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(54) Title: GALECTIN-10 ANTIBODIES

(57) Abstract: The present invention relates to antagonists, particularly antibodies and antigen binding fragments thereof, that bind to the protein galectin-10, particularly human galectin-10. The galectin-10 antagonists disrupt the crystallization of galectin-10 and are therefore useful in methods of preventing and treating diseases and conditions wherein the pathology is linked to the formation/presence of Charcot-Leyden crystals (CLCs).

GALECTIN-10 ANTIBODIES

FIELD OF THE INVENTION

5 The present invention relates to antagonists, particularly antibodies and antigen binding fragments thereof, that bind to the protein galectin-10, particularly human galectin-10. The galectin-10 antagonists, particularly the antibodies and antigen binding fragments of the invention, disrupt the crystallization of galectin-10 and are therefore useful in methods of preventing and treating diseases and conditions wherein the pathology is linked to the 10 formation/presence of Charcot-Leyden crystals (CLCs).

BACKGROUND TO THE INVENTION

Charcot-Leyden crystals (CLCs) were first described in 1853 and are microscopic, colourless 15 crystals found in patients with certain conditions including allergic asthma and parasitic infections. CLCs are frequently observed in human tissues and secretions associated with an eosinophilic inflammatory response. In addition to asthma and parasitic infections, these crystals have been found in patients with cancer, for example myeloid leukemia. Structurally, CLCs accumulate as extracellular hexagonal bipyramidal crystals with a length of 20-40 µm and a 20 width of 2-4 µm. The protein forming these crystals has been identified as galectin-10.

Galectin-10 (also known as Charcot Leyden Crystal Protein) is a small (16.5kDa), hydrophobic, glycan-binding protein expressed abundantly in the bone marrow, primarily by eosinophils (Chua et al. (2012) *PLoS One*. 7(8): e42549). Galectin-10 is also produced to a lesser extent by 25 basophils and Foxp3-positive Tregs (Kubach et al. (2007) *Blood* 110(5): 1550-8). This protein is among the most abundant of eosinophil constituents, representing 7%-10% of total cellular protein. Galectin-10 is only found in humans, it lacks a secretion peptide signal and transmembrane domain, and is secreted under certain conditions by non-classical and novel apocrine mechanisms.

30

Despite abundant reports showing the appearance of CLCs in tissues from patients with eosinophilic disorders, the common view is that these crystals are merely a marker of eosinophil demise.

35 ## SUMMARY OF THE INVENTION

The *in vivo* function of galectin-10 and the significance of CLC formation have remained elusive, particularly because mice do not carry a LGALS10 gene encoding galectin-10. It is reported

herein how galectin-10 crystals can induce a pro-inflammatory response *in vivo* and how this response can be suppressed by the administration of galectin-10 antibodies capable of disrupting galectin-10 crystallization. It is reported herein how galectin-10 antibodies and antigen binding fragments, including IgGs, VHH antibodies and Fabs, can prevent crystallization of galectin-10 and also dissolve pre-existing galectin-10 crystals. Importantly, galectin-10 antibodies were able to dissolve CLCs from patient mucus samples. Taken together, this demonstrates how agents that target galectin-10 crystallization can be used to treat conditions and disorders where the pathology is linked to the presence of CLCs.

In a first aspect, the present invention provides an antagonist that binds to galectin-10, wherein the antagonist binds to an epitope of galectin-10 and thereby shields a crystal packing interface of galectin-10. The antagonist preferably binds to human galectin-10. The present invention further provides an antagonist that binds to galectin-10, which, when bound to soluble galectin-10, inhibits the crystallization of galectin-10. The present invention further provides an antagonist that binds to galectin-10, which, when bound to crystalline galectin-10, promotes the dissolution of crystalline galectin-10.

In certain embodiments, the antagonists that bind to galectin-10 and thereby shield a crystal packing interface of galectin-10 inhibit crystallization of the galectin-10 when bound to soluble galectin-10. Alternatively or in addition, the galectin-10 antagonists, when bound to crystalline galectin-10, may promote dissolution of crystalline galectin-10.

The antagonists of the present invention preferably bind to human galectin-10. In certain embodiments, the antagonist binds to an epitope comprising one or more amino acids from the crystal packing interfaces of galectin-10, preferably human galectin-10. Said epitope may comprise one or more, two or more, three or more, four or more, five or more amino acids selected from the group consisting of: Ser2, Leu3, Leu4, Tyr8, Thr9, Glu10, Ala11, Ala12, Ser13, Thr16, Thr42, Glu43, Met44, Lys45, Asp49, Ile50, Glu68, Tyr69, Gly70, Ala71, Lys73, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Leu96, Pro97, Asp98, Lys99, Gln101, Met103, Gly106, Gln107, Ser108, Ser109, Tyr110, Thr111, Asp113, His114, Arg115, Ile116, Lys117, Ala120, Gln125, Thr133, Lys134, Phe135, Asn136, Val137, Ser138, Tyr139, Leu140 and Lys141. The amino acid residues or positions of galectin-10 are defined with reference to the human protein sequence identified herein as SEQ ID NO: 141.

In certain embodiments, the antagonist binds to an epitope comprising Tyr69 or an epitope comprising an amino acid adjacent to Tyr69. In preferred embodiments, the antagonist binds to an epitope comprising Tyr69. In a further preferred embodiment, the antagonist binds to an epitope comprising Glu68, Tyr69 and Gly70, wherein the amino acid positions are identified with

reference to SEQ ID NO: 141. In certain embodiments, the antagonist binds to an epitope comprising the amino acids Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, His114, Arg115, Ile116, Lys117 and Ala120. In certain embodiments, the antagonist binds to an epitope comprising the amino acids Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, His114, Arg115, Ile116, Lys117, GLu119, Ala120 and Lys122. The epitope may additionally comprise Gln74 and/or Asp98. In certain embodiments, the antagonist binds to an epitope comprising Glu33, Gly59, Arg60 and Lys79. The epitope may additionally comprise Gln74, Gln75 and Glu77. In certain embodiments, the epitope comprises or consists of Leu31, Glu33, Gly59, Arg60, Ser78, Lys79, Asn80, Met81, Pro82 and Gln84. In certain embodiments, the epitope comprises or consists of Glu33, Gly59, Arg60, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Pro82 and Ser109. In certain embodiments, the epitope comprises or consists of Glu33, Gly59, Arg60, Trp72, Gln74, Gln75, Val76, Glu77, Lys79, Asn80, Met81, Pro82, Gln84 and Ser109. In certain embodiments, the epitope comprises or consists of Glu33, Gly59, Arg60, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Pro82, Phe83, Gln84. In certain embodiments, the epitope comprises or consist of Thr42, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, Arg115, Ile116, Lys117, Glu119 and Ala120. In certain embodiments, the epitope comprises or consists of Glu43, Asp49, Glu68, Tyr69, Lys73, Asp98, Asp113, His114, Arg115, Lys117, Glu119 and Ala120. In certain embodiments, the epitope comprises or consists of Asp49, Glu68, Tyr69, Lys73, Gln74, Asp98, Asp113, His114, Arg115, Ile116 and Lys117. In certain embodiments, the epitope comprises or consists of Ser2, Leu3, Leu4, Pro5, Pro7, Tyr8, Thr9, Glu10, Ala11, Lys23, Arg25, Met44, Gly86, Gln87, Glu88, Phe89, Glu90, Asn105, Gln125, Thr133, Lys134 and Phe135. The amino acid positions of galectin-10 are defined with reference to the human protein sequence identified herein as SEQ ID NO: 141.

The galectin-10 antagonist may bind to an epitope consisting of amino acids from the crystal packing interfaces of galectin-10. In such embodiments, the epitope may consist of one or more, two or more, three or more, four or more, five or more amino acids selected from the group consisting of: Ser2, Leu3, Leu4, Tyr8, Thr9, Glu10, Ala11, Ala12, Ser13, Thr16, Thr42, Glu43, Met44, Lys45, Asp49, Ile50, Glu68, Tyr69, Gly70, Ala71, Lys73, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Leu96, Pro97, Asp98, Lys99, Gln101, Met103, Gly106, Gln107, Ser108, Ser109, Tyr110, Thr111, Asp113, His114, Arg115, Ile116, Lys117, Ala120, Gln125, Thr133, Lys134, Phe135, Asn136, Val137, Ser138, Tyr139, Leu140 and Lys141. The amino acid positions of galectin-10 are defined with reference to the human protein sequence identified herein as SEQ ID NO: 141.

Alternatively or in addition, the antagonist may bind to an epitope comprising one or more amino acids from the dimerization interface of galectin-10. In such embodiments, the antagonist may bind to an epitope comprising one or more amino acids selected from the group consisting of:

Pro5, Pro7, Leu27, Ala28, Cys29, Leu31, Asn32, Glu33, Pro34, Tyr35, Gln37, His41, Glu46, Glu47, Gln55, Arg60, Arg61, Arg67, Trp72, Gln75, Trp127, Arg128 and Asp129.

