037; 6038; 6039; 6177; 6178)	LNWYQQKPGKAPKLLIY
72	LFWR2 (ATL_5997, 6004)	LAWYQQKPGQAPRLLIY
73	LFWR2 (ATL_5998; 5999)	LAWYQQKPGKAPKLLIY
74	LFWR2 (ATL_6000)	VSWYQQHPGKAPKLMY
75	LFWR2 (ATL_6001)	LNWYQQKPGKAPNLLIY
76	LFWR2 (ATL_6005)	VSWFQQHPGKAPKLLIY
77	LFWR3 (ATL_5262; 6001; 6002; 6003; 6033; 6034; 6035; 6036; 6037; 6038; 6039; 6177; 6178)	SLQSGVPSRFSGSGSGTDFLTISSLQPEDFATYYC

78	LFWR3 (ATL_5996)	NLETGVPSRFSGSGSGTDFTFTISSLQPEDATYYC
79	LFWR3 (ATL_5997)	SRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC
80	LFWR3 (ATL_5998)	SLQSGVPSRFSGSGSGTDFTLTISSLQAEDVAVYYC
81	LFWR3 (ATL_5999)	SLESGVPSRFSGSGSGTEFTLTISSLQPDDEFATYYC
82	LFWR3 (ATL_6000)	NRPSGVSNRFSGSKSGNTASLTISGLQTEDEAEYYC
83	LFWR3 (ATL_6004)	SRATGIPDRFSGSGSGTDFTLTISSLQPEDFAVYYC
84	LFWR3 (ATL_6005)	KRPSGVPRFSGSKSGNAASLTVSGLQAEDAEADYYC
85	LFWR4 (ATL_5262; 6003; 6004; 6033; 6034; 6035; 6036; 6037; 6038; 6039; 6177; 6178)	FGGGTKVEIK
86	LFWR4 (ATL_5996)	FGQGTKVEIR
87	LFWR4 (ATL_5997; 5998; 5999; 6002)	FGQGTKVEIK
88	LFWR4 (ATL_6000)	FGPGTKVTVL
89	LFWR4 (ATL_6001)	FGPGTKVDIK
90	LFWR4 (ATL_6005)	FGGGTKLTVL
91	ATL_6177 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKG LEWIGYIHNSGTTNQNPSLKSRTISVDKSKNQFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGTLLTVSS
92	ATL_6177 VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAVYYCQQS YSTPLTFGGGTKVEIK
93	ATL_6178 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSISSYFWSWIRQPPGKG LEWIGYIHNSGTTNQNPSLKSRTISVDKSKNQFSLKLSSVTAADT AVYYCARGRGMATLPFDYWGQGTLLTVSS
94	ATL_6178 VL	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFAVYYCQQS YSTPLTFGGGTKVEIK
95	ATL_5338 VH	EVKLDDEGGGLVQGRPMKLSCLVASGFTFSDYWMNWNWRQSPEK GLEWVAQIRNKPNYETYYSDSVKGRFTISRDDSKSSVYLQMNN LRVEDMGIYYCTGSYYGMDYWGQGTSTVTVSS
96	ATL_5338 VL	DVVMTQTPLSLPVSLGDQASISCRSSQSLVNSNGNTYLRWYLQK PGQSPKVLIIYKVSNRVSGVPDRFSGSGSGTDFTLKISRVEAEDLG VYFCSQSTHPVPTFGGGTKLEIK
97	rmUNC5C (N-terminal His and FLAG tag + Amino acids 41-380 of the murine UNC5C sequence, extracellular	GSHHHHHHGSYKDDDDKGSQDDEFFHELPETFPSPPEPLPH FLIEPEEAYIVKNKPNLYCKASPATQIYFKCNSEWHQKDHVVDE RVDETSGLIVREVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTK SRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVE WLKNEDIIDPAEDRNFYITIDHNLIQKARLSDTANYTCVAKNIVAKR

	domain). In-house production from HEK293F cells	KSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRTCTNPA PLNGGAFCEGQSVQKIACTTLCVPDGRWTSWSKWSTCGTECTH WRRRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMQAAPDSDD VALYGS
98	rhUNC5C (N-terminal His and FLAG tag + Amino acids 41- 380 of the human UNC5C sequence, extracellular domain). In-house production from HEK293F cells.	GSHHHHHHGSDYKDDDDKGSQDDDDFFHELPETFPSPPEPLPH FLIEPEEAYIVKNKPNLYCKASPATQIYFKCNSEVWHQKDHIWDE RVDETSGLIVREVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTK SRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVE WLKNEDIIDPVEDRNFYITIDHNLIQKARLSDTANYTCVAKNIVAKR KSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRTCTNPA PLNGGAFCEGQSVQKIACTTLCVPDGRWTPWSKWSTCGTECTH WRRRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMQTAPDSDDV ALYGS
99	>sp O95185 UNC5C_H UMAN Netrin receptor UNC5C OS=Homo sapiens OX=9606 GN=UNC5C PE=1 SV=2	MRKGLRATAARCGGLGYLLQMLVLPALALLSASGTGSAAQDDD FFHELPETFPSPPEPLPHFLIEPEEAYIVKNKPNLYCKASPATQI YFKCNSEVWHQKDHIWDERVDETSGLIVREVSIEISRQQVEELFGP EDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSLE QEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHNLIQK RLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWTEWSVCN SRCGRGYQKRTRTCTNPAPLNGGAFCEGQSVQKIACTTLCVPDG RWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDCDGLVLQS KNCTDGLCMQTAPDSDDVALYGVIVAVIVCLAISVVVALFVYRKN HRDFESDIIDSSALNGGFQPVNIKAARQDLLAVPPDLTSAAAMYR GPVYALHDVSDKIPMTNSPILDPLPNLKIKVYNTSGAVTPQDDLSE FTSKLSPQMTQSLLENEALSLKNQSLARQTDPSCTAFGSFNSLGG HLIVPNSGVSLIPAGAIPQGRVYEMYVTVHRKETMRPPMDDSQT LLTPVVS CGPPGALLTRPVVLTMHHCADPNTEDWKILLKNQAAQG QWEDVVVGEENFTTPCYIQLDAEACHILTENLSTYALVGHSTTK AAAKRLKLAIFGPLCCSSLEYSIRVYCLDDTQDALKEILHLERQMG GQLLEPKALHFKGSTHNLRSLIHDIASLWKSLLAKYQEIPFYH VWSGSQRNHCTFTLERFSLNTVELVCKLCVRQVEGEGQIFQLN CTVSEPTGIDLPLLD PANTITVTGPSAFSIPPIRQKLCSSLDAP QTRGHDWRMLAHKLNLDRLNYFATKSSPTGVILDLWEAQNFPD GNLSMLAAVLEEMGRHETVVS LA AEGQY
100	>sp O08747 UNC5C_M OUSE Netrin receptor UNC5C OS=Mus musculus OX=10090 GN=Unc5c PE=1 SV=1	MRKGLRATAARCGGLGYLLQMLVLPALALLSASGTGSAAQDDE FFHELPETFPSPPEPLPHFLIEPEEAYIVKNKPNLYCKASPATQI YFKCNSEVWHQKDHVVDERVDETSGLIVREVSIEISRQQVEELFG PEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSL EQEVLLQCRPPEGIPVAEVEWLKNEDIIDPAEDRNFYITIDHNLIQK ARLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWTEWSVC NSRCGRGYQKRTRTCTNPAPLNGGAFCEGQSVQKIACTTLCVPD

		GRWTSWSKWSTCGTECTHWRRRECTAPAPKNGGKDCDGLVLQ SKNCTDGLCMQAAPDSDDVALYVGIVIAVTVCLAITVVVALFVYRK NHRDFESDIIDSSALNGGFQPVNIKAARQDLLAVPPDLTSAAMY RGPVYALHDVSDKIPMTNSPILDPLPNLKIKVYNSSGAVTPQDDLA EFSSKLSPQMTQSLENEALNLKNQSLARQTDPSCTAFGTFNSLG GHLIIPNSGVSLIPAGAIPQGRVYEMYVTVHRKENMRPPMEDSQ TLLTPWVSCGPPGALLTRPVILTLHHCADPSTEDWKIQLKNQAVQ GQWEDVVVVGEEENFTTPCYIQLDAEACHILTENLSTYALVGQSTT KAAAKRLKLAIFGPLCCSSLEYSIRVYCLDDTQDALKEVLQLERQM GGQLLEPKALHFKGSIHNLRLSIHDIAHSLWKSLLAKYQEIPFYH IWSGSQRNLHCTFTLERLSLNTVELVCKLCVRQVEGEGQIFQLNC TVSEPTGIDLPLLDPASTITVTGPSAFSIPLRQKLCSSLDAPQT RGHDWRMLAHKLNLDRLNYFATKSSPTGVILDLWEAQNFPDGN LSMLAAVLEEMGRHETVVSLAEEGQY
101	rmUNC5C (Amino acids 41-380 of the murine UNC5C sequence, corresponding to the extracellular domain). In-house production from HEK293F cells.	QDDEFFHELPETFPSPPEPLPHFLIEPEEAYIVKNKPNLYCKAS PATQIYFKCNSEVWHQKDHVDERVDETSGLIVREVSIEISRQQV EELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLG KEVSLEQEVLLQCRPPEGIPVAEVEWLKNEDIIDPAEDRNFYITIDH NLIQKARLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWTE WSVCNSRCGRGYQKRTRCTNPAPLNGGAFCEGQSVQKIACTT LCPVDGRWTSWSKWSTCGTECTHWRRRECTAPAPKNGGKDCD GLVLQSKNCTDGLCMQAAPDSDDVALY
102	rhUNC5C (Amino acids 41- 380 of the human UNC5C sequence, corresponding to the extracellular domain). In-house production from HEK293F cells.	QDDDDFFHELPETFPSPPEPLPHFLIEPEEAYIVKNKPNLYCKAS PATQIYFKCNSEVWHQKDHIVDERVDETSGLIVREVSIEISRQQVE ELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGK EVSLEQEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHN LIQKARLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWTEW SVCNSRCGRGYQKRTRCTNPAPLNGGAFCEGQSVQKIACTTLC PVDGRWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDCDGL VLQSKNCTDGLCMQTAPDSDDVALY
103	Recombinant rat UNC5C (N-terminal His and FLAG tag) In-house production from HEK293F cells.	GSHHHHHHGS DYKDDDDKGSQDDDDFFHELPETFPSPPEPLPH FLIEPEEAYIVKNKPNLYCKAS PATQIYFKCNSEVWHQKDHVDE RVDETSGLIVREVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTK SRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPMAEV EWLKNEDIIDPVEDRNFYITIDHNLIQKARLSDTANYTCVAKNIVAK RKSTTATVIVYVNGGWSTWAEWSVCNSRCGRGYQKRTRCTNP APLNGGAFCEGQSVQKIACTTLC PVDGRWTSWSKWSTCGTECT HWRRRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMQAAPDS DVALYGS
104	Recombinant cyno UNC5C (N-terminal His	GSHHHHHHGS DYKDDDDKGSQDDDDFFHELPETFPSPPEPLPH FLIEPEEAYIVKNKPNLYCKAS PATQIYFKCNSEVWHQKDHIVDE

	and FLAG tag) In-house production from HEK293F cells.	RVDETSGLIVREVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTK SRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVE WLKNEDIIDPVEDRNFYITIDHNLIQARLSDTANYTCVAKNIVAKR KSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRTCTNPA PLNGGAFCEGQSVQKIACTTLCVPDGRWTPWSKWSTCGTECTH WRRRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMQTAPDSDDV ALYGS
105	Human recombinant UNC5A	GSHHHHHHGSYKDDDDKGSQQSATVANPVPGANPDLLPHFLV EPEDVYIVKNKPVLLVCKAVPATQIFFKCNGEWVRQVDHVERST DGSSGLPTMEVRINVSRRQVEKVFGLLEYWCQCVAWSSSGTTK SQKAYIRIAYLRKNFEQEPLAKEVSLEQGIVLPCRPEGIPPAEVE WLRNEDLVDPSPDNVYITREHSLVVRQARLADTANYTCVAKNIV ARRRSASAAVIVYVDGWSWSPWSKWSACGLDCTHWRSSRECSDPA PRNGGEECQGTDLDRNCTSDLCVHTASGPEDVALYGS
106	Human recombinant UNC5B	GSHHHHHHGSYKDDDDKSGTDSGSEVLPSDFPSAPAEPLPY FLQEPQDAYIVKNKPVELRCRAFPATQIFYKCNGEWWSQNDHVT QEGLEATGLRVREVQIEVSRQQVEELFGLEDYWCQCVAWSSA GTTKSRRAYVRIAYLRKNFDQEPLGKEVPLDHEVLLQCRPPEGVP VAEVEWLKNEDVIDPTQDTNFLTIDHNLIIRQARLSDTANYTCVAK NIVAKRRSTTATVIVYVNGGWSSWAEWSPCSNRCGRGWQKRTR TCTNPAPLNGGAFCEGQAFQKTACTTICPVDGAWTEWSKWSAC STECAHWRSRECMAPPPQNGGRDCSGTLLDSKNCTDGLCMQN KKTLSDPNSHLLASGDAALYGS
107	Human recombinant UNC5C version 1	GSQDDDFHHELPETFPSPPEPLPHFLIEPEEAYIVKNKPNLYCK ASPATQIFYKCNSEVWHQKDHIVDERVDETSGLIVREVSIEISRQQ VEELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPL GKEVSLEQEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITID HNLIQARLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWT EWSVCNSRCGRGYQKRTRTCTNPAPLNGGAFCEGQSVQKIACT TLCVPDGRWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDC DGLVLQSKNCTDGLCMQTAPDSDDVALYGSKHHHHHHH
108	Human recombinant UNC5D	GSHHHHHHGSYKDDDDKGSARGTDNGEALPESIPSAPGTLPHF IEEPDDAYIIKSNPIALRCKARPAMQIFFKCNGEWVWHQNEHVSEET LDESSGLKVREVFINTROQVEDFHGPEDYWCQCVAWSHLGTS KSRKASVRIAYLRKNFEQDPQGREVPPIEGMIVLHCRPPEGVPAAE VEWLKNEPIDSEQDENIDTRADHNLIIRQARLSDSGNYTCMAANI VAKRRSLSATVVVYVNGGWSSWTEWSACNVRRCGRGWQKRST CTNPAPLNGGAFCEGMSVQKITCTSLCPVDGSWEVWSEWSVCS PECEHLRIRECTAPPPRNGGKFCEGLSQESENCTDGLCILDKKPL HEIKPQSIENASDGS

109	Human recombinant DCC	GSHHHHHHGS DYKDDDDKGSHLQVTGFQIKAF TALRFLSEPSDA VTMRGGNVLLDCSAESDRGVPVIKWKKGDIHLALGMDERKQQLS NGSLLIQNILHSRHHKPDEGLYQCEASLGDSGSIISRTAKVAVAGP LRFLSQTESVTAFMGDTVLLKCEVIGEPMP TIHWQKNQQDLTPIP GDSRVVVLPSGALQISRLQPGDIGIYRCSARNPASSRTGNEAEVRI LSDPGLHRQLYFLQRPSNVVAIEGKDAVLECCVSGYPPPSFTWLR GEEVIQLRSKKYSLLGGSNLLISNVTDDDSGMYTCVVTYKNENISA SAELTVLVPPWFLNHP SNLYAYESMDIEFECTVSGKPVPTVNWMM KNGDVVIPSDYFQIVGGSNLRILGVVKSDEGFYQCVAENEAGNAQ TSAQLIVPKPAIPSSSVLPSAPRDVVPVLVSSRFVRLSWRPPAEAK GNIQTFTVFFSREGDNRRERALNTTQPGSLQLTVGNLKPEAMYTR VVAYNEWGPGESSQPIKVATQPELQVPGPVENLQAVSTSPTSILIT WEPPAYANGPVQGYRLFCTEVSTGKEQNIEVDGLSYKLEGLKKF TEYSLRFLAYNRYGPGVSTDDITVVTLSDVPSAPPQNVSLVVNS RSIKVSWLPPPSGTQNGFITGYKIRHRKTTRRGEMETLEPNNLWY LFTGLEKGSQYSFQVSAMTVNGTGPPSNWYTAETPENDLDESQV PDQPSSLHVRPQTNCIIMSWTPPLNPNI VVRGYIIGYGVGSPYAET VRVDSKQRYYSIERLESSSHYVISLKAFNNAGEGVPLYESATTRSI TDPTDPVDYYPLDDFPTSVPDLSTPMLPPVGVAVALTHDAVRV SWADNSVPKNQKTSEVRLYTVRWRTSFSASAKYKSEDTTSLSYT ATGLKPNTMYEFSVMVTKNRRSSTWSMTAHATTYEAAPTSAPKD LTVITREGKPRAVIVSWQPPLEANGKITAYILFYTLDKNIPDDWIME TISGDRLTHQIMDLNLD TMYFRIQARN SKGVGPLSDPILFRTLKV EHPDKMANDQGRHGDGGYWPVDTNLIDRSTLNEPPIGQMHPPH GSVTPQKNSNGS
110	Human UNC5C T835M variant	MRKGLRATAARCGLGLGYLLQMLVLPALALLSASGTGSAAQDDD FFHELPETFPSPPEPLPHFLIEPEEAYIVKNKPNLYCKASPATQI YFKCNSEVWHQKDHIVDERVDETSGLIVREVSIEISRQQVEELFGP EDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSLE QEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHNLIKQA RLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWTEWSVCN SRCGRGYQKRTRCTNPAPLNGGAFCEGQSVQKIACTTLCPVDG RWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDCDGLVLQS KNCTDGLCMQTAPDSDDVALYVGIVIAVIVCLAISVVVALFVYRKN HRDFESDIIDSSALNGGFQPVNIKAARQDLLAVPPDLTSAAAMYR GPVYALHDVSDKIPMTNSPILDPLPNLKIKVYNTSGAVTPQDDLSE FTSKLSPQMTQSLENEALSLKNQSLARQTDPSCTAFGSFNSLGG HLIVPNSGVSLIPAGAIPQGRVYEMYVTVHRKETMRPPMDDSQT LLTPVVSCGPPGALLTRPVVLTMHHCADPN TEDWKILLKNQAAQG QWEDVVVVGEENFTTPCYIQLDAEACHILTENLSTYALVGHSTTK AAAKRLKLAIFGPLCCSSLEYSIRVYCLDDTQDALKEILHLERQMG GQLLEPKALHFKGSTHNLRLSIHDIAHSLWKSLLAKYQEIPFYH

		VWSGSQRNHLHCTFTLERFSLNTVELVCKLCVRQVEGEGQIFQLN CTVSEETPIDPLLDPAANTITMVTGPSAFSIPPIRQKLCSSLDAP QTRGHDWRMLAHKLNLDRLNYFATKSSPTGVILDLWEAQNFDP GNLSMLAAVLEEMGRHETVVSLLAAEGQY
111	Human UNC5C fragment ACB_429	GSDPPEPLPHFLIEPEEAYIVKNKPNLYCKASPATQIYFKCNSEW VHQKDHIWDERVDETSGLIVREVSIERSQQVEELFGPEDYWCQC VAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCR PPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHNLIIKQARLSDTANY TCVAKNIVAKRKSTTATVIVYVGSHHHHHH
112	ATL_0006187 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSTGSYYWSWIRQPPGK GLEWIGYTHYSGHTNQNPSSLKSRVTMSVDSSKSQLSLKLTSTVTA DTAVYYCARGRGMATLPFDSWGQGLVTVSS
113	ATL_0006191 VH	QVQLQESGPGLVKPSSETLSLTCTVSGGSIGSYFWSWIRQTPGKG LEWIGYIHYSGSSNYPNPSLKSRVTISVDTFKNQFSLKLTSTVTAADT AVYYCARGRGMATLPFDYWGQGLTVTVSS
114	ATL_0006187 VL;:ATL_0006191 VL (duplicate sequence, SEQ ID NO: 9, 92, 94)	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPK LLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQS YSTPLTFGGGTKVEIK
115	HCDR1_ATL_0006187	GGSTGSYY
116	HCDR1_ATL_0006191	GGSIGSYF
117	HCDR2_ATL_0006187	THYSGHT
118	HCDR2_ATL_0006191	IHYSGSS
119	HCDR3_ATL_0006187	ARGRGMATLPFDS
120	HCDR3_ATL_0006191	ARGRGMATLPFDY
24	LCDR1_ATL_0006187; LCDR1_ATL_0006191	QSISSY
34	LCDR2_ATL_0006187; LCDR2_ATL_0006191	AAS
121	LCDR3_ATL_0006187; LCDR3_ATL_0006191(duplicate sequence SEQ ID : 40)	QQSYSTPLT
122	ATL_0006530 VH	QVQLLETGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGK GLEWVSSISSSSSSIYYADSVKGRFTISRDNKNSLYLQMNSLRA EDTAVYYCARDPYSGYEFVSEPYFDYWGQGLTVTVSS
123	ATL_0006531 VH	EVQLVESGGGVVQPGGSLRLSCAASGFTFDDYAMHWVRQAPGK GLEWVSLISWDGGSTYYADSVKGRFTISRDNKNSLYLQMNSLR AEDTALYYCAKDSIQLWSFDYWGQGLTVTVSS

124	ATL_0006532 VH	QVTLKESGAEVKKPGASVKVSCKASGYTFTGYMHWWVRQAPGQ GLEWMGRINPNSSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLR SDDTAVYYCAREEDCSSTSCYGDADFIDWGQGTMTVTVSS
125	ATL_0006533 VH	QVQLQQSGGGLVKPGGSLRLSCAASGFTFSDYYMSWIRQAPGK GLEWWSYISSSSSYTNYADSVKGRFTISRDNANKNSLYLQMNSLRA EDTAVYYCARGGGGMDVWGQGTMTVTVSS
126	ATL_0006534 VH	QVQLVESGAEVKKPGASVKVSCKASGYTFTGYMHWWVRQAPGQ GLEWMGRINPNSSGGTNYAQKFQGRVTMTRDTSISTAYMELSRLR SDDTAVYYCAREGDDSSGWLGGAFDIWGQGTMTVTVSS
127	ATL_0006535 VH	EVQLLETGGGLVQPGRLRLSCAASGFTFDDYAMHWWVRQAPGK GLEWWSGISWNSGSGIYADSVKGRFTISRDNANKNSLYLQMNSLR AEDTALYYCAKDSYGDYFRGYYYGMDVWGQGTMTVTVSS
128	ATL_0006536 VH	EVQLLESAGGVVQLGRSLRLSCAASGFTFSNYGMHWWVRQAPGK GLEWVAIIYFDGRNKYYAGSVGGRTISRDNANKNTLYLQMDSLRS DDTAVYYCARPLFPWLRVDGLDVWGHGTMTVTVSS
129	ATL_0006537 VH	QVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGK GLEWMGIIPGDSIDTRYSPSFQQQVTISADKSISTAYLQWSSLKA SDTAMYYCARQRRGSGTRGMDVWGQGTMTVTVSS
130	ATL_0006538 VH	QVQLQESGPGLVKPSGTLSTCAVSGGSISSSNWWSWVRQPPG KGLEWIGEIIYHSGSTNYPNPSLKSRTISVDKSKNQFSLKLSSVTAA DTAVYYCARGRGGSYFYDYWGQGTMTVTVSS
131	ATL_0006539 VH	QVQLVESGGGLVQPGGSLRLSCSASGFTFINYAMSWVRQAPGK GLEWVSTISGSGASIYYADSVKGRFTISRDNANKNTVYLQMNSLRA EDTAVYYCAKGAYYDSSGYALDAFDIWGQGTMTVTVSS
132	ATL_0006530 VL	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSA PTTVIYEDNQRPSPGVDRFSGSIDSSSNSASLTISGLKTEDEADYY CQSYDSSNHWFVGGGTKVTVL
133	ATL_0006531 VL	QSALTQPASVSGSPGQSITISCTGARSDVGGYDYVSWFQQHPGK VPKLMIVDNNRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYY CSSFTDSSTYVFGTGKTVTVL
134	ATL_0006532 VL	QSVLTQPHSVSESPGKTVTISCTRSSGSIVSNYVQWYQQRPGSS PTTVIYEDNQRPSPGVDRFSGSIDRSSNSASLTISGLKTEDEGDYY CQSYDKTSRVILFEGGKTVTVL
135	ATL_0006533 VL	NFMLTQPHSVSESPGKTVTISCTGSSGSIASNYVQWYQQRPGSA PTTVIYEDNQRPSPGVDRFSGSIDSSSNSASLTISGLKTEDEADYY CQSYDSSNHWFVGGGTKVTVL
136	ATL_0006534 VL	NFMLTQPHSVLESPGKTVTIPCTRSSGSIASNYVQWYQQRPGSA PTAVIFEDNVRPSGVDRFSGSVDSSSNSASLTISGLKTEDEGDF YCQSYDSSGHVVFVGGGKTVTVL

137	ATL_0006535 VL	QSVLTQPPSVSGAPGQRTISCTGSSSNIGAGYDVHWYQQLPGT APKLLIYGNSNRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYY CQSYDSSLSASYVFGTGKVTVL
138	ATL_0006536 VL	QSALTQPASVSGSPGQSITISCTGTSSDVGNYNLVSQYQYPGK APKLLIYEVIKRPSGISGRFSGSKSGNTASLTISGLQAEDEADYYC SSYTSSSTDYVFGTGKVTVL
139	ATL_0006537 VL	QAGLTQPPSVSVSPGQTASITCSGKKLGDKYTSWYQQKPGQSPL LVIIYQDNKRPSGVPGRFSGSNSGNTATLTISGTQPMDEADYYCQ AWDSSTVIFGGGKVTVL
140	ATL_0006538 VL	QSVLTQPPSASGTPGQRTISCSGSSSNIGSNTVNWYQQVPGTA PKLLIYSNNQRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYC AAWDDSLNGWVFGGKVTVL
141	ATL_0006539 VL	QSVLTQPPSVSVSAGQTASITCSGDMLGKGYASWYQHKPGQSP VLVIYQDARRPSGISERFSGSSSGNTATLTISGTQAMDEADYYCQ AWGSTAVFGGKVTVL
142	HCDR1_ATL_0006530	GFTFSSYS
143	HCDR1_ATL_0006531; HCDR1_ATL_0006535	GFTFDDYA
144	HCDR1_ATL_0006532; HCDR1_ATL_0006534	GYTFTGY
145	HCDR1_ATL_0006533	GFTFSDYY
146	HCDR1_ATL_0006536	GFTFSNYG
147	HCDR1_ATL_0006537	GYSFTSYW
148	HCDR1_ATL_0006538	GGSISSSNW
149	HCDR1_ATL_0006539	GFTFINYA
150	HCDR2_ATL_0006530	ISSSSSYI
151	HCDR2_ATL_0006531	ISWDGGST
152	HCDR2_ATL_0006532; HCDR2_ATL_0006534	INPNSSGT
153	HCDR2_ATL_0006533	ISSSSSYT
154	HCDR2_ATL_0006535	ISWNSSGI
155	HCDR2_ATL_0006536	IYFDGRNK
156	HCDR2_ATL_0006537	IYPGSDT
157	HCDR2_ATL_0006538	IYHSGST
158	HCDR2_ATL_0006539	ISGSGASI
159	HCDR3_ATL_0006530	ARDPYSGYEFVSEPYFDY
160	HCDR3_ATL_0006531	AKDSIQLWSFDY
161	HCDR3_ATL_0006532	AREEDCSSTSCYGDAFDI
162	HCDR3_ATL_0006533	ARGGGGMDV
163	HCDR3_ATL_0006534	AREGDDSSGWLGGAFDI
164	HCDR3_ATL_0006535	AKDSYGDYFRGYYYGMDV

165	HCDR3_ATL_0006536	ARPLFPWLRVDGLDV
166	HCDR3_ATL_0006537	ARQRRGSGTRGMDV
167	HCDR3_ATL_0006538	ARGRGGSYYFDY
168	HCDR3_ATL_0006539	AKGAYYDSSGYALDAFDI
169	LCDR1_ATL_0006530; LCDR1_ATL_0006533; LCDR1_ATL_0006534	SGSIASNY
170	LCDR1_ATL_0006531	RSDVGGYDY
171	LCDR1_ATL_0006532	SGSIVSNY
172	LCDR1_ATL_0006535	SSNIGAGYD
173	LCDR1_ATL_0006536	SSDVGNYNL
174	LCDR1_ATL_0006537	KLGDYK
175	LCDR1_ATL_0006538	SSNIGSNT
176	LCDR1_ATL_0006539	MLGGKY
177	LCDR2_ATL_0006530; LCDR2_ATL_0006532; LCDR2_ATL_0006533; LCDR2_ATL_0006534	EDN
178	LCDR2_ATL_0006531	DVN
179	LCDR2_ATL_0006535	GNS
180	LCDR2_ATL_0006536	EVI
181	LCDR2_ATL_0006537	QDN
182	LCDR2_ATL_0006538	SNN
183	LCDR2_ATL_0006539	QDA
184	LCDR3_ATL_0006530	QSYDSSNHVV
185	LCDR3_ATL_0006531	SSFTDSSTYV
186	LCDR3_ATL_0006532	QSYDKTSRVIL
187	LCDR3_ATL_0006533	QSYDSSNHVV
188	LCDR3_ATL_0006534	QSYDSSGHVV
189	LCDR3_ATL_0006535	QSYDSSLASYSV
190	LCDR3_ATL_0006536	SSYTSSSTDYV
191	LCDR3_ATL_0006537	QAWDSSTVI
192	LCDR3_ATL_0006538	AAWDDSLNGWV
193	LCDR3_ATL_0006539	QAWGSTAV
194	human UNC5C version 2	GSQDDDFHHELPETFPSPPEPLPHFLIEPEEAYIVKNKPVNLYCK ASPATQIYFKCNSEWWHQKDHIWDERVDETSGLIVREVSIEISRQQ VEELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPL GKEVSLEQEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITID HNLIQKARLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWT EWSVCNSRCGRGYQKRTCTNPAPLNGGAFCEGQSVQKIACT

		TLCPVDGRWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDC DGLVLQSKNCTDGLCMQTAPDSDDVALYGSHHHHHH
195	HFWR2_ATL_0006191	WSWIRQTPGKGLEWIGY
196	HFWR3_ATL0006187	NQNPSLKSRVTMSVDSSKSQLSLKLTSVTAADTAVYYC
197	HFWR3_ATL0006191	NYNPSLKSRVTISVDTFKNQFSLKLTSVTAADTAVYYC
198	HFWR4_ATL0006187	WGQGILVIVSS
199	HFWR1_ATL_0006530	QVQLLETGGGLVQPGGSLRLSCAAS
200	HFWR2_ATL_0006530	MNWVRQAPGKGLEWVSS
201	HFWR3_ATL_0006530	YYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
202	HFWR1_ATL_0006531	EVQLVESGGVVPVQPGGSLRLSCAAS
203	HFWR2_ATL_0006531	MHWVRQAPGKGLEWVSL
204	HFWR3_ATL_0006531	YYADSVKGRFTISRDNKNSLYLQMNSLRAEDTALYYC
205	HFWR1_ATL_0006532	QVTLKESGAEVKKPGASVKVSCAS
206	HFWR2_ATL_0006532, 6534	MHWVRQAPGQGLEWMGR
207	HFWR3_ATL_0006532, 6534	NYAQKFQGRVTMTRDTSISTAYMELSRLRSDDTAVYYC
208	HFWR4_ATL_0006532, 6533, 6534, 6535, 6539	WGQGTMTVTVSS
209	HFWR1_ATL_0006533	QVQLQQSGGGLVKPGGSLRLSCAAS
210	HFWR2_ATL_0006533	MSWVRQAPGKGLEWVSY
211	HFWR3_ATL_0006533	NYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYC
212	HFWR1_ATL_0006534	QVQLVESGAEVKKPGASVKVSCAS
213	HFWR1_ATL_0006535	EVQLLETGGGLVQPGRSLRLSCAAS
214	HFWR2_ATL_0006535	MHWVRQAPGKGLEWVSG
215	HFWR3_ATL_0006535	GYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTALYYC
216	HFWR1_ATL_0006536	EVQLLESAGGVVQLGRSLRLSCAAS
217	HFWR2_ATL_0006536	MHWVRQAPGKGLEWVAI
218	HFWR3_ATL_0006536	YYAGSVGGRFTISRDNKNTLYLQMDSLRSDDTAVYYC
219	HFWR4_ATL_0006536	WGHGTMVTVSS
220	HFWR1_ATL_0006537	QVQLVQSGAEVKKPGESLKISCKGS
221	HFWR2_ATL_0006537	IGWVRQMPGKGLEWMGI
222	HFWR3_ATL_0006537	RYSPSFQGGQVTISADKSISTAYLQWSSLKASDTAMYYC
223	HFWR4_ATL_0006537	WGQGTTVTVSS
224	HFWR1_ATL_0006538	QVQLQESGPGLVKPSGTLSTCAVS
225	HFWR2_ATL_0006538	WSWVRQPPGKGLEWIGE
226	HFWR3_ATL_0006538	NYNPSLKSRVTISVDKSKNQFSLKLSSVTAADTAVYYC
227	HFWR1_ATL_0006539	QVQLVESGGGLVQPGGSLRLSCSAS
228	HFWR2_ATL_0006539	MSWVRQAPGKGLEWVST
229	HFWR3_ATL_0006539	YYADSVKGRFTISRDNKNTVYLQMNSLRAEDTAVYYC

230	LFWR1_ATL_0006530, 6533	NFMLTQPHSVSESPGKTVTISCTGS
231	LFWR1_ATL_0006531	QSALTQPASVSGSPGQSITISCTGA
232	LFWR1_ATL_0006532	QSVLTQPHSVSESPGKTVTISCTRS
233	LFWR1_ATL_0006534	NFMLTQPHSVLESPGKTVTIPCTRS
234	LFWR1_ATL_0006535	QSVLTQPPSVSGAPGQRTISCTGS
235	LFWR1_ATL_0006537	QAGLTQPPSVSVSPGQTASITCSGK
236	LFWR1_ATL_0006538	QSVLTQPPSASGTPGQRTISCSGS
237	LFWR1_ATL_0006539	QSVLTQPPSVSVSAGQTASITCSGD
238	LFWR2_ATL_0006530, 6533	VQWYQQRPGSAPTTVIY
239	LFWR2_ATL_0006531	VSWFQQHPGKVPKLMY
240	LFWR2_ATL_0006532	VQWYQQRPGSSPTTVIY
241	LFWR2_ATL_0006534	VQWYQQRPGSAPTAVIF
242	LFWR2_ATL_0006535	VHWYQQLPGTAPKLLIY
243	LFWR2_ATL_0006536	VSWYQQYPGKAPKLLIY
244	LFWR2_ATL_0006537	TSWYQQKPGQSPLLIY
245	LFWR2_ATL_0006538	VNWWYQQVPGTAPKLLIY
246	LFWR2_ATL_0006539	ASWYQHKPGQSPVLVIY
247	LFWR3_ATL_0006530, 6533	QRPSGVPDRFSGSIDSSSNSASLTISGLKTEDEADYYC
248	LFWR3_ATL_0006531	NRPSGVSNRFSGSKSGNTASLTISGLQAEDEADYYC
249	LFWR3_ATL_0006532	QRPSGVPDRFSGSIDRSSSNSASLTISGLKTEDEGDYYC
250	LFWR3_ATL_0006534	VRPSGVPDRFSGSVSDSSSNSASLTISGLKTEDEGDFYC
251	LFWR3_ATL_0006535	NRPSGVPDRFSGSKSGTSASLAITGLQAEDEADYYC
252	LFWR3_ATL_0006536	KRPSGISGRFSGSKSGNTASLTISGLQAEDEADYYC
253	LFWR3_ATL_0006537	KRPSGVPGRFSGSNSGNTATLTISGTQPMDEADYYC
254	LFWR3_ATL_0006538	QRPSGVPDRFSGSKSGTSASLAISGLQSEDEADYYC
255	LFWR3_ATL_0006539	RRPSGISERFSGSSSGNTATLTISGTQAMDEADYYC
256	LFWR4_ATL_0006530, 6533, 6534, 6537, 6538, 6539	FGGGTKVTVL
257	LFWR4_ATL_0006531, 6535, 6536	FGTGTKVTVL
258	LFWR4_ATL_0006532	FGEGTKVTVL
259	Region of UNC5C_HS- o95185-1 that interacts with ATL_5262	YCKASPATQIYFKCNSEWWHQKDHIVDERVDETSGLIVREV
260	Region of UNC5B_Hs- Q8IZJ1-1 that is	RCRAFPATQIYFKCNGEWWSQNDHVTQEGLDEATGLRVREV

	homologous to SEQ ID NO: 259	
261	Region of UNC5A_Hs-Q6ZN44-1 that is homologous to SEQ ID NO: 259	VCKAVPATQIFFKCNGEWVRQVDHVIERSTDGSSGLPTMEV
262	Region of UNC5D_Hs-Q6UXZ4-1 that is homologous to SEQ ID NO: 259	RCKARPAMQIFFKCNGEVWHQNEHVSEETLDESSGLKVREV
263	Recombinant human UNC5C	GSQDDDDFFHELPETFPSPDPEPLPHFLIEPEEAYIVKNKPVNLYCK ASPATQIYFKCNSEVWHQKDHIVDERVDETSGLIVREVSIISRQQ VEELFGPEDYWCQCVAWSSAGTTKSRKAYVRIAYLRKTFEQEPL GKEVSLEQEVLLQCRPPEGIPVAEVEWLKNEDIIDPVEDRNFYITID HNLIKQARLSDTANYTCVAKNIVAKRKSTTATVIVYVNGGWSTWT EWSVCNSRCGRGYQKRTRCTNPAPLNGGAFCEGQSVQKIACT TLCVPDGRWTPWSKWSTCGTECTHWRRRECTAPAPKNGGKDC DGLVLQSKNCTDGLCMQTAPDSDDVALYGSKHHHHHH
264	Recombinant mouse UNC5C	GSHHHHHHGS DYKDDDDKGSQDDFFHELPETFPSPDPEPLPH FLIEPEEAYIVKNKPVNLYCKASPATQIYFKCNSEVWHQKDHVDE RVDETSGLIVREVSIISRQQVEELFGPEDYWCQCVAWSSAGTTK SRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVE WLKNEDIIDPAEDRNFYITIDHNLIKQARLSDTANYTCVAKNIVAKR KSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRCTNPAPL PLNGGAFCEGQSVQKIACTTLCVPDGRWTSWSKWSTCGTECTH WRRRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMAAPDSDD VALYGS
265	Recombinant Cyno UNC5C	GSHHHHHHGS DYKDDDDKGSQDDFFHELPETFPSPDPEPLPH FLIEPEEAYIVKNKPVNLYCKASPATQIYFKCNSEVWHQKDHIVDE RVDETSGLIVREVSIISRQQVEELFGPEDYWCQCVAWSSAGTTK SRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVE WLKNEDIIDPVEDRNFYITIDHNLIKQARLSDTANYTCVAKNIVAKR KSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRCTNPAPL PLNGGAFCEGQSVQKIACTTLCVPDGRWTPWSKWSTCGTECTH WRRRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMQTAPDSDDV ALYGS
266	Recombinant human UNC5A	GSHHHHHHGS DYKDDDDKGSQQSATVANPVPGANPDLLPHFLV EPEDVYIVKNKPVLLVCKAVPATQIFFKCNGEWVRQVDHVIERST DGSSGLPTMEVRINVSRRQQVEKVFGLEEYWCQCVAWSSSGTTK SQKAYIRIAYLRKNFEQEPLAKEVSLEQGIVLPCRPPPEGIPPAEVE WLRNEDLVDP SLDPNVYITREHSLVVRQARLADTANYTCVAKNIV