In certain embodiments, the antagonist is a small molecule, an inhibitory polypeptide or an antibody mimetic. In preferred embodiments, the antagonist is an antibody or antigen binding fragment thereof, as defined elsewhere herein. The antibody may be an immunoglobulin, preferably an immunoglobulin of the IgG class, more preferably IgG1. The antibody may be a VHH antibody. The antibody or antigen binding fragment thereof may be a humanised or germlined variant of a non-human antibody, for example a camelid-derived antibody. The antibody or antigen binding fragment may comprise the CH1 domain, hinge region, CH2 domain and/or CH3 domain of a human IgG, preferably IgG1.

In certain embodiments, the antigen binding fragment is selected from the group consisting of: an antibody light chain variable domain (VL); an antibody heavy chain variable domain (VH); a single chain antibody (scFv); a F(ab')2 fragment; a Fab fragment; an Fd fragment; an Fv fragment; a one-armed (monovalent) antibody; diabodies, triabodies, tetrabodies, or any antigen binding molecule formed by combination, assembly or conjugation of such antigen binding fragments. In preferred embodiments, the antigen binding fragment is a Fab.

The present invention provides, in further aspects, antibodies and antigen binding fragments that bind galectin-10. These antibodies may be defined exclusively with respect to their structural characteristics as described below. In certain embodiments, the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:
(i) HCDR3 comprising or consisting of SEQ ID NO: 3; HCDR2 comprising or consisting of SEQ ID NO: 2; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or consisting of SEQ ID NO: 58; LCDR2 comprising or consisting of SEQ ID NO: 57; LCDR1 comprising or consisting of SEQ ID NO: 56;
(ii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 5; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 61; LCDR2 comprising or consisting of SEQ ID NO: 60; LCDR1 comprising or consisting of SEQ ID NO: 59;
(iii) HCDR3 comprising or consisting of SEQ ID NO: 9; HCDR2 comprising or consisting of SEQ ID NO: 8; HCDR1 comprising or consisting of SEQ ID NO: 7; LCDR3 comprising or consisting of SEQ ID NO: 64; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 62;

(iv) HCDR3 comprising or consisting of SEQ ID NO: 12; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 10; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;

5 (v) HCDR3 comprising or consisting of SEQ ID NO: 15; HCDR2 comprising or consisting of SEQ ID NO: 14; HCDR1 comprising or consisting of SEQ ID NO: 13; LCDR3 comprising or consisting of SEQ ID NO: 70; LCDR2 comprising or consisting of SEQ ID NO: 69; LCDR1 comprising or consisting of SEQ ID NO: 68;

(vi) HCDR3 comprising or consisting of SEQ ID NO: 18; HCDR2 comprising or consisting of SEQ
10 ID NO: 17; HCDR1 comprising or consisting of SEQ ID NO: 16; LCDR3 comprising or consisting of SEQ ID NO: 72; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 71;

(vii) HCDR3 comprising or consisting of SEQ ID NO: 20; HCDR2 comprising or consisting of SEQ ID NO: 19; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or
15 consisting of SEQ ID NO: 75; LCDR2 comprising or consisting of SEQ ID NO: 74; LCDR1 comprising or consisting of SEQ ID NO: 73;

(viii) HCDR3 comprising or consisting of SEQ ID NO: 23; HCDR2 comprising or consisting of SEQ ID NO: 22; HCDR1 comprising or consisting of SEQ ID NO: 21; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1
20 comprising or consisting of SEQ ID NO: 65;

(ix) HCDR3 comprising or consisting of SEQ ID NO: 25; HCDR2 comprising or consisting of SEQ ID NO: 24; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 77; LCDR1 comprising or consisting of SEQ ID NO: 76;

25 (x) HCDR3 comprising or consisting of SEQ ID NO: 28; HCDR2 comprising or consisting of SEQ ID NO: 27; HCDR1 comprising or consisting of SEQ ID NO: 26; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 79;

(xi) HCDR3 comprising or consisting of SEQ ID NO: 31; HCDR2 comprising or consisting of SEQ
30 ID NO: 30; HCDR1 comprising or consisting of SEQ ID NO: 29; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 80;

(xii) HCDR3 comprising or consisting of SEQ ID NO: 33; HCDR2 comprising or consisting of SEQ ID NO: 32; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or
35 consisting of SEQ ID NO: 84; LCDR2 comprising or consisting of SEQ ID NO: 83; LCDR1 comprising or consisting of SEQ ID NO: 82;

(xiii) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 35; HCDR1 comprising or consisting of SEQ ID NO: 34; LCDR3 comprising or

consisting of SEQ ID NO: 87; LCDR2 comprising or consisting of SEQ ID NO: 86; LCDR1 comprising or consisting of SEQ ID NO: 85;
(xiv) HCDR3 comprising or consisting of SEQ ID NO: 38; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 37; LCDR3 comprising or
5 consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 88;
(xv) HCDR3 comprising or consisting of SEQ ID NO: 41; HCDR2 comprising or consisting of SEQ ID NO: 40; HCDR1 comprising or consisting of SEQ ID NO: 39; LCDR3 comprising or consisting of SEQ ID NO: 91; LCDR2 comprising or consisting of SEQ ID NO: 90; LCDR1
10 comprising or consisting of SEQ ID NO: 89;
(xvi) HCDR3 comprising or consisting of SEQ ID NO: 43; HCDR2 comprising or consisting of SEQ ID NO: 42; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 92;
15 (xvii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 44; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 97; LCDR2 comprising or consisting of SEQ ID NO: 96; LCDR1 comprising or consisting of SEQ ID NO: 95;
(xviii) HCDR3 comprising or consisting of SEQ ID NO: 47; HCDR2 comprising or consisting of
20 SEQ ID NO: 46; HCDR1 comprising or consisting of SEQ ID NO: 45; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71;
(xix) HCDR3 comprising or consisting of SEQ ID NO: 50; HCDR2 comprising or consisting of SEQ ID NO: 49; HCDR1 comprising or consisting of SEQ ID NO: 48; LCDR3 comprising or
25 consisting of SEQ ID NO: 96; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 95;
(xx) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 52; HCDR1 comprising or consisting of SEQ ID NO: 51; LCDR3 comprising or consisting of SEQ ID NO: 98; LCDR2 comprising or consisting of SEQ ID NO: 97; LCDR1
30 comprising or consisting of SEQ ID NO: 80; and
(xxi) HCDR3 comprising or consisting of SEQ ID NO: 55; HCDR2 comprising or consisting of SEQ ID NO: 54; HCDR1 comprising or consisting of SEQ ID NO: 53; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71.

35

In certain embodiments, the antibody or antigen binding fragment thereof comprises a combination of a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the following:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 99 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 100 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

5 (ii) a VH comprising the amino acid sequence of SEQ ID NO: 101 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 102 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 103 or an amino acid sequence at 10 least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 104 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 105 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid 15 sequence of SEQ ID NO: 106 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(v) a VH comprising the amino acid sequence of SEQ ID NO: 107 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 108 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% 20 identical thereto;

(vi) a VH comprising the amino acid sequence of SEQ ID NO: 109 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 110 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

25 (vii) a VH comprising the amino acid sequence of SEQ ID NO: 111 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 112 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(viii) a VH comprising the amino acid sequence of SEQ ID NO: 113 or an amino acid sequence 30 at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(ix) a VH comprising the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid 35 sequence of SEQ ID NO: 116 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(x) a VH comprising the amino acid sequence of SEQ ID NO: 117 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

sequence of SEQ ID NO: 118 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xi) a VH comprising the amino acid sequence of SEQ ID NO: 119 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 120 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xii) a VH comprising the amino acid sequence of SEQ ID NO: 121 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 122 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xiii) a VH comprising the amino acid sequence of SEQ ID NO: 123 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 124 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xiv) a VH comprising the amino acid sequence of SEQ ID NO: 125 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 126 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xv) a VH comprising the amino acid sequence of SEQ ID NO: 127 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 128 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xvi) a VH comprising the amino acid sequence of SEQ ID NO: 129 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 130 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xvii) a VH comprising the amino acid sequence of SEQ ID NO: 131 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 132 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xviii) a VH comprising the amino acid sequence of SEQ ID NO: 133 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 134 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xix) a VH comprising the amino acid sequence of SEQ ID NO: 135 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 136 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xx) a VH comprising the amino acid sequence of SEQ ID NO: 137 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and

5 (xxi) a VH comprising the amino acid sequence of SEQ ID NO: 139 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 140 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

10 For embodiments wherein the domains of the antibodies or antigen binding fragments are defined by a particular percentage sequence identity to a reference sequence, the VH and/or VL domains may retain identical CDR sequences to those present in the reference sequence such that the variation is present only within the framework regions.