		ARRRSASAAVIVYVDGWSWSPWSKWSACGLDCTHWRSRECSDDPA PRNGGEECQGTDLDTNRCTSDLCVHTASGPEDVALYGS
267	Recombinant human UNC5B	GSHHHHHHGSDYKDDDDKSGTDSGSEVLPSDFPSAPAEPLPY FLQEPQDAYIVKNKPVELRCRAFPATQIYFKCNGEWSQNDHVT QEGLEATGLRVREVQIEVSRQQVEELFGLLEDYWCQCVAWSSA GTTKSRRAYVRIAYLRKNFDQEPLGKEVPLDHEVLLQCRPEGVP VAEVEWLKNEDVIDPTQDTNFLTIDHNLIRQARLSDTANYTCVAK NIVAKRRSTTATVIVYVNGGWSSWAEWSPCSNRCGRGWQKRTR TCTNPAPLNGGAFCEGQAFQKTACTTICPVDGAWTEWSKWSAC STECAHWRSRECMAPPPQNGGRDCSGTLLDSKNCTDGLCMQN KKTLSDPNSHLLASGDAALYGS
268	Recombinant human UNC5D	GSHHHHHHGSDYKDDDDKGSARGTDNGEALPESIPSAPGTLPHF IEEPDDAYIIKSNPIALRCKARPAMQIFFKNGEWWHQNEHVSEET LDESSGLKVREVFINVTRQQVEDFHGPEYWCQCVAWSHLGTS KSRKASVRIAYLRKNFEQDPQGREVPPIEGMIVLHCRPPEGVPAEE VEWLKNEEPIDSEQDENIDTRADHNLIRQARLSDSGNYTCMAANI VAKRRSLSATVVVYVNGGWSSWTEWSACNVRRCGRGWQKRST CTNPAPLNGGAFCEGMSVQKITCTSLCPVDGSWEVWSEWSVCS PECEHLRIRECTAPPPRNGGKFCEGLSQESENCTDGLCILDKKPL HEIKPQSIENASDGS
269	Recombinant human DCC	GSHHHHHHGSDYKDDDDKGSHLQVTGFQIKAFALTALRFLSEPSDA VTMRGGNVLLDCAESDRGVPVIKWKKGDIHLALGMDERKQQLS NGSLLIQNILHSRHHKPDEGLYQCEASLGDSGSIISRTAKVAVAGP LRFLSQTESVTAFMGDTVLLKCEVIGEPMTIHWQKNQQDLTPIP GDSRVVVLPSGALQISRLQPGDIGYRCSARNPASSRTGNEAEVRI LSDPGLHRQLYFLQRPSNVVAIEGKDAVLECCVSGYPPPSFTWLR GEEVIQLRSKKYSLGGSNLLISNVTDDDSGMYTCVVYKNENISA SAELTVLPPWFLNHPSNLYAYESMDIEFFECTVSGKPVPTVNWMM KNGDVVIPSDYFQIVGGSNLRILGVVKSDEGFYQCVAEENAGNAQ TSAQLIVPKPAIPSSSVLPSAPRDVVPVLVSSRFVRLSWRPPAEAK GNIQTFTVFFSREGDNRERALNTTQPGSLQLTVGNLKPEAMYTR VWAYNEWGPGESSQPIKVATQPELQVPGPVENLQAVSTSPTSILIT WEPPAYANGPVQGYRLFCTEVSTGKEQNIEVDGLSYKLEGLKKF TEYSLRFLAYNRYGPGVSTDDITVVTLSDVPSAPPQNVSLVWNS RSIKVSWLPPPSGTQNGFITGYKIRHRKTTRRGEMETLEPNNLWY LFTGLEKGSQYSFQVSAMTVNGTGPPSNWYTAETPENDLDESQV PDQPSSLHVRPQTNCIIMSWTPPLNPNIIVRGYIIGYGVGSPYAET VRVDSKQRYYSIERLESSHYISLKAFNNAGEGVPLYESATTRSI TDPTDPVDYYPLDDFPTSVPDLSTPMLPPVGVAVALTHDAVRV SWADNSVPKNQKTSEVRLYTVRWRTSFSASAKYKSEDTTSLSYT ATGLKPNTMYEFSVMVTKNRRSSTWSMTAHATTYEAAPTSAPKD

		LTVITREGKPRATIVSWQPPLEANGKITAYILFYTLDKNIPIDDWIME TISGDRLTHQIMDLNLDTMYYFRIQARNNSKGVGPLSDPILFRTLKV EHPDKMANDQGRHGDGGYWPVDNLIDRSTLNEPPIGQMHPPH GSVTPQKNSNGS
270	Recombinant human UNC5C – Ig1 domain only	GSHHHHHHGSSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIF FKIKKTTPLRRLMEAFKARQKEMDSLTFLYDGIEIQADQTPEDLD MEDNDIIEAHREIQIGGGGSQDDDFHELPETFPSPDPEPLPHFLIE PEEAYIVKNKPVNLYCKASPATQIYFKCNSEWWHQKDHIVDERVD ETSGLIVREVSIEISRQQVEELFGPEDYWCQCVAWSSAGTTKSRK AYVRIAGS
271	Recombinant human UNC5C – Ig1 and Ig2 domain	GSDPPEPLPHFLIEPEEAYIVKNKPVNLYCKASPATQIYFKCNSEW VHQKDHIVDERVDETSGLIVREVSIEISRQQVEELFGPEDYWCQC VAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCR PPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHNLIQARLSDTANY TCVAKNIVAKRKSTTATVIVYVGSHHHHHH
272	Recombinant human UNC5C – Ig1, Ig2 and TSP-1 domain.	GSDPPEPLPHFLIEPEEAYIVKNKPVNLYCKASPATQIYFKCNSEW VHQKDHIVDERVDETSGLIVREVSIEISRQQVEELFGPEDYWCQC VAWSSAGTTKSRKAYVRIAYLRKTFEQEPLGKEVSLEQEVLLQCR PPEGIPVAEVEWLKNEDIIDPVEDRNFYITIDHNLIQARLSDTANY TCVAKNIVAKRKSTTATVIVYVNGGWSTWTEWSVCNSRCGRGYQ KRTRTCTNPAPLNGGAFCEGQSVQKIACTTLCVPVGSHHHHHH
273	Recombinant human UNC5C – missing Ig1 domain.	GSRIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVEWLK NEDIIDPVEDRNFYITIDHNLIQARLSDTANYTCVAKNIVAKRKST TATVIVYVNGGWSTWTEWSVCNSRCGRGYQKRTRTCTNPAPLN GGAFCEGQSVQKIACTTLCVPDGRWTPWSKWSTCGTECTHWR RRECTAPAPKNGGKDCDGLVLQSKNCTDGLCMQGSHHHHHH
274	Human UNC5C Ig1 domain (amino acids 62-159)	PHFLIEPEEAYIVKNKPVNLYCKASPATQIYFKCNSEWWHQKDHIV DERVDETSGLIVREVSIEISRQQVEELFGPEDYWCQCVAWSSAGT TKSRKAY
275	Human UNC5C Ig2 domain (amino acids 161-256)	RIAYLRKTFEQEPLGKEVSLEQEVLLQCRPPEGIPVAEVEWLKNE DIIDPVEDRNFYITIDHNLIQARLSDTANYTCVAKNIVAKRKSTTA TVI
276	Human UNC5C TSP1 domain (amino acids 260-314)	NGGWSTWTEWSVCNSRCGRGYQKRTRTCTNPAPLNGGAFCEG QSVQKIACTTLC
277	Human UNC5C TSP2 domain (amino acids 316-368)	DGRWTPWSKWSTCGTECTHWRRECTAPAPKNGGKDCDGLVL QSKNCTDGLCM

Table 1. Sequences referred to in the present disclosure. VH= Heavy chain variable domain; VL=Light chain variable domain; CDR= Complementarity-Determining Region, CDRL refers to CDRs in the light chain variable domain (numbered 1, 2 or 3 from N-ter); CDRH refers to CDRs in the heavy chain variable domain ((numbered 1, 2 or 3 from N-ter)); FWR = Framework region; LFWR refers to an FWR in the light

chain (numbered 1, 2, 3 or 4 from N-ter) whereas HFWR refers to an FWR in the heavy chain (numbered 1, 2, 3 or 4 from N-ter). "ATL_" is used as an antibody identifier.

Examples

These examples demonstrate the identification of potential therapeutic antibodies from a cohort of subjects that have or at considered at risk of developing frontotemporal dementia or Parkinson's disease (PD) (Example 1), as well as their *in vitro* characterisation (Examples 2, 4 and 5) and optimisation (Example 3). Further antibodies were subsequently identified that display similar properties to those of Example 1 and 3 (Example 6), and the potential of these further antibodies to detect soluble UNC5C in biological fluid samples obtained from subjects, such as e.g. subjects treated with a therapeutic that is expected to affect the level of soluble UNC5C in a treated subject, was demonstrated (Examples 7-13).

Materials and methods

Human and mouse UNC5C ELISA

UNC5C recombinant human antigen (Fc-tagged R&D Systems, #1005-UN; or in-house production from HEK293F cells with N-terminal His or FLAG tag (SEQ ID NO: 98) - in-house human antigen was used to confirm results were not driven by Fc tag) or mouse antigen (in-house production from HEK393F cells with N-terminal His or FLAG tag (SEQ ID NO:97 -the expressed murine UNC5C extracellular domain comprises AA 41-380 of the murine UNC5C sequence); rat antigen (SEQ ID NO: 103); or cyno antigen (SEQ ID NO: 104) were directly absorbed to an ELISA plate at 3µg/ml (50µl per well) and incubated overnight at 4°C. The plate was washed with PBS. Plates were blocked with 200µl/well of blocking solution (1% BSA w/v in PBS) for 1 hour at room temperature. Following this, the blocking solution was removed and antibodies to be assessed were diluted in dilution series (1µM to 0.05nM) in blocking solution (1% BSA w/v in PBS) and applied to the plate. Plates were incubated at room temperature for 1 hour. The plate was washed with PBS/0.1% Tween. Anti-Human IgG HRP (Jackson ImmunoResearch; #109-035-097) was added to plate and incubated for 1 hour at room temperature to detect antibody binding. The plate was washed with PBS/0.1% Tween and TMB solution (Life Technology; #002023) added. Plates were incubated for 5 minutes at room temperature prior to the addition of stopping solution (0.5M sulphuric acid). Absorbance was read on Molecular Devices FilterMaxF5 plate reader at 450nm. Analysis was performed using a non-linear fit curve fitting algorithm on GraphPad prism and EC50 calculated.

Target deconvolution using Retrogenix proteomics

The pool of 20 antibodies used in this screen were 18 antibodies identified from FTD sample cohort analysis, one antibody from a Huntington cohort and one positive control anti-PDL1 antibody. The antibodies were mixed in equal proportions (concentrations) to create the pool. The antibody pool was screened at 4ug/ml, so each individual antibody within the pool was screened at 0.2ug/ml. The pool of antibodies was screened for binding against fixed HEK293 cells/slides expressing the "Retrogenix library" comprising duplicate 6019 human plasma membrane proteins, secreted and cell surface tethered human secreted proteins and 397 human heterodimers. Screens were performed in duplicate. Antibody binding is detected using an AlexaFluor647-labelled secondary antibody and scanning slides. UNC5C was identified as a weak binder in one replicate and a very weak binder in the second replicate.

Following this result, each antibody from the pool was tested individually for binding to rhUNC5C protein by ELISA.

Netrin-1 competition assay

5 UNC5C recombinant human antigen (R&D Systems; #1005-UN) or mouse antigen (in-house production) were directly absorbed to ELISA plate at 3µg/ml (50µl per well) and incubated overnight at 4°C. The plate was washed with PBS. Plates were blocked with 200µl/well of blocking solution (1% BSA w/v in PBS) for 1 hour at room temperature. Following this, recombinant human Netrin 1 with C-terminal His tag (R&D Systems; #6419-N1) or vehicle was added to the plate for a pre-incubation step at room temperature for 30 minutes. Netrin 1 concentration was 200µg/ml for single point competition assay and in a dilution range from 200µg/ml to 0.003µg/ml. The assay was set up in replicate wells to be able to detect both ATL_0005262 antibody binding and Netrin 1 binding. ATL_0005262 (at 4µg/ml) was applied to the wells containing Netrin 1 and was incubated at room temperature for 1 hour. The plate was then washed with PBS/0.1% Tween. Anti-Human IgG HRP was added to plate (Jackson ImmunoResearch; #109-035-097) and incubated for 1 hour at room temperature to detect antibody binding to UNC5C. Anti-His-HRP (Invitrogen #MA1-21315-HRP) was added to wells to detect Netrin 1 binding to UNC5C. The plate was washed with PBS/0.1% Tween and TMB solution (LifeTechnology; #002023) added. Plates were incubated for 5 minutes at room temperature prior to the addition of stopping solution (0.5M sulphuric acid). Absorbance read on Molecular Devices FilterMaxF5 plate reader at 450nm.

20 For the reverse experiment to see if Netrin-1 could displace ATL_0005262, UNC5C recombinant human antigen (R&D Systems; #1005-UN) was directly absorbed to ELISA plate at 3µg/ml (50µl per well) and incubated overnight at 4°C. The plate was washed with PBS. Plates were blocked with 200µl/well of blocking solution (1% BSA w/v in PBS) for 1 hour at room temperature. Following this, ATL_0005262 or negative control antibody ATL_0005338 were added to the plates in a 12 point 3-fold dilution curve starting from 666nM. Plates were incubated for 30 minutes at room temperature. Netrin 1 was then added to the wells at a final concentration of 75nM, and incubated at room temperature for 1 hour. The assay was set up in replicate wells to be able to detect both ATL_0005262 antibody binding and Netrin 1 binding. The plate was then washed with PBS/0.1% Tween. Anti-Human IgG HRP was added to plate (Jackson ImmunoResearch; #109-035-097) and incubated for 1 hour at room temperature to detect antibody binding to UNC5C. Anti-His-HRP (Invitrogen #MA1-21315-HRP) was added to wells to detect Netrin 1 binding to UNC5C. The plate was washed with PBS/0.1% Tween and TMB solution (LifeTechnology; #002023) added. Plates were incubated for 5 minutes at room temperature prior to the addition of stopping solution (0.5M sulphuric acid). Absorbance read on Molecular Devices FilterMaxF5 plate reader at 450nm.

35 Netrin-1 competition assay
To determine if Netrin-1 could displace phage-derived antibodies, UNC5C recombinant human antigen (R&D Systems; #1005-UN) was directly absorbed to ELISA plates and incubated overnight at 4°C. The plates were washed with PBS. Plates were blocked with 1 % BSA w/v in PBS blocking solution for 1 hour at room temperature. Following this, the phage-derived antibodies (ATL6530- ATL6539) or negative control antibody ATL_0005338 were added to the plates in a dilution curve ranging from 1 µM to 0.1 pM (final concentration; once Netrin-1 added). Plates were incubated for 30 minutes at room temperature. Netrin-1

(R&D Systems; #6419-N1) was then added to the wells at a final concentration of 75 nM, and incubated at room temperature for 1 hour. The assay was set up in replicate wells to be able to detect both antibody binding UNC5C in the presence and absence of Netrin-1, in addition to Netrin-1 binding UNC5C in the presence of antibody. The plate was then washed with PBS/0.1 % Tween. Anti-human IgG HRP was added to the plates (Jackson ImmunoResearch; #109-035-097) and incubated for 1 hour at room temperature to detect antibody binding to UNC5C. Anti-His-HRP (Invitrogen #MA1-21315-HRP) was added to wells to detect Netrin-1 binding to UNC5C. The plates were washed with PBS/0.1 % Tween and TMB solution (LifeTechnology; #002023) added. Plates were incubated for 5 minutes at room temperature prior to the addition of stopping solution (0.5 M sulphuric acid). Absorbance was read on a Molecular Devices FilterMaxF5 plate reader at 450 nm. Analysis was performed using a non-linear fit curve fitting algorithm on GraphPad prism and EC50 calculated.

Immunohistochemistry

C57BL/6 naïve mouse brains were cryopreserved via snap freezing in OCT, sectioned using a cryostat onto glass slides and stored at -20°C until use. Slides were thawed and air-dried prior to fixation in neutral buffered formalin (10 %) for 10 minutes and washed in PBS-Triton X (0.5 %) for 30 minutes. Hydrophobic barriers were drawn around each tissue section and blocked with appropriate blocking solution (1 % BSA, 10 % FBS, 5 mM EDTA, 0.3 M glycine in PBS-Tx 0.5 %) with addition of either Clusterin (R&D Systems cat. # 2937-HS-050), Netrin-4 (R&D Systems cat. # 1254-N4-025/CF) or Netrin-1 (R&D Systems cat. # 6419-N1-025/CF) [10 nM] for 1 hour. Blocking solution was removed and primary antibodies added in the same blocking buffer (ATLX-1282/ATL_5262 [10 ug/ml]) overnight at 4 degrees. Slides were washed 3 times with PBS-Tx (0.5 %) 5 minutes each, followed by addition of secondary antibody anti-human IgG DyLight® 594 (Abcam cat. # ab97005) [2.5 µg/ml] and DAPI [0.1 µg/ml] (Thermo Scientific cat. # 62248) in respective buffer for 90 minutes at room temperature, washed and mounted with coverslips using VectaShield anti-fade mounting medium (Vector Labs cat. # H-1200-10). Images of slides were acquired using Molecular Devices PICO and analysis performed using FIJI to threshold and measure images, GraphPad t-tests were performed for significance values.

Flow Cytometry

The nucleotide sequences encoding canonical UNC5C (SEQ ID NO: 99) and mutated UNC5C T835M variant (SEQ ID NO: 110) were cloned into the lentiviral transfer vector pCDH-EF1α-MCS-(PGK-GFP-T2A-Puro) (System Biosciences; #CD813A-1) and confirmed with sanger sequencing. Lentiviral particles were produced in the 293T cell line (ATCC; #CRL-3216) using TransIT-VirusGEN Transfection Reagent (Mirus; #MIR 6700) and pPACKH1 HIV Lentivector Packaging Kit (System Biosciences; # LV500A-1). Stable cell lines were generated by transduction of HEK-293 cell line (ECACC; #CRL-1573) with lentiviral particles and subsequent selection of successfully transduced cells with puromycin (Gibco™; # A1113803) at 1 µg/ml. Overexpressing cell lines were monitored for GFP expression and cultured in the presence of selection antibiotic.

For the detection of surface antigens, HEK-293 cells as well as cell lines expressing UNC5C, mutated UNC5C T835M and empty vector (EV) control were harvested using non-enzymatic dissociation buffer (CORNING; cat # 25-056-CI). Cells were then labelled with viability dye eF780 (Invitrogen; #65-0865), washed with FACS buffer (DPBS, Gibco; #14190-169, 2% FBS, LSG; #S-001A-USDA) and incubated with

the antibodies at 100 µg/ml (0.69 nM), in triplicate, for 1 h at 4°C. Cells were then washed twice with the FACS buffer and incubated with the secondary detection anti-IgG APC antibody (clone IS11-3B2.2.3, Miltenyi; # 130-119-772) for 30 minutes at 4°C. Cells were then washed twice, resuspended in FACS buffer and analysed using NovoCyte Penton Flow Cytometer (Agilent). Compensation was performed automatically using NovoExpress software (Agilent) and samples labelled with individual fluorochromes. Detection parameters for each fluorochrome are set out in Table 3 below. Data analysis was performed using FlowJo software (BD).

Fluorochrome	Detection Channel	Excitation	Detection
GFP	B525	488	525/45
APC	R667	637	667/30
eF780	R780	637	780/60

Table 2. Flow cytometry detection parameters

The percentage of APC positive cells (IgG⁺ cells) as well as IgG-APC median fluorescence intensity (MFI) were measured in the population gated on single/viable/cells. The HEK-293 cell line was used as the negative control for APC (IgG⁺) gating and the background signal was subtracted from the results.

Retrogenix Analysis

This was performed by Charles River (UK) using their proprietary Retrogenix Cell Microarray Technology (see www.criver.com/products-services/discovery-services/screening-and-profiling-assays/retrogenix-cell-microarray-technology).

X-ray crystallography

Recombinant human UNC5C was produced in ExpiHEK293 by transient transfection of a construct corresponding to Ig domains 1 and 2 and purified via a C-terminal hexahistidine tag (SEQ ID NO: 111). The Fab fragment of ATL_5262 was produced by transient transfection of ExpiCHO and purified via the C_H1 domain. Both components were purified by SEC, mixed together to form the complex and screened for crystallisation using commercial crystallisation screens. X-ray diffraction data was collected from crystals grown in 22 % PEG Smear Broad, 0.1 M Bicine pH 9.3 (among many other formulations that had a similar composition and pH). Data was autoprocessed with Xia2/Dials and used for molecular replacement with the program Phaser using a truncated "Netrin receptor UNC5C" monomer (UniProt ID D6RE16 AlphaFold model) and a Fab heterodimer (PDB ID 6CNR) as search models. The original data from autoprocessing was reindexed to P6522 (space group of the protein crystal) and the model was taken through several rounds of interactive model rebuilding with the program Coot and refinement with Refmac, and finally manual and automatic solvent building with ARP/wARP, to produce the final model. Data collection and refinement statistics are set out in Table 4. The model contains one molecule each of the Fab heavy and light chains and one molecule of UNC5C in the asymmetric unit. The asymmetric unit is the area within the crystal lattice that contains the molecules that are repeated throughout the crystal. Finding only one copy of each the Fab and antigen, in combination with analysis of how the repeating units interact with each other here show that there are no other biologically relevant interactions e.g. dimerisation of the target.

ATL_5262 Fab – UNC5C Ig1-2	
Wavelength (Å)	0.9537
Resolution range (Å)	63.51 - 2.27 (2.351 - 2.27)
Space group	P 65 2 2
Unit cell	83.9251 Å, 83.9251 Å, 381.065 Å, 90°, 90°, 120°
Total reflections	1491857 (142455)
Unique reflections	38062 (3670)
Multiplicity	39.2 (38.8)
Completeness (%)	99.94 (99.67)
Mean I/sigma(I)	16.60 (0.37)
Wilson B-factor	71.71
R-merge	0.1394 (5.711)
R-meas	0.1413 (5.786)
R-pim	0.02248 (0.9228)
CC1/2 1	-0.373
CC* 1	-0.737
Reflections used in refinement	38042 (3658)
Reflections used for R-free	1924 (201)
R-work	0.2694 (0.4369)
R-free	0.3332 (0.4530)
CC(work)	0.928 (0.288)
CC(free)	0.909 (0.273)
Number of non-hydrogen atoms	4821
Macromolecules	4812
Ligands	0
Solvent	9
Protein residues	622
RMS(bonds)	0.008
RMS(angles)	1.68
Ramachandran favored (%)	87.3
Ramachandran allowed (%)	8.96
Ramachandran outliers (%)	3.75
Rotamer outliers (%)	10.36
Clashscore	15.26
Average B-factor	82.2
Macromolecules	82.26
Solvent	52.57

Table 3. Data collection and refinement statistics. Statistics for the highest-resolution shell are shown in parentheses.

Phage display

A phage library of scFv molecules displayed on an M13 phage was generated by cloning the heavy chain variable region (VH) repertoire of subjects SU_0000656 and SU_0000697 into a sub-library of phagemid

vectors with light chain variable region (VL) sequences from healthy donors. A phage display library of a size 1.3×10^8 clones was generated.

The following reagents were used:

- 5 - Hyper phage (1.5×10^{12} cfu/ml) from Progen, Germany catalogue number PRHYPE, K07deltaplII (used in Phage display and phage ELISA);
- Helper phage (1×10^{11} cfu/ml) from Invitrogen, USA catalogue number 18311-019 (used in Phage display and phage ELISA);
- UNC5C_HUMAN antigen (SEQ ID NO: 194) produced In-house (in house number
- 10 UNC5C_HUMAN_008 (Alchemab) res41-380 Batch#004) (used in Phage Display and Phage ELISA)
- UNC5C_human from R&D Systems, USA catalogue number 1005-UN-050 (used in Phage Display)

Phage ELISA on scFv derived from phage display

- 15 Phages were prepared by culturing each phagemid-carrying TG1 clone from a glycerol stock in 100 μ l of 2TYAG (2TY media supplemented with 100 μ g/ml ampicillin and 2 % glucose) at 37°C with aeration to optical density OD₆₀₀=0.6 followed by rescue with helper or hyper phage added at MOI 10 (Invitrogen, cat: 18311-019 and Progen, cat: PRHYPE, K07deltaplII, respectively) for 1 hour and media change to 2TYAK (2TY media supplemented with 100 μ g/ml ampicillin and 50 mg/ml kanamycin). The cultures were then
- 20 incubated overnight at 25°C with good aeration and next day the phages were separated from bacteria by centrifugation for 10 minutes at 3200 rpm. The phage-containing supernatant was transferred to a new plate and blocked with 3 % milk w/v in PBS.

- UNC5C recombinant human antigen (in-house, UNC5C_HUMAN_008 (Alchemab) res41-380 Batch#004)
- 25 or negative control Lysozyme (MP Biomedicals #195303) were directly absorbed to ELISA plate at 3 μ g/ml (50 μ l per well) and incubated overnight at 4°C. Each antigen-coated plate was washed with PBS and blocked with 200 μ l/well of blocking solution (3 % milk w/v in PBS) for 1 hour at room temperature. Following this, the blocking solution was removed and blocked phage samples to be assessed were applied to the plates. Plates were incubated at room temperature for 1 hour. Each plate was washed with PBS/0.1 %
- 30 Tween and incubated with anti-M13 HRP (Sino Biological, #11973-MM05T-H) for 1 hour at room temperature to detect phage binding. The plates were washed with PBS/0.1 % Tween and TMB solution (Life Technology; #002023) added. Plates were incubated for 5 minutes at room temperature prior to the addition of stopping solution (0.5 M sulphuric acid). Absorbance was read on a Molecular Devices FilterMaxF5 plate reader at 450 nm.

35

Screening IgG binding to UNC5C

- To determine binding of the IgG antibodies to UNC5C, UNC5C_Fc (R&D Systems #1005-UN), or in-house production from HEK293F cells with N-terminal His-tag or negative control Lysozyme (MP Biomedicals #195303) were directly absorbed to an ELISA plate at 1 μ g/ml and incubated overnight at 4°C. The plate
- 40 was washed with PBS. Plates were blocked with 1 % BSA w/v in PBS blocking solution for 1 hour at room temperature. Following this, the blocking solution was removed and antibodies to be assessed were diluted in dilution series (2 μ M to 0.4 pM) in blocking solution (1 % BSA w/v in PBS) and applied to the plate. Plates

were incubated at room temperature for 1 hour. The plate was washed with PBS/0.1 % Tween. Anti-Human IgG HRP (Jackson ImmunoResearch; #109-035-097) was added to the plate and incubated for 1 hour at room temperature to detect antibody binding. The plate was washed with PBS/0.1 % Tween and TMB solution (Life Technology; #002023) added. Plates were incubated for 5 minutes at room temperature prior to the addition of stopping solution (0.5 M sulphuric acid). Absorbance was read on a Molecular Devices FilterMaxF5 plate reader at 450 nm. Analysis was performed using a non-linear fit curve fitting algorithm on GraphPad prism and EC50 calculated.

ELISA – Antigen capture by anti-UNC5C antibody and detection by anti-His-HRP

Nunc Maxisorp 96 well ELISA plates were coated with 5µg/ml ATL6532, ATL6533, ATL6534 or isotype control antibody ATL5338. Antibodies were diluted in PBS and coated at 50µl per well, and incubated overnight at 2-8°C. Plates were washed 3 x with PBS/0.05% Tween (PBS-T) and the plate blocked with 3% BSA in PBS for 1 hour at room temperature. Recombinant human UNC5C with a C-terminal His tag (SEQ ID NO: 263) or irrelevant antigen were diluted in PBS/1% BSA and 50µl added per well. The plates were incubated for 1 hour at room temperature before washing 3 x in PBS-T. His-tagged antigen was detected with 50µl of 1:5000 dilution of anti-His HRP (Invitrogen #MA1-21315-HRP) incubated for 1 hour at room temperature in the dark. Plates were washed twice with PBS before detection with TMB (Pierce #34021), stopped with 0.5M sulphuric acid. Absorbance at 450nm and 570nm were measured on a CLARIOstar Plus plate reader.

ELISA – Sandwich immunoassay format with AF1005 detection

Nunc Maxisorp 96 well ELISA plates were coated with 3µg/ml of ATL6532, ATL6533, ATL6534, ATL6537 or isotype control antibody ATL5338. Antibodies were diluted in PBS and coated at 50µl per well, and incubated overnight at 2-8°C. Plates were washed 3 x with PBS/0.05% Tween (PBS-T) and the plate blocked with 5% chicken serum (VWR #S050H-1000) in PBS for 1 hour at room temperature. Recombinant human UNC5C (SEQ ID NO: 263) or lysozyme were diluted in PBS/5% chicken serum and 50µl added per well. Antigen was incubated for 1 hour at room temperature before washing 3 x in PBS-T. Captured UNC5C was detected with 50µl of 1:1000 dilution of goat polyclonal anti-UNC53 antibody (Biotechnne #AF1005) and incubated for 1 hour at room temperature. Plates were washed 3x with PBS-T then 50µl per well added of 1:10,000 diluted mouse anti-goat polyclonal horseradish peroxidase (HRP)-labelled secondary antibody (Sigma A9452), incubated for 1 hour at room temperature in the dark. Plates were washed twice with PBS before detection with TMB (Pierce 34021), stopped with 0.5M sulphuric acid. Absorbance at 450nm and 570nm were measured on a CLARIOstar Plus plate reader.

ELISA – Antigen down format for domain binding assessment

Nunc Maxisorp 96 well ELISA plates were coated with 3µg/ml of antigen (various truncated versions of UNC5C: full recombinant human UNC5C (SEQ ID NO: 263), recombinant human UNC5C with Ig1 domain only (SEQ ID NO: 270), recombinant human UNC5C with Ig1 and Ig2 domain (SEQ ID NO: 271), recombinant human UNC5C with Ig1, Ig2 and TSP-1 domain (SEQ ID NO: 272), and recombinant human UNC5C missing Ig1 domain only (SEQ ID NO: 273)). Antigen was diluted in PBS and coated at 50µl per well, and incubated overnight at 2-8°C. Plates were washed 3 x with PBS/0.05% Tween (PBS-T) and the plate blocked with 5% chicken serum (VWR #S050H-1000) in PBS for 1 hour at room temperature.

ATL6532, ATL6533, ATL6534, ATL6537 and ATL5338 (isotype control) were each made up in PBS at 10ug/ml to act as detection antibodies. 50µl antibody was added per well and incubated for 1 hour at room temperature before washing 3 x in PBS-T. Rabbit anti-human polyclonal HRP-labelled secondary antibody (Abcam #ab7160) was diluted 1:5000 in PBS and added 50µl per well for 1 hour at room temperature in the dark. Plates were washed twice with PBS before detection with TMB (Pierce #34021), stopped with 0.5M sulphuric acid. Absorbance at 450nm and 570nm were measured on a CLARIOstar Plus plate reader.

Mesoscale Discovery (MSD) Assay – ATL6532 capture with AF1005 detection

MSD Small Spot Streptavidin plates (MSD #L45SA-1) were blocked for 1 hour with 150µl per well of 5% chicken serum, and incubated at room temperature with shaking. Plates were then coated with 3µg/ml biotinylated (avi-tagged) ATL6532 antibody diluted in PBS, 25µl per well and incubated overnight at 2-8°C. Plates were washed 3 x with PBS-T. The antigens tested were: recombinant human UNC5C (SEQ ID NO: 263), recombinant mouse UNC5C (SEQ ID NO: 264), recombinant Cyno UNC5C (SEQ ID NO: 265), recombinant human UNC5A (SEQ ID NO: 266), recombinant human UNC5B (SEQ ID NO: 267), recombinant human UNC5D (SEQ ID NO: 268), recombinant human Netrin receptor DCC (SEQ ID NO: 269), SH-SY5Y lysate, SH-SY5Y media (SH-SY5Y cells express UNC5C and are used here to confirm the assay recognises endogenous UNC5C, not only recombinant; see “SH-SY5Y lysates for endogenous UNC5C Detection” below for preparation). Antigen was diluted in PBS and added 25µl per well. Plates were incubated for 2 hours at room temperature, with shaking. Plates were washed 3 x with TBS-T and goat polyclonal anti-UNC5C detection antibody (Biotechne #AF1005) added at 0.5µg/ml diluted in PBS, 25µl per well incubated at room temperature for 1 hour with shaking. Plates were washed 3 x with TBS-T. Donkey anti-goat polyclonal sulfo-tagged secondary antibody (MSD #R32AG-1) at 0.25µg/ml was added 25µl per well and incubated for 1 hour at room temperature with shaking. Plates were washed 3 x with TBS-T and 150µl of 2 x Read buffer T (MSD #R92TC-3; a Tris-based buffer containing tripropylamine (TPA) as a co-reactant for light generation) was added per well. Plates were read on an MSD QuickPlex SQ120MM. To test impact of ATLX1282 on assay performance, all steps were performed as described above, except that recombinant human UNC5C (and ATLX1282 (10µg/ml ATLX1282, 100µg/ml ATLX1282, or 100µg/ml ATL5338 (isotype control)) were combined just prior to antigen incubation.

MSD – performance in plasma, serum and artificial CSF

Plasma from 6 healthy individuals was obtained from NHS Blood and Transplant (NHS BT). An additional 5 healthy individual donor samples were pooled (Pool 1). A commercial plasma pool (pool 2) was obtained from CosmoBio (#KOJ-12250210) and a commercial serum pool from TebuBio (#088HSER-ABP100ml). Artificial cerebrospinal fluid (CSF) was purchased from Biotechne (#3525) and 2% FBS added before use. To test any impact of matrix interference on assay recovery, recombinant human UNC5C (SEQ ID NO: 263) was spiked into PBS and each of the above biological matrices and signal compared. For the 10% matrix experiments UNC5C was spiked at 10x the desired final concentration into PBS and each neat matrix, and then diluted 1 in 10. For the 50% plasma experiment, UNC5C was spiked at 2 x desired final concentration in PBS or neat plasma then diluted 1 in 2.