15 In certain embodiments, the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:

(i) HCDR3 comprising or consisting of SEQ ID NO: 162; HCDR2 comprising or consisting of SEQ ID NO: 161; HCDR1 comprising or consisting of SEQ ID NO: 160; LCDR3 comprising or
20 consisting of SEQ ID NO: 179; LCDR2 comprising or consisting of SEQ ID NO: 178; LCDR1 comprising or consisting of SEQ ID NO: 177;

(ii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 182; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1
25 comprising or consisting of SEQ ID NO: 180;

(iii) HCDR3 comprising or consisting of SEQ ID NO: 168; HCDR2 comprising or consisting of SEQ ID NO: 167; HCDR1 comprising or consisting of SEQ ID NO: 166; LCDR3 comprising or consisting of SEQ ID NO: 185; LCDR2 comprising or consisting of SEQ ID NO: 184; LCDR1 comprising or consisting of SEQ ID NO: 183;

30 (iv) HCDR3 comprising or consisting of SEQ ID NO: 171; HCDR2 comprising or consisting of SEQ ID NO: 170; HCDR1 comprising or consisting of SEQ ID NO: 169; LCDR3 comprising or consisting of SEQ ID NO: 187; LCDR2 comprising or consisting of SEQ ID NO: 186; LCDR1 comprising or consisting of SEQ ID NO: 180;

(v) HCDR3 comprising or consisting of SEQ ID NO: 174; HCDR2 comprising or consisting of
35 SEQ ID NO: 173; HCDR1 comprising or consisting of SEQ ID NO: 172; LCDR3 comprising or consisting of SEQ ID NO: 189; LCDR2 comprising or consisting of SEQ ID NO: 188; LCDR1 comprising or consisting of SEQ ID NO: 180;

(vi) HCDR3 comprising or consisting of SEQ ID NO: 176; HCDR2 comprising or consisting of SEQ ID NO: 175; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 192; LCDR2 comprising or consisting of SEQ ID NO: 191; LCDR1 comprising or consisting of SEQ ID NO: 190; and

5 (vii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 193; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1 comprising or consisting of SEQ ID NO: 180.

10 In certain embodiments, the antibody or antigen binding fragment thereof comprises a combination of a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the following:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 194 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

15 sequence of SEQ ID NO: 195 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 196 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 197 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99%

20 identical thereto;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 198 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 199 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

25 (iv) a VH comprising the amino acid sequence of SEQ ID NO: 200 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 201 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(v) a VH comprising the amino acid sequence of SEQ ID NO: 202 or an amino acid sequence at

30 least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 203 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(vi) a VH comprising the amino acid sequence of SEQ ID NO: 204 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

35 sequence of SEQ ID NO: 205 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and

(vii) a VH comprising the amino acid sequence of SEQ ID NO: 206 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

sequence of SEQ ID NO: 207 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

For embodiments wherein the domains of the antibodies or antigen binding fragments are defined by a particular percentage sequence identity to a reference sequence, the VH and/or VL domains may retain identical CDR sequences to those present in the reference sequence such that the variation is present only within the framework regions.

In certain embodiments, the antibody is a VHH antibody comprising CDR sequences selected from the group consisting of:
(i) CDR3 comprising or consisting of SEQ ID NO: 210; CDR2 comprising or consisting of SEQ ID NO: 209; CDR1 comprising or consisting of SEQ ID NO: 208;
(ii) CDR3 comprising or consisting of SEQ ID NO: 213; CDR2 comprising or consisting of SEQ ID NO: 212; CDR1 comprising or consisting of SEQ ID NO: 211;
(iii) CDR3 comprising or consisting of SEQ ID NO: 216; CDR2 comprising or consisting of SEQ ID NO: 215; CDR1 comprising or consisting of SEQ ID NO: 214;
(iv) CDR3 comprising or consisting of SEQ ID NO: 219; CDR2 comprising or consisting of SEQ ID NO: 218; CDR1 comprising or consisting of SEQ ID NO: 217;
(v) CDR3 comprising or consisting of SEQ ID NO: 222; CDR2 comprising or consisting of SEQ ID NO: 221; CDR1 comprising or consisting of SEQ ID NO: 220;
(vi) CDR3 comprising or consisting of SEQ ID NO: 225; CDR2 comprising or consisting of SEQ ID NO: 224; CDR1 comprising or consisting of SEQ ID NO: 223;
(vii) CDR3 comprising or consisting of SEQ ID NO: 228; CDR2 comprising or consisting of SEQ ID NO: 227; CDR1 comprising or consisting of SEQ ID NO: 226;
(viii) CDR3 comprising or consisting of SEQ ID NO: 231; CDR2 comprising or consisting of SEQ ID NO: 230; CDR1 comprising or consisting of SEQ ID NO: 229;
(ix) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
(x) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xi) CDR3 comprising or consisting of SEQ ID NO: 238; CDR2 comprising or consisting of SEQ ID NO: 237; CDR1 comprising or consisting of SEQ ID NO: 232;
(xii) CDR3 comprising or consisting of SEQ ID NO: 241; CDR2 comprising or consisting of SEQ ID NO: 240; CDR1 comprising or consisting of SEQ ID NO: 239;
(xiii) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xiv) CDR3 comprising or consisting of SEQ ID NO: 244; CDR2 comprising or consisting of SEQ ID NO: 243; CDR1 comprising or consisting of SEQ ID NO: 242;

(xv) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
(xvi) CDR3 comprising or consisting of SEQ ID NO: 247; CDR2 comprising or consisting of SEQ ID NO: 246; CDR1 comprising or consisting of SEQ ID NO: 245; and
5 (xvii) CDR3 comprising or consisting of SEQ ID NO: 249; CDR2 comprising or consisting of SEQ ID NO: 248; CDR1 comprising or consisting of SEQ ID NO: 217.

In certain embodiments, the antibody is a VHH antibody wherein the VHH domain comprises or consists of the amino acid sequence represented by any one of SEQ ID NOs: 250, 251, 252,
10 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265 or 266, or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

For embodiments wherein the VHH domains are defined by a particular percentage sequence identity to a reference sequence, the VHH domain may retain identical CDR sequences to those
15 present in the reference sequence such that the variation is present only within the framework regions.

The invention further provides an antibody or antigen binding fragment thereof, which binds to the same epitope as the antibodies or antigen binding fragments defined herein with reference to
20 specific SEQ ID NOs. Also provided are isolated polynucleotides encoding the antibodies or antigen binding fragments thereof, including polynucleotides encoding the VH and/or VL domains of the antibodies and antigen binding fragments described herein. The invention further provides an expression vector comprising the afore-mentioned polynucleotides operably linked to regulatory sequences which permit expression of the antibody, antigen binding fragment,
25 variable heavy chain domain or variable light chain domain in a host cell or cell-free expression system. Also provided are host cells or cell-free expression systems containing the afore-mentioned expression vectors.

The present invention also provides a pharmaceutical composition comprising an antagonist in
30 accordance with the first aspect of the invention, particularly an antibody or antigen binding fragment thereof, and at least one pharmaceutically acceptable carrier or excipient.

Further provided is an antagonist in accordance with the first aspect of the invention, particularly an antibody or antigen binding fragment thereof, or a pharmaceutical composition in accordance
35 with the invention for use as a medicament.

In a further aspect, the present invention provides a method of treating a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of

an antagonist according to the first aspect of the invention, particularly an antibody or antigen binding fragment thereof, or a pharmaceutical composition in accordance with the invention. The antagonist, antibody, antigen binding fragment or pharmaceutical composition may be administered to treat a disease or condition associated with the presence or formation of
5 galectin-10 crystals. In certain embodiments, the disease or condition is selected from: asthma; chronic rhinosinusitis; celiac disease; helminth infection; gastrointestinal eosinophilic inflammation; cystic fibrosis (CF); allergic bronchopulmonary aspergillosis (ABPA); Churg-Straus vasculitis; chronic eosinophilic pneumonia; and acute myeloid leukemia. In preferred embodiments, the antagonists, antibodies, antigen binding fragments or pharmaceutical
10 compositions are administered to treat asthma.

The present invention also provides use of an antagonist according to the first aspect of the invention, particularly an antibody or antigen binding fragment thereof, for the detection of galectin-10 in a sample obtained from a patient. The antagonist, antibody or antigen binding
15 fragment is preferably used for the detection of crystalline galectin-10. The patient sample may be a sputum sample.

The invention also provides a kit comprising a galectin-10 antagonist in accordance with the first aspect of the invention, preferably a galectin-10 antibody or antigen binding fragment thereof,
20 and optionally instructions for use.