Antigens used in ELISA and MSD

Antigen name	Amino acid sequence	Use
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Recombinant human UNC5C	SEQ ID NO: 263	Antigen in ELISA and MSD
Recombinant mouse UNC5C	SEQ ID NO: 264	Antigen in ELISA and MSD
Recombinant Cyno UNC5C	SEQ ID NO: 265	Antigen in ELISA and MSD
Recombinant human UNC5A	SEQ ID NO: 266	Antigen in ELISA and MSD to prove specificity of assay for UNC5C.
Recombinant human UNC5B	SEQ ID NO: 267	Antigen in ELISA and MSD to prove specificity of assay for UNC5C.
Recombinant human UNC5D	SEQ ID NO: 268	Antigen in ELISA and MSD to prove specificity of assay for UNC5C.
Recombinant human Netrin receptor DCC	SEQ ID NO: 269	Antigen in ELISA and MSD to prove specificity of assay for UNC5C.
Recombinant human UNC5C with Ig1 domain only	SEQ ID NO: 270	Antigen in ELISA – to identify binding domain
Recombinant human UNC5C with Ig1 and Ig2 domain	SEQ ID NO: 271	Antigen in ELISA – to identify binding domain
Recombinant human UNC5C with Ig1, Ig2 and TSP-1 domain.	SEQ ID NO: 272	Antigen in ELISA – to identify binding domain
Recombinant human UNC5C missing Ig1 domain only	SEQ ID NO: 273	Antigen in ELISA – to identify binding domain

Table 5. Recombinant proteins used for ELISA and MSD experiments. All antigens above were manufactured in house.

SH-SY5Y lysates for endogenous UNC5C Detection

SH-SY5Y cells were cultured to 90% confluence in a T125cm flask. Media was removed and cells washed with ice cold PBS. 2.5ml of lysis buffer (50mM Tris-HCl (15568025, Invitrogen), 50mM NaCl (24740011, Invitrogen), 1% Triton-X (T9284, Sigma) with protease inhibitor (Abcam #ab65621) was added to the flask. Cells in lysis buffer were scraped and transferred into an Eppendorf tube. Lysate was sonicated on ice for 20 minutes and then left to stand until debris settled. Clear supernatant was removed into a fresh tube, aliquoted and frozen at -80°C.

MSD – Measurement of soluble UNC5C in healthy control, rheumatoid arthritis, ALS, RBD, PD and AD patient plasma samples

MSD Small Spot Streptavidin plates (MSD #L45SA-1) were blocked for 1 hour with 150ul per well of 5% chicken serum, room temperature incubation with shaking. Plates were then coated with 3ug/ml biotinylated (avi-tagged) ATL6532 diluted in PBS, 25ul per well and incubated for 2 hours at room temperature with shaking. Plates were washed 3 x with TBS-T. Plasma samples were diluted 1 in 2 or 1 in 4 in PBS/5% chicken serum and 25ul of diluted sample added per well. Spiked plasma QC controls with known concentrations of UNC5C and SH-SY5Y lysates as endogenous positive controls were included on each plate. Plates were incubated for 2 hours at room temperature, with shaking. Plates were washed 3 x with TBS-T and goat polyclonal anti-UNC5C detection antibody (Biotechne #AF1005) added at 0.5ug/ml diluted in PBS/5% chicken serum, 25ul per well and incubated at room temperature for 1 hour with shaking. Plates were washed 3 x with TBS-T. Anti-goat sulfo detection antibody (MSD #R32AG-1) at 0.25ug/ml in PBS/5% chicken serum was added 25ul per well and incubated for 1 hour at room temperature with shaking. Plates were washed 3 x with TBS-T and 150ul of 2 x Read buffer T (MSD #R92TC-3, a Tris-based buffer containing tripropylamine (TPA) as a co-reactant for light generation) was added per well. Plates were read on an MSD QuickPlex SQ120MM.

Total protein levels were measured by bicinchoninic acid (BCA) assay (ThermoFisher/Pierce #23225) as per kit instructions and soluble UNC5C levels were normalised to total protein levels.

MSD assay of samples from cynomolgus monkeys administered anti-UNC5C antibody ATLX1282

- 5 Cynomolgus monkeys were dosed once per week with 10, 30 or 100mg/kg ATLX1282, 2 animals per dose group. Following day 1 and day 22 dosing, serum samples were collected at 1, 6, 24, 48, 72 or 168 hours post dose. Blood was collected into a serum separator tube, inverted several times to mix and allowed to clot at room temperature for at least 30 minutes. Samples were then centrifuged, and serum collected. Serum was frozen at -80°C prior to analysis. The MSD assay was performed as described above for measurement of human samples except that QC samples were made in corresponding appropriate matrix (cynomolgus serum).

MSD assay of samples from rNLS mice administered anti-UNC5C antibody ATLX1282

- 15 NEFH-iTA × hTDP-43ΔNLS bigenic mice (rNLS mice) were treated with 60mg/kg isotype control antibody or 6, 20 or 60mg/kg of ATLX1282. Mice were dosed twice a week from age 5 weeks until age 12 weeks. At 12 weeks of age terminal bleeds were collected into K2EDTA tubes and processed to plasma by centrifugation for 15 minutes at 3,000g at 5°C in a refrigerated centrifuge. Plasma was stored at -80°C prior to analysis. The MSD assay was performed as described above for measurement of human samples except that QC samples were made in corresponding appropriate matrix (mouse plasma). Significance by Kruskal-Wallis test (non-normal distribution).

EXAMPLE 1 – Convergence analysis

- Convergent sequence clusters derived from the antibody repertoire of resilient groups of individuals can be used to identify disease-specific antibody sequences. The present inventors sought to identify candidate protective antibodies from Frontotemporal dementia (FTD)-resilient individuals from a cohort of FTD patients (Genetic FTD Initiative (GENFI), www.genfi.org/). FTD encompasses several types of dementia impacting frontal and temporal lobes of the brain, and major genetic risk factors are GRN and C9orf72 mutations.

- 30 Resilience to FTD was defined as individuals not developing disease despite being within the top 33% of the cumulative age of onset of frontotemporal dementia and the presence of GRN or C9orf72 mutations. From the total cohort of over 1000 individuals, the 22 most highly resilient individuals were selected. Sequencing of the antibody repertoires of these resilient patients revealed a convergent heavy chain variable (VH) sequence among three highly resilient individuals (See Figure 1 for workflow used to identify convergent VH sequences from the FTD dataset). The probability of all three individuals producing this antibody by chance is extremely low. It is likely that this particular sequence was highly selected for and thus advantageous to the individual.

- 40 The VH sequence was subsequently paired with a VL using a transformer-based model comprising an encoder-decoder model trained on a corpus of paired VH-VL sequences. Further details on how such a

model may be trained and used is provided in WO2022/223451. The trained model takes a VH sequence as input and generates a single complementary VL sequence as output.

5 The resulting antibody, ATL_5262 (ATL_0005262) was included in a proteomics array for target deconvolution, identifying the Netrin receptor UNC5C as its target (data not shown). Binding of ATL_5262 to UNC5C was confirmed by ELISA (**Figure 2**).

10 Interestingly, UNC5C antibodies were subsequently identified across repertoires derived from subjects who are resilient to neurodegeneration (**Figure 3**). In particular, the heavy chain sequence of ATL_5262 was used as a probe to search similar sequences in an unpublished repertoire database derived from neurodegeneration. Sequences with high homology were found in a resilient Alzheimer's disease subject (individual showing slow cognitive progression); a subject with resilience for Parkinson's disease (prodromal REM sleep behaviour disorder (RBD) but no Parkinson's diagnosis); three subjects with resilience for FTD (carriers of high-risk mutations and older age but no progression to FTD); and resilient
15 centenarians with no symptoms of neurodegeneration (**Figure 3**). Sequences were also found in cognitively healthy controls – predominantly individuals over 60 years of age. Notably there is an absence of UNC5C antibodies in so-called "Progressors" from these cohorts, with exception to SU_0001278 who has a diagnosis of Parkinson's disease and Mild Cognitive Impairment. PD subject SU_0001278 is 'Unclassified' since they were initially included in the cohort as a healthy control, but subsequently declassified since they
20 have suspected REM sleep behaviour disorder. Since they have no PD diagnosis they are 'potentially resilient'. All the sequences identified, referred to herein as "ATL_5262 homologues", have the same V and J gene as ATL5262 VH and at most 2AA mismatches across the junction region. **Figure 7** shows a VH sequence alignment of representative ATL_5262 homologous sequences ATL_6187 and ATL_6191 (SEQ ID NO: 112-113). The representative VH sequences of the ATL_5262 homologues were subsequently
25 paired with the VL of ATL_5262 (SEQ ID NO: 9) and produced as IgG1 Fc NULL antibodies. The VH of these antibodies had at least 80% sequence identity with the sequence of the VH of ATL_5262 (in particular: 6187: 84.87%, 6191:89.92%- for reference the 5262 derivative antibody 6178 has 94.96% VH sequence identity with the VH of ATL_5262, all determined using Clustal). Antibodies 6187 and 6191 both had EC50 for binding to EC50 below 10^{-7} (determined by ELISA). These antibodies have 0 or 1 mutation in the VH
30 CDR3 compared to ATL_5262 (at most 1 mutation at position 117 in IMGT numbering, Y117S) and up to 3 mutations in each of the VH CDR2 and CDR1 compared to ATL_5262. Both ATL_6178 and ATL_6191 had a mutated variable region and IGHG1 constant region, which indicates that class-switch recombination and affinity maturation through somatic hypermutation have occurred, and thus that most likely there has been a germinal centre-induced immune response to antigen (see **Table 6**). These highly mutated class
35 switched sequences are likely from memory B cells, which are typically more specific to the antigen than a naïve B cell.

The fact that UNC5C antibodies could be found in resilient individuals in these cohorts highlights the relevance of UNC5C as a therapeutic target in various neurodegenerative conditions.

40

The identified homologues were subsequently tested for binding to UNC5C by ELISA and the results are shown in **Table 9**.

Antibody ID	Subject	Disease status	Sex (age)	Sequence features	AA mismatches (vs ATL5262 full VH)
ATL6187(*))	SU_0000448	Cognitively healthy control	Female (62)	Highly mutated and IGHG1-class switched	18
ATL6191 (*)	SU_0001278	PD-unclassified	Male (63)	Mutated and IGHG1-class switched	12

Table 6. Characteristics of ATL_5262 homologues. Highly mutated: >20 mutations (indicative of a response to antigen exposure). (*) indicate antibodies with an ELISA binding profile similar to ATL5262.

EXAMPLE 2 – Competition with Netrin 1

To determine whether ATL_5262 competes for the same binding site as the UNC5C ligand, Netrin 1, a competition assay was performed.

Briefly, ELISA plates were coated with 45nM UNC5C and pre-incubated with 3µM Netrin 1 for 30 minutes prior to adding 27nM ATL_5262, or a negative control anti-fluorescein antibody labelled “ATL_5338”. **Figure 4A** shows that Netrin 1 could be detected in wells containing UNC5C alone, UNC5C and ATL5262, and UNC5C and ATL_5338, indicating that Netrin 1 inhibits binding of ATL_5262 to UNC5C. **Figure 4B** shows that ATL_5262 could only be detected in wells not preincubated with Netrin 1, confirming that ATL_5262 binds to UNC5C and this binding is inhibited by Netrin 1. **Figure 4B** further shows that the control antibody ATL_5338 does not bind to UNC5C.

To determine whether Netrin 1 inhibits ATL_5262 binding to UNC5C in a concentration-dependent manner, a 12-point dilution series of Netrin 1, starting at 3µM, was performed and binding of ATL_5262 to UNC5C was assessed using ELISA (**Figure 5**). **Figure 5** shows that Netrin 1 inhibits binding of ATL_5262 to UNC5C in a concentration-dependent manner.

To test whether Netrin 1 is able to compete with ATL_5262 already bound to UNC5C, ELISA plates were coated with 45nM UNC5C and preincubated with a 12-point dilution series of ATL_5262, starting at 666nM for 30 minutes prior to adding 75nM Netrin 1 (**Figure 6**). **Figure 6** shows that Netrin 1 is able to outcompete ATL_5262 binding as indicated by the “ATL_0005262 +Netrin 1 curve” being shifted to the right compared with “ATL_0005262 alone”, i.e. only at high concentrations of ATL_5262, can ATL_5262 partly outcompete Netrin 1. Similarly, only at the highest concentration of ATL_5262 was a small decrease in Netrin signal detected. As expected, no binding signal was observed for wells incubated with UNC5C and control antibody ATL_5338.

Together, these data show that Netrin 1 inhibits binding of ATL_5262 to UNC5C in a concentration-dependent manner, indicating that ATL_5262 competes for the same binding site in UNC5C as Netrin 1. ATL_5262 may thus act as a Netrin 1 mimic. To the best of the inventors’ knowledge, the antibodies described herein are the first antibodies that bind UNC5C and compete with binding of netrin 1.

EXAMPLE 3 – Antibody variants

To identify lead antibodies with optimal development characteristics, variants of the VH (SEQ ID NO: 1) and VL sequence (SEQ ID NO: 9) of ATL_5262 were generated and their binding potency to UNC5C was tested using direct ELISA for binding to human UNC5C and mouse UNC5C.

In order to generate VH variants, the ATL_5262 sequence was compared with the VH germline sequence and found to have five amino acid substitutions compared with the germline sequence. Reverting sequences to germline typically reduces immunogenicity and improves antibody expression and stability. Therefore, one VH variant tested was the germline reversed VH framework sequence, ATL_6033 (SEQ ID NO: 2). To identify if any specific reversion to germline was detrimental to antibody expression or binding activity partially germline reversed VH framework sequences were generated with 4 out of 5 germline mutations - that is all germline reversions minus one position remaining identical to the VH of ATL_5262 (ATL_6034 (SEQ ID NO:3); ATL_6035 (SEQ ID NO: 4); ATL_6036 (SEQ ID NO: 5); ATL_6037 (SEQ ID NO: 6); ATL_6038 (SEQ ID NO: 7). In addition, a VH sequence of ATL_5262 with liability removal mutation T82K (using IMGT numbering) (ATL_6039) (SEQ ID NO: 8) was also generated, (see Table 7). All antibodies were expressed using the ATL_5262 VL chain (SEQ ID NO: 9).

All antibodies tested were IgG1 variants L234A/L235A (LALA), i.e. IgG1 variants comprising L234A/L235A substitutions reducing binding to the IgG Fc receptors FcγRI, FcγRII and FcγRIII as well as to complement component C1q, reducing Fc-mediated toxicity (Lund J, et al. 1991).

Antibodies (AA seq numbering)	EC50 (M)	IGMT numbering
ATL_0006033 (full germline)	4.77E-08	
ATL_0006034 (germline - T35)	9.81E-08	germline – T40
ATL_0006035 (germline - R44)	9.74E-08	germline – R49
ATL_0006036 (germline - Q59)	5.38E-09	germline – Q67
ATL_0006037 (germline - I71)	5.51E-08	germline – I80
ATL_0006038 (germline - H77)	4.82E-08	germline – H86
ATL_0006039 (T73K)	1.09E-09	T82K
ATL_0005262	3.48E-09	

Table 7. EC50 values for VH variant antibodies tested in ELISA.

Reversing the entire ATL_5262 VH sequence to germline increases the EC50, and therefore reduces potency, over 10-fold compared with ATL_5262 (Table 7 and Figure 8). All variants with germline reversion Q67Y (IMGT) (ATL_6033; 6034; 6035; 6037; and 6038) show reduced binding activity in ELISA (Figure 8), reflected in an increased EC50 value. It is therefore desirable to keep glutamine (Q) at IMGT position 67 so as to maintain the degree of UNC5C binding observed for ATL_5262. The inventors further observed that mutating IMGT position 82 of the VH to a lysine (K) in order to reduce the risk of aspartate (T) isomerisation, enhances binding of the resulting antibody (ATL_6039) to UNC5C about 3-fold compared

with ATL_5262 (Figure 8). These data therefore indicate that IMGT residues 67 and 82 are important for UNC5C binding. These mutations were subsequently combined in the VHs of ATL_6177 (SEQ ID NO: 91) and ATL_6178 (SEQ ID NO: 93), with both of these mutating IMGT position 82 (T82K) and retaining Q67. In addition, ATL_6178 (SEQ ID NO: 93) also removes an additional potential sequence liability by mutating Asparagine (N) at position 58 IMGT, which is part of a predicted Asparagine deamidation site in CDR2 (NS motif), to Tyrosine (Y) (N58Y). By combining beneficial mutations, ATL_6177 and ATL_6178 are expected to have improved stability and binding potency compared with other ATL_5262 variants.

The tested VH variants show similar binding profiles for human (Figure 9A) and murine UNC5C (Figure 9B), whilst showing minimal binding to lysozyme at the highest concentration of antibody used (Figure 9C).

UNC5C exists in monomeric form in the absence of its ligand netrin 1, and in dimeric form when it binds to netrin-1. To test whether ATL_5262 binds both monomeric and dimeric UNC5C, an ELISA was performed using Fc-tagged dimeric rhUNC5C and His-tagged monomeric rhUNC5C. Similar binding profiles were observed for binding to the dimeric Fc-tagged rhUNC5 (Figure 10A) and monomeric his-tagged rhUNC5C (Figure 10B), and this was reflected in similar EC50 values (Table 8) indicating that ATL_5262 is capable of binding to both monomeric and dimeric UNC5C.

EC50 (M)	IGMT numbering	rhUNC5C_Fc (n=2)	rhUNC5C_His (n=1)
ATL_0006033 (full germline)		1.90E-07	2.65E-07
ATL_0006034 (germline - T35)	germline – T40	2.10E-07	5.08E-07
ATL_0006035 (germline - R44)	germline – R49	1.28E-07	2.12E-07
ATL_0006036 (germline - Q59)	germline – Q67	1.16E-08	3.70E-08
ATL_0006037 (germline - I71)	germline – I80	8.71E-08	1.48E-07
ATL_0006038 (germline - H77)	germline – H86	1.04E-07	3.02E-07
ATL_0006039 (T73K)	T82K	1.90E-09	9.78E-09
ATL_0005262 parental		5.84E-09	1.40E-08
ATL_0006002-001 (VL variant)		8.178E-09	2.126E-08

Table 8. EC50 values for VH variant antibodies tested in ELISA using Fc-tagged or His-tagged UNC5C.

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Antibody variants of ATL_5262 with different light chains were also generated and tested.

Further candidate VLs were obtained by a two-step process. First, 600 different VLs were computationally synthesised for the VH of ATL_5262 using a modified version of the model described in WO2022/223451, where encoder and decoder models were replaced with a pretrained AntiBERTa model as described in Leem at al., 2022. As a second step, the 600 different VH-VL pairs were prioritised using a separate AntiBERTa model, described in GB 2215218.5 filed 14 October 2022. In brief, the model from GB2215218.5

25

takes a VH-VL pair as input (natural or synthetic) and computes the probability that the two chains would form a pairing.

10 VLs were selected for synthesis based on the predicted probability from the model (selecting candidate
5 VLs with high predicted pairing probability with the VH of ATL_5262), low sequence liability content, and
sequence diversity. VL variants tested included the VL of ATL_5996 (SEQ ID NO: 10); ATL_5997 (SEQ ID
NO: 11); ATL_5998 (SEQ ID NO: 12); ATL_5999 (SEQ ID NO: 13); ATL_6000 (SEQ ID NO: 14); ATL_6001
(SEQ ID NO: 15); ATL_6002 (SEQ ID NO: 16); ATL_6003 (SEQ ID NO:17); ATL_6004 (SEQ ID NO: 18);
ATL_6005 (SEQ ID NO:19). All of the above VL variants antibodies include the VH of ATL_5262.

10 The different light chain variants were screened for binding to rhUNC5C and rmUNC5C by ELISA and
compared with ATL_5262. The results show ATL_6002 (same VH as ATL_0005262 but different VL)
binding to rhUNC5C in a very similar way to ATL_0005252 (**Figure 11A**). Similar results were also observed
for binding to rmUNC5C (**Figure 11B**). Variant ATL_6003 demonstrated reduced binding (approximately
15 10-fold) to both rhUNC5C and rmUNC5C.

This data demonstrates that other VL pairings, for example using the VL of ATL_6002, are possible that
maintain the functionality of the VH described herein. The data further demonstrates that the initial VH-VL
pairing obtained (resulting in ATL_5262) was already very good.

20 To measure the binding potency of antibodies to UNC5C protein, antibodies were assessed in a direct
ELISA format for binding to human UNC5C. In addition, a selection of these antibodies was also screened
for binding to mouse, cyno, and rat UNC5C in a direct ELISA format. Lysozyme was run as a control antigen
and showed no discernible binding to any of the antibodies assessed at a concentration below 1µM. The
25 anti-fluorescein antibody ATL-5338 was used as a negative isotype control and showed no binding to any
of the UNC5C antigens assessed to antibody concentrations <1µM. In addition, some of the antibodies
were assessed in an additional assay to assess their ability to compete with Netrin 1 for binding to UNC5C.
The results of these tests are shown in **Table 9**.

	Antibody	Human UNC5C EC50 (M) UNC5C (R&D Systems; #1005-UN)	Mouse UNC5C EC50 (M) (inhouse antigen)	Cyno UNC5C EC50 (M) (inhouse antigen)	Rat UNC5C ELISA EC50 (inhouse antigen)	Netrin 1 induced shift in binding curve
Alternative light chain	ATL_0005996	>0.000001	>0.000001	n/a	n/a	n/a
	ATL_0005997	>0.000001	>0.000001	n/a	n/a	n/a
	ATL_0005998	Binding at 0.000001	Binding at 0.00000107 but	n/a	n/a	n/a

		07 but top of curve not reached	top of curve not reached			
	ATL_0005999	>0.000001	>0.000001	n/a	n/a	n/a
	ATL_0006000	>0.000001	>0.000001	n/a	n/a	n/a
	ATL_0006001	Binding at 0.00000107 but top of curve not reached	Binding at 0.00000107 but top of curve not reached	n/a	n/a	n/a
	ATL_0006002	2.45E-08	6.537E-08	n/a	n/a	Yes
	ATL_0006003	9.5e-8	3.072E-07	n/a	n/a	n/a
	ATL_0006004	>0.000001	>0.000001	n/a	n/a	n/a
	ATL_0006005	>0.000001	>0.000001	n/a	n/a	n/a
Germline and development	ATL_0006033	4.77E-08	1.26E-07	n/a	n/a	n/a
	ATL_0006034	9.81E-08	1.94E-07	n/a	n/a	n/a
	ATL_0006035	9.74E-08	1.24E-07	n/a	n/a	n/a
	ATL_0006036	5.38E-09	1.74E-08	n/a	n/a	Yes
	ATL_0006037	5.51E-08	7.99E-08	n/a	n/a	n/a
	ATL_0006038	4.82E-08	1.17E-07	n/a	n/a	n/a
	ATL_0006039	1.09E-09	2.55E-09	Binding at 0.00000000411523 but bottom of curve not reached	Binding at 0.00000000411523 but bottom of curve not reached	Yes
Combo	ATL_0006177	1.36E-08		n/a		Yes
	ATL_0006178	9.62E-09	5.956E-09	1.578E-08	7.218E-09	Yes
5262 homologues	ATL_0006187	2.24E-08	n/a	n/a	n/a	n/a
	ATL_0006191	4.52E-09	Binding at 0.00000000411523 but bottom of curve not reached	Binding at 0.00000000411523 but bottom of curve not reached	Binding at 0.00000000411523 but bottom of curve not reached	n/a

Table 9. Binding potency of listed antibodies to human, mouse, and cyno UNC5C and ability to compete with netrin-1.

ATL_0005262, ATL_0005998, ATL_0006001, ATL_0006002, ATL_0006003, ATL_0006033,
5 ATL_0006034, ATL_0006035, ATL_0006036, ATL_0006037, ATL_0006038, ATL_0006039,
ATL_0006177, ATL_0006178, ATL_0006187, ATL_0006191 all demonstrated binding to human
UNC5C with various potencies (see Table 9). For ATL_0006001 and ATL_6039, a signal at least 3x the
level of lysosome control was observed, but a full dose response curve could not be obtained and so an
EC50 cannot be reported. In both cases the data therefore shows that the antibodies do bind the target. In
10 the case of ATL_6039 an EC50 could not be obtained because the antibody was very potent and a lower
concentration range would need to be tested to get an EC50. This data means that antibody certainly binds,
just cannot report potency number.

ATL_0005262, ATL_0005998, ATL_0006001, ATL_0006002, ATL_0006003, ATL_0006178,
15 ATL_0006191 showed binding to mouse UNC5C. ATL_0005262, ATL_0006178 and ATL_0006191 were
demonstrated binding to cyno UNC5C. ATL_0005262, ATL_0006178 and ATL_0006191 were tested and
demonstrated binding to rat UNC5C. In addition, Netrin 1 competed with ATL_0005262, ATL_0006002,
ATL_0006036, ATL_0006039, ATL_0006177 and ATL_0006178 for binding to human UNC5C.

20 These data demonstrate that at least ATL_0006178 (also referred to herein as ATLX-1282) exhibits binding
to human, mouse, rat, and cyno UNC5C, and is capable of competing with netrin-1 for binding to UNC5C.

EXAMPLE 4 – Binding to cellular UNC5C

To assess the ability of ATL_6178 (also referred to herein as ATLX-1282) and ATL5262 to bind to UNC5C
25 in its natural central nervous system (CNS) context, antibody binding to frozen mouse brain sections was
assessed.

ATLX-1282 (ATL_6178) and ATL_5262 demonstrated a similar binding pattern, and this was blocked by
10 nM Netrin-1, but not by similar sized proteins such as clusterin and Netrin-4 (which do not bind to
30 UNC5C), as shown in Figures 13A-D. This is in keeping with the *in vitro* data described above showing
the ability of ATLX-1282 and ATL_5262 to bind to UNC5C selectively.

To verify binding of antibodies to UNC5C expressed on the cell surface, antibodies were assessed in a flow
cytometry assay. The canonical human UNC5C isoform (O95185) and UNC5C T835M variant linked to AD
35 (VAR_081368) were constitutively overexpressed in a HEK-293 cell line using a lentiviral system.
Antibodies were then tested for binding using flow cytometry. The cell line transduced with the empty vector
(EV) and an antibody specific for an unrelated target expressed in relevant IgG format (control ATL) were
used as negative controls.

ATL5262 and ATLX-1282 showed increased binding to HEK-293 cells overexpressing UNC5C and HEK-293 cells overexpressing UNC5C T835M relative to cells transfected with an empty vector control (Figures 14A and B).

5 These data demonstrate that ATL5262 and ATLX-1282 can bind to UNC5C and the UNC5C T835M variant linked to AD (VAR_081368) when they are in a natural cell membrane context. This data shows that the antibodies bind to the Alzheimer's disease associated form of UNC5C. The antibodies described herein were shown to bind to the extracellular region of UNC5C and this mutation affects the intracellular region. Therefore, all antibodies described are expected to bind to the AD relevant form of UNC5C.

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EXAMPLE 5 – Determination of crystal structure of ATL 5262 Fab fragment bound to human UNC5C Ig domains

To better understand the binding of ATL_5262 to UNC5C, an X-ray crystal structure was obtained for a complex containing a Fab fragment of ATL_5262 bound to the immunoglobulin (Ig) domains of human
15 UNC5C. A cartoon view of the full Fab-UNC5C complex is shown in **Figure 15A**, and close-up views of the specific interactions between the proteins in **Figures 15B-E**. A summary of the inter-chain contacts is set out in **Table 10** below, with details of the specific amino acid interactions in **Table 11**.

Inter-chain interaction	Buried surface area, Å ²	H-Bonds	Salt Bridges
VH:UNC5C	397.9	5	2
VL:UNC5C	238.5	4	0
VH:VL	1816.9	17	2

Table 10. ATL_5262 Fab:UNC5C interchain contacts summary

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VH	UNC5C	VL
Thr64	Thr89	
Gly63	Gln90	
Thr65	Gln102	
Ser74	Lys103	
Asn66	Val107	Thr114
	Asp108	Thr114
Tyr55, Asn66, Arg108, Met110	Glu109	Ser109, T114
	Arg110	Tyr108, Ser109
	Val111	Tyr38, Ser107, Tyr108
Arg108, Met110	Ile118	
Tyr55, Thr64, Thr65	Arg120	

Table 11. Summary of inter-chain interactions within 4.0 Å, including polar contacts and hydrophobic interactions.

Figure 15F shows a protein alignment of human UNC5 family members B, A and D to region of human UNC5C Ig-like domain 1 that interacts with ATL_5262. Identical sequences highlighted in red. Amino acids

of UNC5C involved in the interaction to ATL_5262 and summarised in **Table 11** are indicated by arrows. These data indicate that specificity of ATL_5262 to UNC5C over the other UNC5 family members may be driven by amino acids that are different between UNC5C and the other UNC5 proteins.

5 **EXAMPLE 6 – further antibodies**

Phage display selections were carried out to discover additional functional and resilient patient-derived antibodies for UNC5C. Phage display libraries were generated from VH repertoires of patients who carried the original antibody ATL_0005262, and a VL repertoire from healthy donors. Phage display selections were done by panning on two UNC5C antigens. A subset of enriched antibodies was screened by phage
10 ELISA on UNC5C antigen and 10 antibodies were chosen for IgG conversion.

Ten phage-derived IgGs (ATL_6530 to ATL_6539) were screened for binding to UNC5C by ELISA and the EC50 values estimated from UNC5C binding curves are listed in **Table 12** below. All antibodies showed binding to UNC5C with high affinity. ATL_6178 was also included for reference. **Figure 16** shows an alignment of the VH and VL sequences of these antibodies.

Antibody Batch	Assay Type	Assay Orientation	EC50 (M)
Y_0001389-001 (ATL_6530)	ELISA	Antigen Down	3.90E-11
Y_0001390-001 (ATL_6531)	ELISA	Antigen Down	1.30E-11
Y_0001391-001 (ATL_6532)	ELISA	Antigen Down	2.80E-12
Y_0001392-001 (ATL_6533)	ELISA	Antigen Down	9.20E-12
Y_0001393-001 (ATL_6534)	ELISA	Antigen Down	1.00E-11
Y_0001394-001 (ATL_6535)	ELISA	Antigen Down	2.40E-08
Y_0001395-001 (ATL_6536)	ELISA	Antigen Down	1.10E-09
Y_0001396-001 (ATL_6537)	ELISA	Antigen Down	7.10E-10
Y_0001397-001 (ATL_6538)	ELISA	Antigen Down	5.50E-10
Y_0001398-001 (ATL_6539)	ELISA	Antigen Down	1.10E-08
Y_0001282-003 (ATL_6178)	ELISA	Antigen Down	8.00E-09

15 **Table 12.** EC50 values from UNC5C binding curves. Antigen= UNC5C (R&D Systems; #1005-UN) for all data in this table.

Next, these antibodies were screened to determine if they were able to compete for binding to UNC5C with recombinant Netrin 1. UNC5C was coated down on ELISA plates and a titration of each antibody individually was pre-incubated with UNC5C protein prior to the addition of netrin 1 at a set concentration. In each case
20 the binding of each antibody and netrin 1 was determined using secondary detection antibodies specific for each reagent. If the presence of netrin 1 was able to shift the antibody binding curve to the right (i.e. require a higher concentration of antibody to see a similar level of signal for binding to UNC5C), this was observed as competition in the assay. Furthermore, if presence of antibody was able to reduce the signal of Netrin1 binding detected in the assay, this was also observed as competition (see **Table 13**). All antibodies
25 displayed competition with Netrin-1.

	Antibody (Antigen Binding)	Ab Top Concentration (M)	Ab Bottom Conc (M)	Competitor Conc (M)
1	Y_0001389-001 (ATL_6530)	1e-6	1e-13	7.5e-8
2	Y_0001390-001 (ATL_6531)	1e-6	1e-13	7.5e-8

3	Y_0001391-001 (ATL_6532)	1e-6	1e-13	7.5e-8
4	Y_0001392-001 (ATL_6533)	1e-6	1e-13	7.5e-8
5	Y_0001393-001 (ATL_6534)	1e-6	1e-13	7.5e-8
6	Y_0001394-001 (ATL_6535)	1e-6	1e-13	7.5e-8
7	Y_0001395-001 (ATL_6536)	1e-6	1e-13	7.5e-8
8	Y_0001396-001 (ATL_6537)	1e-6	1e-13	7.5e-8
9	Y_0001397-001 (ATL_6538)	1e-6	1e-13	7.5e-8
10	Y_0001398-001 (ATL_6539)	1e-6	1e-13	7.5e-8
11	Y_0001282-003 (ATL_6178)	1e-6	1e-13	7.5e-8

Table 13. Competition of antibodies with Netrin 1 binding. Ab=antibody, Conc=concentration. Antigen= UNC5C (R&D Systems; #1005-UN); Competitor (Antigen)= Netrin 1 (R&D Systems; #6419-N1) for all data in this table.

	Antibody (Antigen Binding)	Hit (Competition Observed)	Notes
1	Y_0001389-001 (ATL_6530)	Yes	shift in bind curve profile vs what is seen in absence of Netrin 1
2	Y_0001390-001 (ATL_6531)	Yes	shift in bind curve profile vs what is seen in absence of Netrin 1
3	Y_0001391-001 (ATL_6532)	Yes	No shift in bind curve profile vs what is seen in absence of Netrin 1; Netrin 1 binding signal is reduced, suggesting ATL6532 is particularly potent and out competes Netrin 1 for binding in this assay setup
4	Y_0001392-001 (ATL_6533)	Yes	shift in bind curve profile vs what is seen in absence of Netrin 1
5	Y_0001393-001 (ATL_6534)	Yes	shift in bind curve profile vs what is seen in absence of Netrin 1
6	Y_0001394-001 (ATL_6535)	Yes	Shift in bind curve profile vs what is seen in absence of Netrin 1
7	Y_0001395-001 (ATL_6536)	Yes	Shift in bind curve profile vs what is seen in absence of Netrin 1
8	Y_0001396-001 (ATL_6537)	Yes	shift in bind curve profile vs what is seen in absence of Netrin 1
9	Y_0001397-001 (ATL_6538)	Yes	shift in bind curve profile vs what is seen in absence of Netrin 1
10	Y_0001398-001 (ATL_6539)	Yes	Shift in bind curve profile vs what is seen in absence of Netrin 1
11	Y_0001282-003 (ATL_6178)	Yes	Shift in bind curve profile vs what is seen in absence of Netrin 1

5 **Table 13 (continued).** Competition of antibodies with Netrin 1 binding.

This example thus identifies further antibodies that bind UNC5C with high affinity and are able to compete with Netrin-1 for UNC5C binding and are therefore expected to have similar properties to ATL_5262 and ATLX-1282.

EXAMPLE 7 – ELISA capture and detection of UNC5C

Engagement of UNC5C may lead to changes in circulating levels of soluble UNC5C. The present inventors postulated that measuring these levels in a biological sample obtained from a patient may therefore have utility as a biomarker of target engagement for UNC5C binding therapeutic antibodies, and sought to develop an immunoassay using the high affinity antibodies against UNC5C generated via phage display (see Example 6 above).

The inventors tested the performance of two of the phage display-derived antibodies (ATL6532 and ATL6534) in capturing recombinant human UNC5C with a C-terminal His tag using an ELISA experiment with detection by anti-His HRP. ATL5338 was used as isotype control. The results demonstrate that both ATL6532 and ATL6534 can be used to capture His-tagged recombinant UNC5C in this assay, and that capture of His-tagged recombinant UNC5C by ATL6532 results in a much higher assay signal than capture by ATL6534 (**Figure 17A**). Increasing coating concentration of ATL6534 results in an acceptable assay signal (**Figure 17B**). Together, these results indicate that both ATL6532 and ATL6534 can act as capture antibodies for UNC5C, and that ATL6532 is superior to ATL6534 in terms of sensitivity.

The inventors also used the same experimental approach to compare the capture of recombinant human UNC5C with a C-terminal His tag by ATL6532 and ATL6533. ATL5338 was used as isotype control, and a His-tagged irrelevant antigen was used as a negative control antigen. The results demonstrate that capture of His-tagged UNC5C by ATL6532 and ATL6533 and detection with an anti-His HRP results in a good titratable signal for both antibodies, with ATL6532 providing greater sensitivity than ATL6533 (**Figure 18A**). There was no signal from the irrelevant His tagged antigen, demonstrating the specificity of ATL6532 and ATL6533 for UNC5C (**Figure 18B**).

EXAMPLE 8 – ELISA – Sandwich immunoassay format with AF1005 detection

The inventors further tested the performance of four of the phage display-derived antibodies (ATL6532, ATL6533, ATL6534, and ATL6537) as the capture antibody in a sandwich immunoassay using goat polyclonal anti-UNC5C antibody (Biotechne #AF1005) as the detection antibody and mouse anti-goat horseradish peroxidase (HRP) antibody (Sigma A9452) as the secondary antibody. ATL5338 was used as an isotype control. Lysozyme was used as a negative control antigen.

As shown in Table 14 below, in this immunoassay format only ATL6532 and ATL6533 capture resulted in a signal above blank (mean of blank + 3 SD), with ATL6532 capture resulting in the higher signal. ATL6532 and ATL6533 were taken forward to assess performance with a wider range of UNC5C concentrations. The results from the assessment with a wider range of concentration shows that titratable signal is observed with both ATL6532 and ATL6533 capture antibodies, with greater signal and sensitivity observed with ATL6532 capture (**Figure 19A**). There was no signal from the lysozyme antigen control, again demonstrating the specificity of ATL6532 and ATL6533 for UNC5C (**Figure 19B**).