BRIEF DESCRIPTION OF DRAWINGS

25 **Figure 1: Production of recombinant Gal10 crystals resembling in vivo CLC crystals**
His-tagged Gal10 was expressed in E coli. (A) SDS Page of His-tagged Gal10 before and after addition of TEV protease to remove the His-Tag. (B) Multi-angle laser scattering (MALLS) reveals a molecular mass of appr. 40 kDa, representing a dimeric form of His-tagged Gal10 in solution. (C) After cleavage by TEV protease, the protein solution spontaneously crystallizes into
30 needle shaped crystals. (D) Original drawing of Charcot (1853) describing the various shapes of crystals seen in the airways of asthmatics. (E) Snapshots of various forms of crystals taken form a fluorescently labeled batch of recombinant Gal10 crystals. All macroscopic forms of crystals originally described by Charcot and von Leyden are found.

35 **Figure 2: Isolation and crystal structure of *in vivo* grown CLC crystals of sinusitis patients**
(A) Mucus samples were collected during surgery of patients with chronic rhinosinusitis and nasal polyps (CRSwNP). Polyp tissue is shown here. (B) Immunostaining for Gal10 shows copious amounts of crystalline material in the allergic sticky mucin. Similar mucus is also found

in the airways of asthmatics and ABPA patients. (C) *Ex vivo* obtained crystal mounted for X-ray diffraction studies. (D) X-ray diffraction pattern of a patient derived crystal. (E) Crystal structure of patient derived CLC crystal reveals a dimeric nature. (F) comparison of the obtained *ex vivo* crystal structure to recombinantly-produced Gal10 crystals and published recrystallized CLC
5 crystals obtained from a human eosinophil cell line (AML14.3D10) reveals complete similarity (root mean square of distance (RMSD) difference of 0.2 Angstrom), showing that the recombinant Gal10 crystals are biosimilar to CLCs.

Figure 3: Creation of crystallization resistant Gal10 muteins by detailed analysis of the
10 **crystal packing interface of Gal10 crystals**
(A) and (B) are close-up views of various amino acids closely involved in the crystal packing interface between two adjacent Gal10 dimers. Highlighted amino acids were selected for a mutational analysis and creation of muteins. (C) Spontaneous crystallization experiment of wild-type and mutein Gal10 protein after removal of the His tag by TEV protease. The Tyr69Glu
15 mutein was completely resistant to autocrystallization and was used throughout the paper as crystallization resistant soluble Gal10 mutein. (D) X-ray structure of the Gal10 Tyr69Glu mutein. This structure was used to model the scattering profile in a small-angle X-ray scattering in solution (SAXS) experiment of Tyr69Glu mutein in solution. (E) The experimental data of the SAXS experiment overlap with the modeled data, essentially showing that the mutein forms a
20 dimer in solution. (F) Overlap of the structures of wild type with Y69E Gal10 mutant based on the SAXS data.

Figure 4: Innate airway inflammation induced by Gal10 crystals
C57Bl/6 mice were injected intratracheally with Gal10 crystals, soluble Gal10mut or with control
25 PBS. (a) Number of neutrophils (left panel) and monocytes (right panel) recovered from the lungs 6 and 24 hours after the treatment. (b) Levels of IL-6 (left panel) and TNFα (right panel) in the bronchoalveolar lavage 6 and 24 hours after the treatment. (c) Levels of IL-1β and CCL-2 in the lung 6 and 24 hours after the treatment. NS implies a p value >0.12; * implies a p value <0.033; ** implies a p value <0.002; *** implies a p value <0.0002; **** implies a p value <0.0001.

30

Figure 5: Innate inflammation induced by Gal10 crystals does not depend on the NLRP3 inflammasome
Nlrp3-deficient (left panel) and Caspase1/11-deficient mice (right panel) (deficient mice are referred to as -/-), and their wild type littermates (referred to as +/+) were injected intratracheally
35 with Gal10 crystals or with control PBS. The number of neutrophils recovered from digested lungs of the different mouse strains was determined 6 and 24 hours after the treatment. NS implies a p value >0.12; * implies a p value <0.033; ** implies a p value <0.002; *** implies a p value <0.0002; **** implies a p value <0.0001.

Figure 6: Innate inflammation induced by Gal10 crystals does not depend on TLR4

Toll-like receptor 4 (TLR4)-deficient and their wild type (WT) littermates were injected intratracheally with Gal10 crystals or with control PBS. The number of neutrophils recovered from digested lungs of the different mouse strains was determined 24 hours after the treatment. NS implies a p value >0.12; * implies a p value <0.033; ** implies a p value <0.002; *** implies a p value <0.0002; **** implies a p value <0.0001.

Figure 7: Gal10 crystals boost human asthma features in a humanized model of the disease

(A) Experimental setup illustrating the dosing regimen of house dust mite (HDM) extracts and the different forms of Galectin 10. Peripheral blood mononuclear cells (PBMCs) were injected intraperitoneally. House dust mite (HDM) extracts, Charcot-Leyden crystals (CLC) and the mutated Galectin 10 ($Gal10^{mut}$) were all administered intratracheally. (B) Number of human $CD45^{+}$ leukocytes recovered from the left lungs of mice treated as described under (A). (C) Levels of human IgE measured in the serum of mice treated as described under (A).

Figure 8: Prevention of Gal10 autocrystallization *in vitro* by addition of Gal10 antibodies

(A) Gal10 was allowed to autocrystallize by removal of the HIS tag via TEV protease. This assay was performed in the presence of various Gal10-specific scFv-Fc antibody clones or irrelevant scFv-Fc antibodies, and crystal formation was observed in a crystallization robot. (B) overview of the activity of various scFv-Fc and IgG1 antibodies.

Figure 9: Time lapse images of crystal dissolution by 4 clones of IgG1 antibodies

(A) To study if antibodies can also dissolve existing crystals, clones were added to *in vitro* grown Gal10 crystals, and observed using spinning disk confocal microscopy. The 4 clones all completely dissolved crystals within 90 minutes, whereas irrelevant isotype antibody did not. (B) Kinetic dissolution curve of crystals upon addition of crystal-dissolving antibodies. The total are of refractive crystalline material in the high power view of the spinning disk microscope was integrated and normalized to 1 prior to addition of the crystals.

Figure 10: Crystal structure of Fab fragments of crystal dissolving clones in complex with Gal10

Crystals of a mixture of Fab-fragments and recombinant Gal10 were formed using a crystallization robot, and subsequently analyzed by X-ray diffraction (A-C). The Gal10 crystal structure is depicted as a cartoon model (black). The Light Chain (LC) and Heavy Chain (HC) of the Fab fragments are shown in surface mode with the LC colored white and the HC colored dark grey. (D-F) The three clones from which co-crystallization structure could be obtained all target the crucial Tyr69 residue of Gal10.

Figure 11: Solubilization of CLC crystals in the mucus of CRSwNP patients by antibodies
Fresh sticky allergic mucin of CRSwNP patients was collected during routine sinus surgery, and stored for 2 days prior to addition of crystal dissolving antibodies or isotype control. Crystals could be readily identified in the fresh mucus of patients due to their highly diffractive properties
5 in a spinning disk confocal microscope. Upon addition of crystal dissolving antibodies, the crystals dissolved overnight.

Figure 12: Proof of concept that solubilizing Gal10 crystals *in vivo* reduce key features of asthma in a humanized mouse model
10 (A) Experimental setup illustrating the dosing regimen of house dust mite (HDM) extracts, Galectin 10 crystals and of antibodies. Peripheral blood mononuclear cells (PBMCs) were injected intraperitoneally. House dust mite (HDM) extracts, Galectin 10 crystals, 1D11 antibodies and control antibodies were all administered intratracheally. (B) Hematoxylin Eosin staining of lung sections, (C) Levels of human IgE measured in the serum, (D) Mucin Muc5ac mRNA
15 expression in lungs of mice treated as described under (A), (E) Investigator-blinded quantitative image analysis of number of inflammatory cells extending into a 500 µm perimeter region from the basement membrane, expressed per length of basement membrane, (F) Bronchoconstriction measured as dynamic airway resistance (Rrs) after inhalation of increasing concentrations of the bronchoconstrictor methacoline.

20

Figure 13: Screening of scFv periplasmic extracts by ELISA
The galectin-10 binding capacity of scFv periplasmic extracts was determined by binding ELISA as described herein. Absorbance was measured at 450 nm (reference at 620 nm). For each periplasmic Master plate (PMP), a blank control and negative control (periplasmic extract binding
25 to irrelevant target) were included. The raw data (OD values) were plotted on GraphPad Prism 7.01. A binder was defined as an scFv showed a binding capacity higher than 0.5 OD value on ELISA binding.

Figure 14: Screening of scFv periplasmic extracts using BLI technology
30 The galectin-10 binding capacity of selected scFv periplasmic extracts was analyzed on BLI technology by using an Octet Red96. A capture approach was used, where human and cynomolgus (WGS or REF isoforms) galectin-10-His were immobilized on anti-His1K biosensors before being incubated with diluted selected scFv periplasmic extracts. The off-rate of each scFv clone plotted on GraphPad Prism 7.01.

35

Figure 15: Crystal dissolution by Gal10 IgG1 antibodies
To study if Gal10 antibodies can dissolve existing crystals, clones were added to *in vitro* grown recombinant human Gal10 crystals, and observed using spinning disk confocal microscopy. The

8 clones all completely dissolved crystals over the time-course studied, whereas irrelevant isotype antibody did not.

Figure 16: Crystal structure of Fab fragments of crystal dissolving clones in complex with Gal10

Crystals of a mixture of Fab-fragments and recombinant Gal10 were formed using a crystallization robot, and subsequently analyzed by X-ray diffraction.

Figure 17: Crystal dissolution by Gal10 IgG1 antibodies and Fab fragments

CLC dissolution experiments using pre-formed recombinant human CLCs were carried out over a time-course in the presence of Gal10 mAbs and Gal10 Fabs. The dissolution of crystals was observed using spinning disk confocal microscopy.

Figure 18: Screening of VHH periplasmic extracts by ELISA

The galectin-10 binding capacity of the VHH periplasmic extracts was measured by binding ELISA as described herein. Absorbance was measured at 450 nm (reference at 620 nm). For each periplasmic Master plate (PMP), a blank control and negative control (VHH periplasmic extract binding to irrelevant target) were included. The raw data (OD values) were plotted on GraphPad Prism 7.01.

Figure 19: Screening of VHH periplasmic extracts using BLI technology

The binding capacity of the selected VHH periplasmic extracts was analysed on BLI technology by using an Octet Red96. For this purpose, a capture approach was used, where human and cynomolgus (WGS or YRT isoforms) galectin-10-His were immobilized on anti-His1K biosensors before being incubated with diluted selected VHH periplasmic extracts. The off-rate (1/s) and response (nm) of each VHH clone were plotted on GraphPad Prism 7.01.

Figure 20: Crystal dissolution by Gal10 VHH antibodies

CLC dissolution experiments using pre-formed recombinant human CLCs were carried out over a time-course in the presence of Gal10 VHH antibodies. The dissolution of crystals was observed using spinning disk confocal microscopy.

DETAILED DESCRIPTION

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one skilled in the art in the technical field of the invention.

“Antagonist” – As used herein, the term “antagonist” means any agent capable of binding to galectin-10 and shielding a crystal packing interface. By shielding or “obscuring” a crystal packing interface, the function of the antagonist is to disrupt crystallization of galectin-10 molecules. Antagonists in accordance with the present invention will typically bind specifically or “specifically bind” to galectin-10. The term “specifically bind” refers to the ability of an antagonist to preferentially bind to its target, in this case galectin-10. Agents capable of binding to protein targets, particularly agents capable of exhibiting binding specificity for a given protein target, are known to those skilled in the art. Such agents include but are not limited to small molecule inhibitors, biological molecules including inhibitory peptides, and antibody mimetics such as affibodies, affilins, affitins, adnectins, atrimers, evasins, DARPins, anticalins, avimers, fynomers, versabodies and duocalins. Preferred antagonists in accordance with the present invention are antibodies and antigen binding fragments thereof.

“Antibody” or “Immunoglobulin”- As used herein, the term "immunoglobulin" includes a polypeptide having a combination of two heavy and two light chains whether or not it possesses any relevant specific immunoreactivity. "Antibodies" refer to such assemblies which have significant known specific immunoreactive activity to an antigen of interest (herein galectin-10). The term “galectin-10 antibodies” is used herein to refer to antibodies which exhibit immunological specificity for the galectin-10 protein, including human galectin-10, and in some cases species homologues thereof. Antibodies and immunoglobulins comprise light and heavy chains, with or without an interchain covalent linkage between them. Basic immunoglobulin structures in vertebrate systems are relatively well understood.

The generic term “immunoglobulin” comprises five distinct classes of antibody that can be distinguished biochemically. All five classes of antibodies are within the scope of the present invention. The following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, immunoglobulins comprise two identical light polypeptide chains of molecular weight approximately 23,000 Daltons, and two identical heavy chains of molecular weight 53,000-70,000. The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

The light chains of an antibody are classified as either kappa or lambda (κ,λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host

cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (e.g., γ1-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA, IgD or IgE, respectively. The immunoglobulin subclasses (isotypes) e.g., IgG1, IgG2, IgG3, IgG4, IgA1, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention.

As indicated above, the variable region of an antibody allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain of an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three complementary determining regions (CDRs) on each of the VH and VL chains.

The term “antibody” as used herein is also intended to encompass “VHH antibodies” or “Heavy-chain only antibodies”.

“VHH antibodies” – As used herein the term “VHH antibody” or “heavy-chain only antibody” refers to a type of antibody produced only by species of the *Camelidae* family, which includes camels, llama, alpaca. Heavy chain-only antibodies or VHH antibodies are composed of two heavy chains and are devoid of light chains. Each heavy chain has a variable domain at the N-terminus, and these variable domains are referred to as “VHH” domains in order to distinguish them from the variable domains of the heavy chains of the conventional heterotetrameric antibodies *i.e.* the VH domains, described above.

“Galectin-10” - As used herein, the term “galectin-10” (or Gal10 or Gal-10) refers to the small, hydrophobic glycan binding protein that autocrystallizes to form Charcot-Leyden crystals. Galectin-10 is also referred to as Charcot-Leyden crystal protein (CLCP), eosinophil lysophospholipase and lysolecithin acylhydrolase. The term “galectin-10” is broad enough to cover the human protein and any species homologues. The amino acid sequence of the full-length human galectin-10 is represented by SEQ ID NO: 141 (see below). This sequence corresponds to the sequence deposited in the UniProt database as human galectin-10, accession number Q05315. Also encompassed within the term “galectin-10” are naturally occurring variants of the human sequence, for example the Ala→Val variant on position 28.

SEQ ID NO: 141

```
1        10         20         30         40         50
MSLLPVPYTE AASLSTGSTV TIKGRPLACF LNEPYLQVDF HTEMKEESDI
        60         70         80         90        100
VFHFQVCFGR RVVMNSREYG AWKQQVESKN MPFQDGQEFE LSISVLPDKY
       110        120        130        140
QVMVNGQSSY TFDHRIKPEA VKMVQVWRDI SLTKFNVSYL KR
```

"Galectin-10 crystals" or **"Charcot-Leyden crystals"** – the terms "galectin-10 crystals", "Charcot-Leyden crystals" and "CLCs" are used herein interchangeably to refer to crystals formed of galectin-10. The crystals formed by galectin-10 are typically bi-pyramidal hexagonal crystals and are approximately 20-40 µm in length and approximately 2-4 µm width. These crystals have been associated with eosinophilic inflammatory disorders.

"**Crystal packing interface**" – a "crystal packing interface" of galectin-10 refers to a set of amino acids forming a surface patch on galectin-10 that contacts one or more neighbouring galectin-10 molecules in the crystalline lattice. CLCs have different crystal packing interfaces and the amino acids forming these crystal packing interfaces have been characterised as: Ser2, Leu3, Leu4, Tyr8, Thr9, Glu10, Ala11, Ala12, Ser13, Thr16, Thr42, Glu43, Met44, Lys45, Asp49, Ile50, Glu68, Tyr69, Gly70, Ala71, Lys73, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Leu96, Pro97, Asp98, Lys99, Gln101, Met103, Gly106, Gln107, Ser108, Ser109, Tyr110, Thr111, Asp113, His114, Arg115, Ile116, Lys117, Ala120, Gln125, Thr133, Lys134, Phe135, Asn136, Val137, Ser138, Tyr139, Leu140 and Lys141, wherein the positions are defined with reference to SEQ ID NO: 141 above.

"**Epitope**" – As used herein, the term "epitope" means the region of the galectin-10 protein to which the antagonist binds. An antagonist will typically bind to its respective galectin-10 epitope via a complementary binding site on the antagonist. The epitope to which the antagonist binds will typically comprise one or more amino acids from the full-length galectin-10 protein. The epitope may include amino acids that are contiguous in the galectin-10 protein i.e. a linear epitope or may include amino acids that are non-contiguous in the galectin-10 protein i.e. a conformational epitope.

"Binding Site" - As used herein, the term "binding site" comprises a region of a polypeptide which is responsible for selectively binding to a target antigen of interest (e.g. galectin-10). Binding domains comprise at least one binding site. Exemplary binding domains include an antibody variable domain. The antibody molecules of the invention may comprise a single binding site or multiple (e.g., two, three or four) binding sites.

"Derived From" - As used herein the term "derived from" a designated protein (e.g. a camelid antibody or antigen binding fragment thereof) refers to the origin of the polypeptide or amino acid sequence. In one embodiment, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide is a CDR sequence or sequence related thereto. In one embodiment, the amino acid sequence which is derived from a particular starting polypeptide is not contiguous. For example, in one embodiment, one, two, three, four, five, or six CDRs are derived from a starting antibody. In one embodiment, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide or amino acid sequence has an amino acid sequence that is essentially identical to that of the starting sequence, or a portion thereof wherein the portion consists of at least 3-5 amino acids, at least 5-10 amino acids, at least 10-20 amino acids, at least 20-30 amino acids, or at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the starting sequence. In one embodiment, the one or more CDR sequences derived from the starting antibody are altered to produce variant CDR sequences, e.g. affinity variants, wherein the variant CDR sequences maintain target antigen binding activity.