Absorbance 450nm - 570nm - duplicate wells.

	ATL_5338		ATL_6532		ATL_6533		ATL_6534		ATL_6537	
10ng/ml UNC5C	0.376	0.366	1.117	1.308	0.661	0.72	0.582	0.602	0.431	0.397
0ng/ml UNC5C	0.42	0.447	0.449	0.488	0.501	0.487	0.508	0.539	0.468	0.419

Table 14. Absorbance measurements from Sandwich format immunoassay with AF1005 detection.

EXAMPLE 9 – ELISA – Antigen down format for domain binding assessment

To determine which domain of UNC5C the tested phage display-derived antibodies bound to, the inventors used an antigen down format ELISA experiment. In this experiment ELISA plates were coated with various truncated versions of UNC5C: full recombinant human UNC5C ("Full Human ECD"; SEQ ID NO: 263), recombinant human UNC5C with Ig1 domain only ("No Ig2, TSP1 or TSP2"; SEQ ID NO: 270), recombinant human UNC5C with Ig1 and Ig2 domain ("No TSP1 or TSP2; SEQ ID NO: 271), recombinant human UNC5C with Ig1, Ig2 and TSP-1 domain ("No TSP2"; SEQ ID NO: 272), and recombinant human UNC5C missing Ig1 domain only ("No Ig1"; SEQ ID NO: 273). ATL6532, ATL6533, ATL6534, and ATL6537 were used as detection antibodies.

The results in **Figure 20** show that ATL6532, ATL6533 and ATL6534 all lose binding to UNC5C when only the Ig1 domain is present, proving that these antibodies bind elsewhere on the molecule (i.e. these antibodies bind the Ig2, TSP1, and/or TSP2 domains of UNC5C). ATL6537 however shows strongest binding to the Ig1 domain alone and no binding when the Ig1 domain is not present, demonstrating that its binding site is within the Ig1 domain.

ATL6532 is not impacted by loss of Ig1, TSP1 or TSP2 domains, suggesting it binds the Ig2 domain. ATL6533 loses all binding when both TSP domain are missing and shows reduced binding when only TSP2 is missing, suggesting it binds somewhere within the TSP domains and possibly needs both TSP domains for full binding. ATL6534 on this occasion showed poor binding even to full length extracellular domain so the binding location is not determinable from these results.

As Netrin and some therapeutic antibodies (e.g. ATLX1282) also bind within the Ig1 domain, antibodies which bind outside of the Ig1 domain (i.e. antibodies which bind the Ig2, TSP1, and/or TSP2 domains of UNC5C) are less likely to compete for the binding. Therefore antibodies which bind outside of the Ig1 domain are most suitable for an UNC5C biomarker assay to determine the effect of a therapeutic anti-UNC5C antibody which binds the Ig1 domain of UNC5C on the level of soluble UNC5C in a subject.

Therefore, these data indicate that ATL6532 and ATL6533 have the best domain-binding characteristics of the tested antibodies for use in a biomarker assay and of these two antibodies, ATL6532 has the best results across a range of ELISA formats (Examples 7-9).

EXAMPLE 10 – Mesoscale Discovery (MSD) assay to detect soluble UNC5C in biological fluid samples

Based on the results from Examples 7-9, the ATL6532 antibody was taken forward for testing as a capture antibody in MSD format (Mesoscale Discovery) using goat polyclonal anti-UNC5C antibody (Biotechnique #AF1005) as the detection antibody, and anti-goat sulfo antibody (MSD #R32AG-1) as the secondary antibody.

To test the potential utility of the MSD assay across multiple species, the inventors compared detection of recombinant human UNC5C (SEQ ID NO: 263), recombinant mouse UNC5C (SEQ ID NO: 264), and recombinant Cyno UNC5C (SEQ ID NO: 265). The results showed that the MSD assay format recognises mouse and cyno UNC5C to the same degree as human UNC5C, demonstrating that the assay is suitable for use in multiple species (**Figure 21**).

To determine the specificity of the assay in MSD format for UNC5C over close family members, the inventors compared detection of recombinant human UNC5C (SEQ ID NO: 263), recombinant human UNC5A (SEQ ID NO: 266), recombinant human UNC5B (SEQ ID NO: 267), recombinant human UNC5D (SEQ ID NO: 268), recombinant human Netrin receptor DCC (SEQ ID NO: 269). In addition, to confirm the assay recognises endogenous UNC5C, not only recombinant UNC5C, SH-SY5Y lysate, and SH-SY5Y media (SH-SY5Y cells express UNC5C) were included in the experiment.

The results showed that the assay detects UNC5C in a dose-dependent manner, but does not detect closely related family members UNC5A, UNC5B, UNC5D or DCC. This confirms that the assay is specific for UNC5C over close family members. In addition, the detection of SH-SY5Y lysate, but not SH-SY5Y media confirms that the assay recognises endogenous UNC5C, not only recombinant UNC5C (**Figure 22**).

EXAMPLE 11 – ATLX1282 does not prevent detection of UNC5C by ATL6532

To test the ability of the MSD assay to detect UNC5C in the presence of high concentrations of a therapeutic anti-UNC5C antibody, the inventors performed the MSD assay with three different concentrations of recombinant human UNC5C antigen (SEQ ID NO: 263), each tested alone, and with the addition of 10µg/ml ATLX1282, 100µg/ml ATLX1282, or 100µg/ml ATL5338 (isotype control).

The results show that the ability of the assay to detect UNC5C is not diminished by the presence of high concentrations of ATLX1282 (**Figure 23**). Without wishing to be bound by any particular theory, this is likely because ATLX1282 binds to the Ig1 domain of UNC5C while ATL6532 binds the Ig2 domain (Example 9 above). These results demonstrate the potential utility of this assay as a biomarker and/or companion diagnostic assay for measuring circulating UNC5C levels in the blood of patients dosed with a therapeutic anti-UNC5C antibody such as ATLX1282, for example by performing this assay on biological fluid samples obtained from said patients.

EXAMPLE 12 – MSD Assay performance in plasma, serum and artificial CSF

To further demonstrate the applicability of the MSD assay for testing biological fluid samples obtained from patients, the inventors tested the performance of the MSD assay in different relevant biological matrices to investigate the potential impact of matrix interference on assay recovery. Specifically, the assay was tested using PBS (control), plasma samples from healthy individuals, serum from a commercial serum pool, and artificial cerebrospinal fluid (CSF), each spiked with recombinant human UNC5C (SEQ ID NO: 263).

The results showed that the assay performs well in multiple relevant biological matrices, including 50% plasma, as the detection in the different biological matrices was comparable to the detection in PBS, indicating no significant matrix interference (**Figure 24**). These results further demonstrate the potential utility of this assay in testing biological fluid samples obtained from patients.

EXAMPLE 13 – MSD Assay of UNC5C in healthy control and ALS patient plasma samples

To demonstrate the performance of the MSD assay in testing biological fluid samples obtained from patients, the inventors used the assay to compare the levels of soluble UNC5C in plasma samples obtained from amyotrophic lateral sclerosis (ALS) patients with plasma samples obtained from healthy controls.

Specifically, plasma samples obtained from 82 healthy controls and 150 ALS patients, with similar age and sex distribution, were measured in the soluble UNC5C MSD assay. ALS samples were obtained from 3 separate clinical cohorts and contained a range of mild, moderate and severe diagnoses and Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS) scores. Total protein levels in the plasma samples were measured using a bicinchoninic acid (BCA) assay and soluble UNC5C levels were normalised to total protein levels to account for variation between subjects.

The results showed that soluble UNC5C levels (normalised to total protein levels) are significantly higher in plasma samples from ALS patients than in healthy controls (**Figure 25**). These results thus support the use of soluble UNC5C levels as a biomarker to assess the presence and/or severity of neurodegenerative diseases such as ALS. These results also further demonstrate the utility of this assay in testing biological fluid samples obtained from subjects, for example to identify subjects with increased levels of soluble UNC5C relative to healthy controls, who may benefit from treatment with a therapeutic compound or composition that affects the level of soluble UNC5C.

Stratification of these results by subject resiliency to ALS reveals a link between subject resiliency status and sUNC5C levels (**Figure 26A**). The subjects were categorised as resilient, semi-resilient, progressor, or unknown, based on their decline in the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R) score over time (slope) and years of survival after symptom onset. Individuals with steep slopes and short survival were categorised as progressors, those with shallow slopes and longer survival were categorised as resilient. Patients with too few ALSFRS-R scores to accurately calculate a slope were categorised as 'unknown'. Exemplary ALSFRS-R slopes for each resilience category are shown in **Figure 26B**. These results thus support the use of soluble UNC5C levels as a biomarker to assess whether a subject is likely to be resilient to a neurodegenerative disease such as ALS.

EXAMPLE 14 – MSD Assay of UNC5C in animals administered anti-UNC5C antibody ATLX1282

To assess the performance of the MSD assay as a target engagement biomarker assay for subjects treated with a therapeutic compound or composition that binds soluble UNC5C, the assay was used to measure soluble UNC5C in plasma or serum samples obtained from animals treated with anti-UNC5C antibody ATLX1282.

Cynomolgous monkeys and NEFH-ITA × hTDP-43ΔNLS bigenic mice (rNLS mice; an ALS mouse model) were administered ATLX1282. Plasma or serum samples were obtained from the animals following administration of ATLX1282 and the MSD assay was used to assess the level of sUNC5C in the samples (see Materials and Methods).

The results show that the assay detects higher levels of sUNC5C in subjects following treatment with ATLX1282 (Figure 27 and Figure 28). The inventors postulate that this may be due to ATLX1282 increasing the half-life of soluble UNC5C. These results therefore demonstrate the utility of the MSD assay as a target engagement biomarker assay for therapeutic compounds or compositions that bind soluble UNC5C, such as anti-UNC5C antibodies.

EXAMPLE 15 – MSD Assay of UNC5C in healthy control, Rheumatoid Arthritis, ALS, RBD, PD, Preclinical AD, and Symptomatic AD patient plasma samples

To further demonstrate the use of soluble UNC5C as a biomarker for detecting the presence and/or severity of a range of neurodegenerative diseases in biological fluid samples obtained from subjects, the inventors used the assay to compare the levels of soluble UNC5C in plasma samples obtained from patients with ALS, REM sleep Behavioural Disorder (RBD), PD, and AD (including both preclinical and symptomatic AD patients). RBD is considered a prodromal stage for Parkinson's Disease with a phenoconversion rate of 73.5% after 12 years (Lee et al, 2023). The NIA-AA AT(N) framework defines individuals positive for AD biomarkers amyloid beta and tau as preclinical Alzheimer's Disease (Jack et al 2018).

Plasma samples obtained from healthy controls were also tested, as well as plasma samples obtained from patients with rheumatoid arthritis (RA). Samples from Rheumatoid Arthritis patients were included to demonstrate that increased soluble UNC5C is specific to neurodegenerative diseases/disorders. In total, plasma from 93 healthy controls, 150 ALS patients (see Example 13 above), 20 RA patients, 25 RBD patients, 81 PD patients and 22 AD patients were measured in the soluble UNC5C MSD assay.

The results show soluble UNC5C levels were not significantly higher in Rheumatoid Arthritis samples compared to healthy controls, indicating that this is a neurodegeneration specific marker (Figure 29; Table 15). Soluble UNC5C levels were higher in all neurological conditions tested, including early stage preclinical or prodromal neurodegenerative disease, demonstrating the utility of soluble UNC5C as a biomarker for a broad range of neurodegenerative conditions, both symptomatic/clinical and preclinical/prodromal.

Disease	Median pg sUNC5C/mg	Kruskal-Wallis vs Healthy		
	Total protein	Adjusted P-Value	Significant?	Summary

Healthy Control	10.05	N/A	N/A	N/A
RA	12.27	0.7868	No	ns
ALS	15.21	<0.0001	Yes	****
RBD	18.89	<0.0001	Yes	****
PD	16.85	<0.0001	Yes	****
AD	16.81	<0.0001	Yes	****

Table 15. Summary of soluble UNC5C concentrations in plasma samples obtained from patients with a range of neurological diseases, rheumatoid arthritis patients, and healthy controls.

Taken together, the results from Examples 7-15 demonstrate that capture of UNC5C by anti-UNC5C antibodies, such as ATL6532, and detection with a polyclonal anti-UNC5C antibody (e.g. goat polyclonal anti-UNC5C detection antibody (Biotechne #AF1005) provides a sensitive, specific assay that can detect recombinant human, mouse and cyno UNC5C as well as endogenous UNC5C in cell lysates and human plasma and serum samples. These results also demonstrate that anti-UNC5C antibodies which bind a domain of UNC5C other than the Ig1 domain (i.e. anti-UNC5C antibodies which bind the Ig2, TSP1, and/or TSP2 domains of UNC5C, e.g. the Ig2 domain) find particular utility in such assays, as they do not compete with netrin binding or any therapeutic antibody which binds the Ig1 domain of UNC5C, such as ATLX1282. ATL6532 is an example of such an antibody which binds the Ig2 domain of UNC5C and has excellent sensitivity for capturing UNC5C across multiple assay formats tested herein.

More generally, these Examples support the use of soluble UNC5C as a biomarker for detecting the presence and/or severity and/or resilience status of a neurodegenerative disease in a subject (both preclinical/prodromal and clinical/symptomatic neurodegenerative disease), for example ALS, RBD, PD, AD and further support the detection of soluble UNC5C levels as a target engagement biomarker assay for subjects treated with a therapeutic compound or composition that binds soluble UNC5C.

References

All documents cited herein are incorporated by reference in their entirety.

Ackerman, S L, and B B Knowles. "Cloning and mapping of the UNC5C gene to human chromosome 4q21-q23." *Genomics* vol. 52,2 (1998): 205-8. doi:10.1006/geno.1998.5425

Bang, Jee et al. "Frontotemporal dementia." *Lancet* (London, England) vol. 386,10004 (2015): 1672-82. doi:10.1016/S0140-6736(15)00461-4

Bang, Jee et al. "Frontotemporal dementia." *Lancet* (London, England) vol. 386,10004 (2015): 1672-82. doi:10.1016/S0140-6736(15)00461-4

Boyer, Nicholas P, and Stephanie L Gupton. "Revisiting Netrin-1: One Who Guides (Axons)." *Frontiers in cellular neuroscience* vol. 12 221. 31 Jul. 2018, doi:10.3389/fncel.2018.00221

Burrell, James R et al. "Motor neuron dysfunction in frontotemporal dementia." *Brain : a journal of neurology* vol. 134,Pt 9 (2011): 2582-94. doi:10.1093/brain/awr195

Chen, Guiqin et al. "Netrin-1 receptor UNC5C cleavage by active δ -secretase enhances neurodegeneration, promoting Alzheimer's disease pathologies." *Science advances* vol. 7,16 eabe4499. 16 Apr. 2021, doi:10.1126/sciadv.abe4499

Chen, Guiqin et al. "UNC5C Receptor Proteolytic Cleavage by Active AEP Promotes Dopaminergic Neuronal Degeneration in Parkinson's Disease." *Advanced science* (Weinheim, Baden-Wuerttemberg, Germany) vol. 9,7 (2022): e2103396. doi:10.1002/adv.202103396

- Cunningham, B C, and J A Wells. "High-resolution epitope mapping of hGH-receptor interactions by alanine-scanning mutagenesis." *Science (New York, N.Y.)* vol. 244,4908 (1989): 1081-5. doi:10.1126/science.2471267
- 5 Dun, Xin-Peng, and David B Parkinson. "Role of Netrin-1 Signaling in Nerve Regeneration." *International journal of molecular sciences* vol. 18,3 491. 24 Feb. 2017, doi:10.3390/ijms18030491
- Glasgow, Stephen D et al. "Guiding synaptic plasticity: Novel roles for netrin-1 in synaptic plasticity and memory formation in the adult brain." *The Journal of physiology* vol. 599,2 (2021): 493-505. doi:10.1113/JP278704
- 10 Golde, Todd E. "Disease-Modifying Therapies for Alzheimer's Disease: More Questions than Answers." *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* vol. 19,1 (2022): 209-227. doi:10.1007/s13311-022-01201-2
- Hashimoto, Yuichi et al. "An Alzheimer Disease-linked Rare Mutation Potentiates Netrin Receptor Uncoordinated-5C-induced Signaling That Merges with Amyloid β Precursor Protein Signaling." *The Journal of biological chemistry* vol. 291,23 (2016): 12282-93. doi:10.1074/jbc.M115.698092
- 15 Hardiman, Orla et al. "Amyotrophic lateral sclerosis." *Nature reviews. Disease primers* vol. 3 17071. 5 Oct. 2017, doi:10.1038/nrdp.2017.71
- Jack et al. NIA-AA 'Research Framework: Toward a biological definition of Alzheimer's disease.' *Alzheimers Dement.* 2018 Apr;14(4):535-562. doi: 10.1016/j.jalz.2018.02.018. PMID: 29653606; PMCID: PMC5958625
- 20 Jasmin, Mélissa et al. "Netrin-1 and its receptor DCC modulate survival and death of dopamine neurons and Parkinson's disease features." *The EMBO journal* vol. 40,3 (2021): e105537. doi:10.15252/embj.2020105537
- Ju, Ting et al. "Decreased Netrin-1 in Mild Cognitive Impairment and Alzheimer's Disease Patients." *Frontiers in aging neuroscience* vol. 13 762649. 16 Feb. 2022, doi:10.3389/fnagi.2021.762649
- 25 Knopman, David S et al. "Alzheimer disease." *Nature reviews. Disease primers* vol. 7,1 33. 13 May. 2021, doi:10.1038/s41572-021-00269-y
- Llambi, F et al. "Netrin-1 acts as a survival factor via its receptors UNC5H and DCC." *The EMBO journal* vol. 20,11 (2001): 2715-22. doi:10.1093/emboj/20.11.2715
- 30 Lee et al. 'REM Sleep Behavior Disorder and Its Possible Prodromes in General Population Prevalence, Polysomnography Findings, and Associated Factors' *Neurology.* 2023 Dec 4;101(23):e2364-e2375. doi: 10.1212/WNL.0000000000207947. PMID: 37816644; PMCID: PMC10752649
- Leem, Jinwoo et al. "Deciphering the language of antibodies using self-supervised learning." *Patterns (New York, N.Y.)* vol. 3,7 100513. 18 May. 2022, doi:10.1016/j.patter.2022.100513
- 35 Leonardo, E D et al. "Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors." *Nature* vol. 386,6627 (1997): 833-8. doi:10.1038/386833a0
- Lund, J et al. "Human Fc gamma RI and Fc gamma RII interact with distinct but overlapping sites on human IgG." *Journal of immunology (Baltimore, Md. : 1950)* vol. 147,8 (1991): 2657-62
- Li, Quan et al. "The role of UNC5C in Alzheimer's disease." *Annals of translational medicine* vol. 6,10 (2018): 178. doi:10.21037/atm.2018.04.43
- 40 Obien, Marie Engelene J et al. "Revealing neuronal function through microelectrode array recordings." *Frontiers in neuroscience* vol. 8 423. 6 Jan. 2015, doi:10.3389/fnins.2014.00423
- Panza, Francesco et al. "Development of disease-modifying drugs for frontotemporal dementia spectrum disorders." *Nature reviews. Neurology* vol. 16,4 (2020): 213-228. doi:10.1038/s41582-020-0330-x
- 45 Phukan, Julie et al. "Cognitive impairment in amyotrophic lateral sclerosis." *The Lancet. Neurology* vol. 6,11 (2007): 994-1003. doi:10.1016/S1474-4422(07)70265-X