"Camelid-Derived" - In certain preferred embodiments, the antibodies of the invention comprise framework amino acid sequences and/or CDR amino acid sequences derived from a camelid conventional antibody or a VHH antibody raised by active immunisation of a camelid. However, antibodies of the invention comprising camelid-derived amino acid sequences may be engineered to comprise framework and/or constant region sequences derived from a human amino acid sequence (i.e. a human antibody) or other non-camelid mammalian species. For example, a human or non-human primate framework region, heavy chain portion, and/or hinge portion may be included in the galectin-10 antibodies. In one embodiment, one or more non-camelid amino acids may be present in the framework region of a "camelid-derived" antibody, e.g., a camelid framework amino acid sequence may comprise one or more amino acid mutations in which the corresponding human or non-human primate amino acid residue is present. Moreover, camelid-derived VH and VL domains, or humanised variants thereof, may be linked to the constant domains of human antibodies to produce a chimeric molecule, as described elsewhere herein.

"Conservative amino acid substitution" - A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine,

isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide may be replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

"Heavy chain portion" - As used herein, the term "heavy chain portion" includes amino acid sequences derived from the constant domains of an immunoglobulin heavy chain. A polypeptide comprising a heavy chain portion comprises at least one of: a CH1 domain, a hinge (e.g., upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. In one embodiment, an antibody or antigen binding fragment of the invention may comprise the Fc portion of an immunoglobulin heavy chain (e.g., a hinge portion, a CH2 domain, and a CH3 domain). In another embodiment, an antibody or antigen binding fragment of the invention may lack at least a portion of a constant domain (e.g., all or part of a CH2 domain). In certain embodiments, at least one, and preferably all, of the constant domains are derived from a human immunoglobulin heavy chain. For example, in one preferred embodiment, the heavy chain portion comprises a fully human hinge domain. In other preferred embodiments, the heavy chain portion comprises a fully human Fc portion (e.g., hinge, CH2 and CH3 domain sequences from a human immunoglobulin).

In certain embodiments, the constituent constant domains of the heavy chain portion are from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide may comprise a CH2 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 or IgG4 molecule. In other embodiments, the constant domains are chimeric domains comprising portions of different immunoglobulin molecules. For example, a hinge may comprise a first portion from an IgG1 molecule and a second portion from an IgG3 or IgG4 molecule. As set forth above, it will be understood by one of ordinary skill in the art that the constant domains of the heavy chain portion may be modified such that they vary in amino acid sequence from the naturally occurring (wild-type) immunoglobulin molecule. That is, the polypeptides of the invention disclosed herein may comprise alterations or modifications to one or more of the heavy chain constant domains (CH1, hinge, CH2 or CH3) and/or to the light chain constant region domain (CL). Exemplary modifications include additions, deletions or substitutions of one or more amino acids in one or more domains.

"Chimeric" - A "chimeric" protein comprises a first amino acid sequence linked to a second amino acid sequence with which it is not naturally linked in nature. The amino acid sequences may normally exist in separate proteins that are brought together in the fusion polypeptide or they may normally exist in the same protein but are placed in a new arrangement in the fusion

polypeptide. A chimeric protein may be created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship. Exemplary chimeric antibodies of the invention include fusion proteins comprising camelid-derived VH and VL domains, or humanised variants thereof, fused to the constant domains of a human antibody, e.g. human IgG1, IgG2, IgG3 or IgG4.

“Variable region” or “variable domain” - The terms "variable region" and "variable domain" are used herein interchangeably and are intended to have equivalent meaning. The term "variable" refers to the fact that certain portions of the variable domains VH and VL differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its target antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called "hypervariable loops" in each of the VL domain and the VH domain which form part of the antigen binding site. The first, second and third hypervariable loops of the VLambda light chain domain are referred to herein as L1(λ), L2(λ) and L3(λ) and may be defined as comprising residues 24-33 (L1(λ), consisting of 9, 10 or 11 amino acid residues), 49-53 (L2(λ), consisting of 3 residues) and 90-96 (L3(λ), consisting of 5 residues) in the VL domain (Morea *et al.*, Methods 20:267-279 (2000)). The first, second and third hypervariable loops of the VKappa light chain domain are referred to herein as L1(κ), L2(κ) and L3(κ) and may be defined as comprising residues 25-33 (L1(κ), consisting of 6, 7, 8, 11, 12 or 13 residues), 49-53 (L2(κ), consisting of 3 residues) and 90-97 (L3(κ), consisting of 6 residues) in the VL domain (Morea *et al.*, Methods 20:267-279 (2000)). The first, second and third hypervariable loops of the VH domain are referred to herein as H1, H2 and H3 and may be defined as comprising residues 25-33 (H1, consisting of 7, 8 or 9 residues), 52-56 (H2, consisting of 3 or 4 residues) and 91-105 (H3, highly variable in length) in the VH domain (Morea *et al.*, Methods 20:267-279 (2000)).

Unless otherwise indicated, the terms L1, L2 and L3 respectively refer to the first, second and third hypervariable loops of a VL domain, and encompass hypervariable loops obtained from both Vkappa and Vlambda isotypes. The terms H1, H2 and H3 respectively refer to the first, second and third hypervariable loops of the VH domain, and encompass hypervariable loops obtained from any of the known heavy chain isotypes, including γ, ε, δ, α or μ.

The hypervariable loops L1, L2, L3, H1, H2 and H3 may each comprise part of a "complementarity determining region" or "CDR", as defined below. The terms "hypervariable loop" and "complementarity determining region" are not strictly synonymous, since the hypervariable loops (HVs) are defined on the basis of structure, whereas complementarity determining regions (CDRs) are defined based on sequence variability (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health,

Bethesda, MD., 1983) and the limits of the HVs and the CDRs may be different in some VH and VL domains.

The CDRs of the VL and VH domains can typically be defined as comprising the following amino acids: residues 24-34 (LCDR1), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable domain, and residues 31-35 or 31-35b (HCDR1), 50-65 (HCDR2) and 95-102 (HCDR3) in the heavy chain variable domain; (Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Thus, the HVs may be comprised within the corresponding CDRs and references herein to the "hypervariable loops" of VH and VL domains should be interpreted as also encompassing the corresponding CDRs, and vice versa, unless otherwise indicated.

The more highly conserved portions of variable domains are called the framework region (FR), as defined below. The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β-sheet configuration, connected by the three hypervariable loops. The hypervariable loops in each chain are held together in close proximity by the FRs and, with the hypervariable loops from the other chain, contribute to the formation of the antigen binding site of antibodies. Structural analysis of antibodies revealed the relationship between the sequence and the shape of the binding site formed by the complementarity determining regions (Chothia *et al.*, J. Mol. Biol. 227: 799-817 (1992)); Tramontano *et al.*, J. Mol. Biol, 215:175-182 (1990)). Despite their high sequence variability, five of the six loops adopt just a small repertoire of main-chain conformations, called "canonical structures". These conformations are first of all determined by the length of the loops and secondly by the presence of key residues at certain positions in the loops and in the framework regions that determine the conformation through their packing, hydrogen bonding or the ability to assume unusual main-chain conformations.

"CDR" - As used herein, the term "CDR" or "complementarity determining region" means the non-contiguous antigen binding sites found within the variable region of both heavy and light chain polypeptides. These particular regions have been described by Kabat *et al.*, J. Biol. Chem. 252, 6609-6616 (1977) and Kabat *et al.*, Sequences of protein of immunological interest. (1991), and by Chothia *et al.*, J. Mol. Biol. 196:901-917 (1987) and by MacCallum *et al.*, J. Mol. Biol. 262:732-745 (1996) where the definitions include overlapping or subsets of amino acid residues when compared against each other. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth for comparison. Preferably, the term "CDR" is a CDR as defined by Kabat based on sequence comparisons.

Table 1: CDR definitions

	CDR Definitions		
	Kabat[1]	Chothia[2]	MacCallum[3]
V_H CDR1	31-35	26-32	30-35
V_H CDR2	50-65	53-55	47-58
V_H CDR3	95-102	96-101	93-101
V_L CDR1	24-34	26-32	30-36
V_L CDR2	50-56	50-52	46-55
V_L CDR3	89-97	91-96	89-96

[1]Residue numbering follows the nomenclature of Kabat *et al*., supra

[2]Residue numbering follows the nomenclature of Chothia *et al*., supra

[3]Residue numbering follows the nomenclature of MacCallum *et al*., supra

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"Framework region" - The term "framework region" or "FR region" as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (e.g., using the Kabat definition of CDRs). Therefore, a variable region framework is between about 100-120 amino acids in length but includes only those amino acids outside of the CDRs. For the specific
10 example of a heavy chain variable domain and for the CDRs as defined by Kabat *et al*., framework region 1 corresponds to the domain of the variable region encompassing amino acids 1-30; framework region 2 corresponds to the domain of the variable region encompassing amino acids 36-49; framework region 3 corresponds to the domain of the variable region encompassing amino acids 66-94, and framework region 4 corresponds to the domain of the variable region
15 from amino acids 103 to the end of the variable region. The framework regions for the light chain are similarly separated by each of the light chain variable region CDRs. Similarly, using the definition of CDRs by Chothia *et al*. or McCallum *et al*. the framework region boundaries are separated by the respective CDR termini as described above. In preferred embodiments the CDRs are as defined by Kabat.