- Poliak, Sebastian et al. "Synergistic integration of Netrin and ephrin axon guidance signals by spinal motor neurons." *eLife* vol. 4 e10841. 3 Dec. 2015, doi:10.7554/eLife.10841
- Pokinko, Matthew et al. "Resilience to amphetamine in mouse models of netrin-1 haploinsufficiency: role of mesocortical dopamine." *Psychopharmacology* vol. 232,20 (2015): 3719-29. doi:10.1007/s00213-015-4032-9
- Prince, M et al (2014) Dementia UK: Update Second Edition report produced by King's College London and the London School of Economics for the Alzheimer's Society.
- Rajasekharan, Sathyanath, and Timothy E Kennedy. "The netrin protein family." *Genome biology* vol. 10,9 (2009): 239. doi:10.1186/gb-2009-10-9-239
- 10 Renaud, Jean-Paul et al. "Cryo-EM in drug discovery: achievements, limitations and prospects." *Nature reviews. Drug discovery* vol. 17,7 (2018): 471-492. doi:10.1038/nrd.2018.77
- Rosso, Sonia M et al. "Frontotemporal dementia in The Netherlands: patient characteristics and prevalence estimates from a population-based study." *Brain : a journal of neurology* vol. 126,Pt 9 (2003): 2016-22. doi:10.1093/brain/awg204
- 15 Smith, Alec S T et al. "Human Induced Pluripotent Stem Cell-Derived TDP-43 Mutant Neurons Exhibit Consistent Functional Phenotypes Across Multiple Gene Edited Lines Despite Transcriptomic and Splicing Discrepancies." *Frontiers in cell and developmental biology* vol. 9 728707. 29 Sep. 2021, doi:10.3389/fcell.2021.728707
- 20 Spijkers, Xandor M et al. "A directional 3D neurite outgrowth model for studying motor axon biology and disease." *Scientific reports* vol. 11,1 2080. 22 Jan. 2021, doi:10.1038/s41598-021-81335-z
- Zamani, Elham et al. "Netrin-1 protects the SH-SY5Y cells against amyloid beta neurotoxicity through NF- κ B/Nrf2 dependent mechanism." *Molecular biology reports* vol. 47,12 (2020): 9271-9277. doi:10.1007/s11033-020-05996-1

Claims:

1. Use of an antibody which specifically binds to UNC5C protein or a fragment thereof for detecting the level of soluble UNC5C in a biological fluid sample obtained from a subject.
2. A method for detecting the level of soluble UNC5C in a biological fluid sample obtained from a subject,
5 wherein the method comprises contacting the biological fluid sample with an antibody or antibody fragment thereof which specifically binds to UNC5C protein or a fragment thereof.
3. A method of determining the effect of a therapeutic compound or composition that affects the level of soluble UNC5C in a subject who has been administered the therapeutic compound or composition,
10 wherein the method comprises determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject.
4. A method of detecting the presence and/or severity of a neurodegenerative disease in a subject and/or determining whether a subject is likely to be resilient to a neurodegenerative disease, the method comprising:
(a) determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject;
15 (b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C, wherein a higher level of soluble UNC5C in the biological fluid sample relative to the reference level of soluble UNC5C is indicative of the presence of the neurodegenerative disease and/or indicative of the severity of the neurodegenerative disease and/or indicative of the subject being unlikely to be resilient to the neurodegenerative disease.
- 20 5. A method of selecting a subject for treatment with a therapeutic compound or composition that affects the level of soluble UNC5C, or for participating in a clinical trial of a therapeutic compound or composition that affects the level of soluble UNC5C, the method comprising:
(a) determining a level of soluble UNC5C in a biological fluid sample previously obtained from the subject;
(b) comparing the level of soluble UNC5C and a reference level of soluble UNC5C; and
25 (c) selecting the subject for treatment or for participating in the clinical trial when the level of soluble UNC5C in the biological fluid sample is higher than the reference level of soluble UNC5C.
6. The method according to any one of claims 3-5, wherein determining the level of soluble UNC5C in the biological fluid sample previously obtained from the subject comprises contacting the biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof, optionally wherein
30 the probe is an antibody or fragment thereof.
7. The use according to claim 1, or the method according to claim 2 or 6, wherein the antibody comprises a heavy chain variable domain (VH) with the following CDRs:
CDRH1 comprising amino acid sequence SEQ ID NO: 144, 145, or 147;
CDRH2 comprising amino acid sequence SEQ ID NO: 152, 153, or 156; and
35 CDRH3 comprising amino acid sequence SEQ ID NO: 161, 162, 163, or 166;
optionally wherein the antibody comprises a VH with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO: 144, 152, 161); ATL_6533 (SEQ ID NO: 145, 153, 162); ATL_6534 (SEQ ID NO: 144, 152, 163); ATL_6537 (SEQ ID NO: 147, 156, 166);

optionally wherein the antibody comprises a VH with the CDRs of antibody ATL_6532 (SEQ ID NO: 144, 152, 161).

8. The use according to claim 1 or claim 7, or the method according to claims 2 or 6-7, wherein the antibody has a heavy chain variable domain (VH) with the following framework sequences:

HFWR1 of SEQ ID NO: 205, 209, 212, or 220;

HFWR2 of SEQ ID NO: 206, 210, or 221;

HFWR3 of SEQ ID NO: 207, 211, or 222; and

HFWR4 of SEQ ID NO: 208 or 223;

optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of any of the antibodies ATL_6532 (SEQ ID NO:205, 206, 207, 208); ATL_6533 (SEQ ID NO:209, 210, 211, 208); ATL_6534 (SEQ ID NO:212, 206, 207,208); ATL_6537 (SEQ ID NO:220, 221, 222, 223);

optionally wherein the antibody has a heavy chain variable domain (VH) with the framework sequences of antibody ATL_6532 (SEQ ID NO:205, 206, 207, 208).

9. The use according to any of claims 1 or 7-8, or the method according to claims 2 or 6-8, wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532 or ATL_6533, optionally wherein the antibody has a heavy chain variable domain (VH) with the CDRs and/or the framework sequences of ATL_6532.

10. The use according to any of claims 1 or 7-9, or the method according to claims 2 or 6-9, wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 124 (ATL_0006532 VH); 125 (ATL_0006533 VH); 126 (ATL_0006534 VH); 129 (ATL_0006537 VH);

optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 124 (ATL_6532 VH); SEQ ID NO: 125 (ATL_6533 VH);

optionally wherein the antibody has a heavy chain variable domain (VH) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 124 (ATL_6532 VH).

11. The use according to any of claims 1 or 7-10, or the method according to claims 2 or 6-10, wherein the antibody comprises a light chain variable domain (VL) with the following CDRs:

CDRL1 comprising amino acid sequence SEQ ID NO: 169, 171, or 174;

CDRL2 comprising amino acid sequence SEQ ID NO: 177 or 181; and

CDRL3 comprising amino acid sequence SEQ ID NO: 186, 187, 188, or 191;

optionally wherein the antibody comprises a VL with the CDRs of any of the antibodies ATL_6532 (SEQ ID NO:171, 177, 186); ATL_6533 (SEQ ID NO:169, 177, 187); ATL_6534 (SEQ ID NO:169, 177, 188); ATL_6537 (SEQ ID NO:174, 181, 191);

optionally wherein the antibody comprises a VL with the CDRs of antibody ATL_6532 (SEQ ID NO:171, 177, 186).

12. The use according to any of claims 1 or 7-11, or the method according to claims 2 or 6-11, wherein the antibody has a light chain variable domain (VL) with the following framework sequences:

LFWR1 of SEQ ID NO: 230, 232, 233, or 235;

LFWR2 of SEQ ID NO: 240, 241, 238, or 244;

LFWR3 of SEQ ID NO: 247, 249, 250, or 253; and

LFWR4 of SEQ ID NO: 256 or 258;

optionally wherein the antibody comprises a VL with the FWRs of any of the antibodies ATL_6532 (SEQ ID NO:232, 240, 249, 258); ATL_6533 (SEQ ID NO:230, 241, 247, 256); ATL_6534 (SEQ ID NO:233, 238, 250, 256); ATL_6537 (SEQ ID NO:235, 244, 253, 256);

optionally wherein the antibody comprises a VL with the FWRs of antibody ATL_6532 (SEQ ID NO:232, 240, 249, 258).

13. The use according to any of claims 1 or 7-12, or the method according to claims 2 or 6-12, wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from SEQ ID NOs: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533); SEQ ID NOs: 136 (ATL_0006534); SEQ ID NO: 139 (ATL_0006537);

optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with a sequence selected from: SEQ ID NO: 134 (ATL_0006532); SEQ ID NO: 135 (ATL_0006533);

optionally wherein the antibody has a light chain variable domain (VL) comprising a sequence that has at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 95% sequence identity with SEQ ID NO: 134 (ATL_0006532).

14. The use according to any of claims 1 or 7-13, or the method according to claims 2 or 6-13, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain, TSP type-1 (TSP1) domain, and/or TSP type-2 (TSP2) domain of UNC5C protein.

15. The use, or method according to claim 14, wherein the antibody specifically binds the second immunoglobulin (Ig2) domain of UNC5C protein.

16. The use according to any of claims 1 or 7-15, or the method according to claims 2 or 6-15, wherein the antibody does not compete or cross compete for binding to UNC5C with an antibody comprising the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282).

17. The use according to any of claims 1 or 7-16, or the method according to claims 2 or 6-16, wherein the antibody competes or cross competes for binding to UNC5C with an antibody comprising the heavy

chain variable domain (VH) set forth in SEQ ID NO: 124 (ATL_6532) and the light chain variable domain (VL) set forth in SEQ ID NO: 134 (ATL_6532).

18. The use according to any of claims 1 or 7-17, or the method according to claims 2 or 6-17, wherein the antibody binds human, mouse and/or cynomolgus monkey UNC5C protein.

19. The use according to any of claims 1 or 7-18, or the method according to claims 2 or 6-18, wherein the antibody selectively binds to UNC5C over other one or more netrin receptors, optionally wherein the antibody selectively binds to UNC5C over one or more (or all of): DCC, UNC5A, UNC5B, UNC5D.

10

20. The use according to any of claims 1 or 7-19, or the method according to claims 2-19, wherein the subject is a mammal, optionally wherein the subject is a mouse, monkey, or human, optionally wherein the subject is a human.

15 21. The use according to any of claims 1 or 7-20, or the method according to claims 2-20, wherein the biological fluid sample is blood or cerebrospinal fluid (CSF), optionally wherein a blood sample is selected from a whole blood, plasma or serum sample.

22. The use or method according to claim 21, wherein the subject is a patient who has been treated with a therapeutic compound or composition that alters the concentration of soluble UNC5C in the subject's blood and/or CSF, optionally wherein the therapeutic compound is an anti-UNC5C antibody.

23. The use according to any of claims 1 or 7-22 or the method according to claims 2-22, wherein the subject is a patient who has been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is: (i) a prodromal or preclinical stage neurodegenerative disease; or (ii) a symptomatic or clinical stage neurodegenerative disease.

24. The use according to claim 23, or the method according to claims 4 or 6-23, wherein the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), Parkinson's disease (PD), or a prodromal stage thereof, optionally wherein the neurodegenerative disease is ALS.

25. The use according to any of claims 1 or 7-24, wherein the use comprises contacting the biological fluid sample with the antibody.

35

26. The use according to claim 25 or the method according to any of claims 2 or 6-25, wherein the antibody or antibody fragment is used as a capture antibody in an immunoassay, optionally wherein the immunoassay is a sandwich ELISA or a meso scale discovery (MSD) assay.

40 27. The use or method according to claim 26, further comprising:

detecting UNC5C bound by the capture antibody using a detection probe, wherein the detection probe binds UNC5C, optionally wherein the detection probe is a detection antibody, UNC5C binding protein or ligand, wherein:

- (i) the detection probe carries a detectable label; or
- 5 (ii) the detection probe is detected using a secondary antibody which binds the detection probe, wherein the secondary antibody carries a detectable label; and
- detecting the detectable label to determine the level of soluble UNC5C in the biological fluid sample.

28. The use or method according to claim 27, wherein the detection antibody is a non-human antibody

10 which binds UNC5C;

optionally wherein the detection antibody is a polyclonal antibody;

optionally wherein the detection antibody is a goat polyclonal antibody.

29. The use or method according to claim 27 or claim 28, wherein the secondary antibody is a non-

15 human polyclonal antibody which binds to antibodies of the species from which the detection antibody is derived;

optionally wherein the detection antibody is a goat antibody, and the secondary antibody is an anti-goat polyclonal antibody;

optionally wherein the secondary antibody is a mouse anti-goat polyclonal antibody or a donkey anti-goat

20 polyclonal antibody.

30. The use or method according to any one of claims 27 to 29, wherein the detectable label is selected from the group consisting of a fluorescent, chemiluminescent, electrochemiluminescent, radioactive or enzymatically active label;

25 optionally wherein the detectable label is an electrochemiluminescent or enzymatically active label;

optionally wherein the detectable label is a horseradish peroxidase (HRP) enzyme or a Sulfo-tag.

31. The use or method according to claim 30, wherein the detectable label is an electrochemiluminescent label, optionally a Sulfo-tag, wherein detecting the detectable label comprises:

30 (i) applying an electrical current to the electrochemiluminescent label; and

 (ii) measuring the luminescence.

32. The use or method according to claim 30, wherein the detectable label is a horseradish peroxidase (HRP) enzyme, wherein detecting the detectable label comprises:

35 (i) contacting the horseradish peroxidase (HRP) enzyme with TMB (3, 3', 5, 5'-tetramethylbenzidine); and

 (ii) measuring the ultraviolet (UV) absorption at 450nm and/or 570nm.

33. The use according to any of claims 22-32, or the method according to any one of claims 3 or 5-32,

40 wherein the therapeutic compound is an anti-UNC5C antibody.

34. The use or method according to claim 33, wherein the therapeutic anti-UNC5C antibody binds the first immunoglobulin (Ig1) domain of UNC5C protein.

35. The use or method according to claim 33 or claim 34, wherein the therapeutic anti-UNC5C antibody comprises a VH with the CDRs of antibody ATLX1282 (SEQ ID NO: 20, 22, 23) and a VL with the CDRs of antibody ATLX1282 (SEQ ID NO: 24, 34, 40).

36. The use or method according to any one of claims 33-35, wherein the therapeutic anti-UNC5C antibody comprises the heavy chain variable domain (VH) set forth in SEQ ID NO: 93 (ATLX1282) and the light chain variable domain (VL) set forth in SEQ ID NO: 94 (ATLX1282), optionally wherein the therapeutic anti-UNC5C antibody is ATLX1282.

37. A kit comprising:

(i) a capture antibody, wherein the capture antibody specifically binds to UNC5C protein or a fragment thereof; and

(ii) a detection probe, wherein the detection probe binds UNC5C, optionally wherein the detection probe carries a detectable label, wherein the detection probe comprises an anti-UNC5C antibody, UNC5C binding protein or UNC5C ligand, and/or wherein the capture antibody is different from the detection antibody, optionally further comprising:

(iii) a secondary antibody which binds the detection antibody, wherein the secondary antibody carries a detectable label.

38. The kit according to claim 37, wherein the capture antibody has the features defined in any of claims 7-19 and/or wherein the detection antibody has the features defined in claim 28, and/or wherein the secondary antibody has the features defined in claim 29, and/or wherein the detectable label has the features defined in claim 30, and/or wherein the detectable label is a horseradish peroxidase (HRP) enzyme and the kit further comprises TMB (3, 3', 5, 5'-tetramethylbenzidine), and/or wherein the capture antibody is immobilised to a solid support, and/or wherein the kit further comprises means to measure a total protein level in a sample..

39. The use according to any of claims 1 or 7-36, or the method according to any of claims 2-3 or 6-36, comprising: (a) determining a first level of soluble UNC5C in a first biological fluid sample previously obtained from the subject, wherein the first biological fluid sample was obtained prior to administration of a therapeutic compound or composition to the subject; (b) determining a second level of soluble UNC5C, optionally wherein the second level of soluble UNC5C is a reference level or a level soluble UNC5C in a second biological fluid sample previously obtained from the subject, wherein the second biological fluid sample was obtained after administration of the therapeutic compound or composition to the subject; and (c) comparing the first level of soluble UNC5C and the second level of soluble UNC5C to determine the effect of the therapeutic compound or composition on the level of soluble UNC5C.

40. The use or method according to claim 39, wherein determining the first level of soluble UNC5C comprises contacting said first biological fluid sample previously obtained from the subject with a probe

which specifically binds to UNC5C protein or a fragment thereof, and/or wherein determining the second level of soluble UNC5C comprises contacting said second biological fluid sample with a probe which specifically binds to UNC5C protein or a fragment thereof, optionally wherein the probe is an antibody or fragment thereof.

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41. The method according to any of claims 4-36 or the method or use according to claims 39 or 40, wherein: determining a level of soluble UNC5C in a biological fluid sample comprises determining a level of total protein in the first biological fluid sample; and comparing levels of soluble UNC5C comprises comparing levels of soluble UNC5C normalised to the level of total protein in the respective biological fluid sample.

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42. The method or use according to claim 41, wherein the levels of total protein are determined using a copper-based protein assay, a fluorescence-based protein assay, or a dye-based protein assay, optionally wherein the levels of total protein are determined using a bicinchoninic acid (BCA) assay.

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43. The method according to any of claims 4-36 or the method or use according to claims 39-42, wherein a reference level of soluble UNC5C is a level of soluble UNC5C associated with one or more biological fluid samples from respective healthy controls, wherein the healthy control is a subject who has not been diagnosed with a neurodegenerative disease, optionally wherein the neurodegenerative disease is selected from amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), Alzheimer's disease (AD), or Parkinson's disease (PD).

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