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In naturally occurring antibodies, the six CDRs present on each monomeric antibody are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding site as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the heavy and light variable domains show less inter-molecular
25 variability in amino acid sequence and are termed the framework regions. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, these framework regions act to form a scaffold that provides for positioning the six CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding site formed by the positioned CDRs defines a surface
30 complementary to the epitope on the immunoreactive antigen. This complementary surface

promotes the non-covalent binding of the antibody to the immunoreactive antigen epitope. The position of CDRs can be readily identified by one of ordinary skill in the art.

“Hinge region” - As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux K.H. *et al.* J. Immunol. 161:4083-90 1998). Antibodies of the invention comprising a “fully human” hinge region may contain one of the hinge region sequences shown in Table 2 below.

Table 2: Human hinge sequences

IgG	Upper hinge	Middle hinge	Lower hinge
IgG1	EPKSCDKTHT (SEQ ID NO:142)	CPPCP (SEQ ID NO:143)	APELLGGP (SEQ ID NO: 144)
IgG3	ELKTPLGDTTHT (SEQ ID NO: 145)	CPRCP $(EPKSCDTPPPCPRCP)_3$ (SEQ ID NO: 146)	APELLGGP (SEQ ID NO: 147)
IgG4	ESKYGPP (SEQ ID NO: 148)	CPSCP (SEQ ID NO: 149)	APEFLGGP (SEQ ID NO: 150)
IgG42	ERK (SEQ ID NO: 151)	CCVECPPPCP (SEQ ID NO: 152)	APPVAGP (SEQ ID NO: 153)

“CH2 domain” - As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, e.g., from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system, Kabat EA *et al.* Sequences of Proteins of Immunological Interest. Bethesda, US Department of Health and Human Services, NIH. 1991). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

“Fragment” - The term “fragment”, as used in the context of antibodies of the invention, refers to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain. The term “antigen binding fragment” refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen binding (i.e., specific binding to galectin-10). As used herein, the term “fragment” of an antibody

molecule includes antigen binding fragments of antibodies, for example, an antibody light chain variable domain (VL), an antibody heavy chain variable domain (VH), a single chain antibody (scFv), a F(ab')2 fragment, a Fab fragment, an Fd fragment, an Fv fragment, a one-armed (monovalent) antibody, diabodies, triabodies, tetrabodies or any antigen binding molecule formed by combination, assembly or conjugation of such antigen binding fragments. The term "antigen binding fragment" as used herein is further intended to encompass antibody fragments selected from the group consisting of unibodies, domain antibodies and nanobodies. Fragments can be obtained, e.g., via chemical or enzymatic treatment of an intact or complete antibody or antibody chain or by recombinant means.

"Fab" - A "Fab" or "Fab fragment" refers to a molecule composed of a heavy chain and light chain wherein the light chain consists of the VL domain and the one constant domain (CL, Cκ or Cλ) and the heavy chain consists of the VH domain and the CH1 domain only. A Fab fragment is typically one arm of a Y-shaped immunoglobulin molecule. A Fab fragment can be generated from an immunoglobulin molecule by the action of the enzyme papain. Papain cleaves immunoglobulin molecules in the region of the hinge so as yield two Fab fragments and a separate Fc region.

"scFv" or "scFv fragment" – An scFv or scFv fragment means a single chain variable fragment. An scFv is a fusion protein of a VH domain and a VL domain of an antibody connected via a linker.

"Valency"- As used herein the term "valency" refers to the number of potential target binding sites in a polypeptide. Each target binding site specifically binds one target molecule or specific site on a target molecule. When a polypeptide comprises more than one target binding site, each target binding site may specifically bind the same or different molecules (e.g., may bind to different ligands or different antigens, or different epitopes on the same antigen).

"Specificity" - The term "specificity" refers to the ability to bind (e.g., immunoreact with) a given target, e.g. galectin-10. A polypeptide may be monospecific and contain one or more binding sites which specifically bind a target or a polypeptide may be multispecific and contain two or more binding sites which specifically bind the same or different targets.

"Synthetic" - As used herein the term "synthetic" with respect to polypeptides includes polypeptides which comprise an amino acid sequence that is not naturally occurring. For example, non-naturally occurring polypeptides which are modified forms of naturally occurring polypeptides (e.g., comprising a mutation such as an addition, substitution or deletion) or which comprise a first amino acid sequence (which may or may not be naturally occurring) that is linked

in a linear sequence of amino acids to a second amino acid sequence (which may or may not be naturally occurring) to which it is not naturally linked in nature.

“Engineered” - As used herein the term “engineered” includes manipulation of nucleic acid or polypeptide molecules by synthetic means (e.g. by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques). Preferably, the antibodies of the invention are engineered, including for example, humanized and/or chimeric antibodies, and antibodies which have been engineered to improve one or more properties, such as antigen binding, stability/half-life or effector function.

“Modified antibody” - As used herein, the term “modified antibody” includes synthetic forms of antibodies which are altered such that they are not naturally occurring, e.g., antibodies that comprise at least two heavy chain portions but not two complete heavy chains (such as, domain deleted antibodies or minibodies); multispecific forms of antibodies (e.g., bispecific, trispecific, etc.) altered to bind to two or more different antigens or to different epitopes on a single antigen); heavy chain molecules joined to scFv molecules and the like. scFv molecules are known in the art and are described, e.g., in US patent 5,892,019. In addition, the term “modified antibody” includes multivalent forms of antibodies (e.g., trivalent, tetravalent, etc., antibodies that bind to three or more copies of the same antigen). In another embodiment, a modified antibody of the invention is a fusion protein comprising at least one heavy chain portion lacking a CH2 domain and comprising a binding domain of a polypeptide comprising the binding portion of one member of a receptor ligand pair.

The term “modified antibody” may also be used herein to refer to amino acid sequence variants of the antibodies of the invention as structurally defined herein. It will be understood by one of ordinary skill in the art that an antibody may be modified to produce a variant antibody which varies in amino acid sequence in comparison to the antibody from which it was derived. For example, nucleotide or amino acid substitutions leading to conservative substitutions or changes at "non-essential" amino acid residues may be made (e.g., in CDR and/or framework residues). Amino acid substitutions can include replacement of one or more amino acids with a naturally occurring or non-natural amino acid.

“Humanising substitutions” - As used herein, the term “humanising substitutions” refers to amino acid substitutions in which the amino acid residue present at a particular position in the VH or VL domain of an antibody (for example a camelid-derived galectin-10 antibody) is replaced with an amino acid residue which occurs at an equivalent position in a reference human VH or VL domain. The reference human VH or VL domain may be a VH or VL domain encoded by the

human germline. Humanising substitutions may be made in the framework regions and/or the CDRs of the antibodies, defined herein.

"Humanised variants" - As used herein the term "humanised variant" refers to a variant antibody which contains one or more "humanising substitutions" compared to a reference antibody, wherein a portion of the reference antibody (e.g. the VH domain and/or the VL domain or parts thereof containing at least one CDR) has an amino acid derived from a non-human species, and the "humanising substitutions" occur within the amino acid sequence derived from a non-human species.

"Germlined variants" - The term "germlined variant" is used herein to refer specifically to "humanised variants" in which the "humanising substitutions" result in replacement of one or more amino acid residues present at a particular position (s) in the VH or VL domain of an antibody (for example a camelid-derived galectin-10 antibody) with an amino acid residue which occurs at an equivalent position in a reference human VH or VL domain encoded by the human germline. It is typical that for any given "germlined variant", the replacement amino acid residues substituted *into* the germlined variant are taken exclusively, or predominantly, from a single human germline-encoded VH or VL domain. The terms "humanised variant" and "germlined variant" are often used interchangeably herein. Introduction of one or more "humanising substitutions" into a camelid-derived (e.g. llama derived) VH or VL domain results in production of a "humanised variant" of the camelid (llama)-derived VH or VL domain. If the amino acid residues substituted in are derived predominantly or exclusively from a single human germline-encoded VH or VL domain sequence, then the result may be a "human germlined variant" of the camelid (llama)-derived VH or VL domain.

"Affinity variants" - As used herein, the term "affinity variant" refers to a variant antibody which exhibits one or more changes in amino acid sequence compared to a reference antibody, wherein the affinity variant exhibits an altered affinity for the target antigen in comparison to the reference antibody. For example, affinity variants will exhibit a changed affinity for galectin-10, as compared to the reference galectin-10 antibody. Preferably the affinity variant will exhibit *improved* affinity for the target antigen, e.g. galectin-10, as compared to the reference antibody. Affinity variants typically exhibit one or more changes in amino acid sequence in the CDRs, as compared to the reference antibody. Such substitutions may result in replacement of the original amino acid present at a given position in the CDRs with a different amino acid residue, which may be a naturally occurring amino acid residue or a non-naturally occurring amino acid residue. The amino acid substitutions may be conservative or non-conservative.

"High human homology" - An antibody comprising a heavy chain variable domain (VH) and a light chain variable domain (VL) may be considered as having high human homology if the VH domains and the VL domains, taken together, exhibit at least 90% amino acid sequence identity to the closest matching human germline VH and VL sequences. Antibodies having high human homology may include antibodies comprising VH and VL domains of native non-human antibodies which exhibit sufficiently high % sequence identity to human germline sequences, including for example antibodies comprising VH and VL domains of camelid conventional antibodies, as well as engineered, especially humanised or germlined, variants of such antibodies and also "fully human" antibodies.

In one embodiment the VH domain of the antibody with high human homology may exhibit an amino acid sequence identity or sequence homology of 80% or greater with one or more human VH domains across the framework regions FR1, FR2, FR3 and FR4. In other embodiments the amino acid sequence identity or sequence homology between the VH domain of the polypeptide of the invention and the closest matching human germline VH domain sequence may be 85% or greater, 90% or greater, 95% or greater, 97% or greater, or up to 99% or even 100%.

In one embodiment the VH domain of the antibody with high human homology may contain one or more (e.g. 1 to 10) amino acid sequence mis-matches across the framework regions FR1, FR2, FR3 and FR4, in comparison to the closest matched human VH sequence.

In another embodiment the VL domain of the antibody with high human homology may exhibit a sequence identity or sequence homology of 80% or greater with one or more human VL domains across the framework regions FR1, FR2, FR3 and FR4. In other embodiments the amino acid sequence identity or sequence homology between the VL domain of the polypeptide of the invention and the closest matching human germline VL domain sequence may be 85% or greater 90% or greater, 95% or greater, 97% or greater, or up to 99% or even 100%.

In one embodiment the VL domain of the antibody with high human homology may contain one or more (e.g. 1 to 10) amino acid sequence mis-matches across the framework regions FR1, FR2, FR3 and FR4, in comparison to the closest matched human VL sequence.

B. Galectin-10 Antagonists

In a first aspect, the present invention provides an antagonist which binds to galectin-10, wherein the antagonist binds to an epitope of galectin-10 and thereby shields a crystal packing interface of galectin-10. The present invention further provides an antagonist that binds to galectin-10,

which, when bound to soluble galectin-10, inhibits the crystallization of galectin-10. The present invention further provides an antagonist that binds to galectin-10, which, when bound to crystalline galectin-10, promotes the dissolution of crystalline galectin-10. The antagonists of the invention preferably bind to human galectin-10.

The protein galectin-10 is a relatively small (16.5kDa) glycan-binding protein. Galectin-10 proteins form dimers in solution and can also form insoluble hexagonal bipyramidal crystals. These crystals were first observed in patients with allergic asthma and parasitic infections, and are otherwise known as Charcot-Leyden crystals (or CLCs).

The antagonists of the present invention bind to an epitope of galectin-10. The epitope may be a linear epitope i.e. it may consist of two or more consecutive amino acids in the galectin-10 primary protein sequence. Alternatively, the epitope may be a conformational epitope comprising or consisting of two or more amino acids that are not located adjacent to each other in the galectin-10 primary protein sequence. For embodiments in which the antagonist binds to a conformational epitope, the two or more amino acids of the epitope will typically be located in close proximity within the 3-dimensional structure of the galectin-10 protein. The epitopes to which the galectin-10 antagonists of the invention bind may comprise or consist of at least two amino acids, at least three amino acids, at least four amino acids, at least five amino acids, at least six amino acids, at least seven amino acids. In certain embodiments, the epitopes to which the galectin-10 antagonists bind comprise or consist of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids.

The antagonists of the invention bind to an epitope of galectin-10 and thereby shield a crystal packing interface of galectin-10. As defined elsewhere herein, a crystal packing interface of galectin-10 is a surface patch of amino acids that contacts one or more neighbouring galectin-10 molecules in the crystalline lattice. By binding to an epitope of galectin-10 that serves to shield a crystal packing interface of galectin-10, the antagonists of the invention disrupt the crystallization of galectin-10. It follows, that the antagonists of the invention may shield a crystal packing interface fully or partially, provided that the antagonist disrupts the crystallization of galectin-10. In certain embodiments, the antagonists, when bound to soluble galectin-10, inhibit crystallization of galectin-10. In certain embodiments, the antagonists, when bound to crystalline galectin-10, promote dissolution of galectin-10.

The antagonistic properties of the galectin-10 antagonists described herein may be measured in accordance with the assays described herein. For example, galectin-10 antagonists, including galectin-10 antibodies, may be incubated with soluble galectin-10 under experimental conditions that favour galectin-10 crystallization and the ability of the antagonists to inhibit this process may

be measured. The inhibitory activity of the galectin-10 antagonists may be measured relative to a control, for example an antagonist that does not bind to galectin-10. The inhibitory activity of the galectin-10 antagonists may also be measured relative to a control that is a galectin-10 binding molecule without crystallization inhibitory activity. The galectin-10 antagonists may 5 inhibit crystallization of galectin-10 by 100% relative to control, by 90% relative to control, by 80% relative to control, by 70% relative to control.

Alternatively, galectin-10 antagonists, including galectin-10 antibodies and antigen binding fragments, may be incubated with pre-formed galectin-10 crystals and the ability of the 10 antagonists to dissolve the crystals may be measured over a suitable time-course. The galectin-10 crystals may be recombinant crystals formed from recombinant galectin-10 produced *in vitro*. Alternatively, the galectin-10 crystals may be crystals obtained from a patient sample, for example crystals obtained from polyps within the nasal or sinus cavities of a patient. In certain embodiments, the galectin-10 antagonists of the invention may be capable of dissolving pre-15 formed galectin-10 crystals over a period of up to 10 hours, up to 12 hours, up to 14 hours, up to 16 hours, up to 18 hours, up to 20 hours. The galectin-10 antagonists may dissolve the crystals completely i.e. by 100%. Alternatively, the galectin-10 antagonists may dissolve the crystals such that over 50% of the crystals are dissolved, over 60% or the crystals are dissolved, over 70% of the crystals are dissolved, over 80% of the crystals are dissolved, over 90% of the 20 crystals are dissolved over the time-course.

In certain embodiments, the galectin-10 antagonist shields a crystal packing interface by binding to an epitope comprising one or more amino acids from the crystal packing interfaces of galectin-10. The amino acids of galectin-10 that form the crystal packing interfaces are typically identified 25 as: Ser2, Leu3, Leu4, Tyr8, Thr9, Glu10, Ala11, Ala12, Ser13, Thr16, Thr42, Glu43, Met44, Lys45, Asp49, Ile50, Glu68, Tyr69, Gly70, Ala71, Lys73, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Leu96, Pro97, Asp98, Lys99, Gln101, Met103, Gly106, Gln107, Ser108, Ser109, Tyr110, Thr111, Asp113, His114, Arg115, Ile116, Lys117, Ala120, Gln125, Thr133, Lys134, Phe135, Asn136, Val137, Ser138, Tyr139, Leu140 and Lys141, wherein the positions 30 are defined with reference to SEQ ID NO: 141. Thus, in certain embodiments, the galectin-10 antagonists of the invention bind to an epitope comprising one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more amino acids selected from the group consisting of: Ser2, Leu3, Leu4, Tyr8, Thr9, Glu10, Ala11, Ala12, Ser13, Thr16, Thr42, Glu43, Met44, Lys45, Asp49, Ile50, Glu68, Tyr69, Gly70, 35 Ala71, Lys73, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Leu96, Pro97, Asp98, Lys99, Gln101, Met103, Gly106, Gln107, Ser108, Ser109, Tyr110, Thr111, Asp113, His114, Arg115, Ile116, Lys117, Ala120, Gln125, Thr133, Lys134, Phe135, Asn136, Val137, Ser138, Tyr139, Leu140 and Lys141. In certain embodiments, the epitope consists entirely of amino

acids from the crystal packing interfaces of galectin-10. For example, the epitope may consist of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids from the crystal packing interfaces of galectin-10. Alternatively, the epitope may comprise amino acids from the crystal packing interfaces and additionally comprise at least one amino acid from outside the amino
5 acids of the crystal packing interfaces of galectin-10.

In preferred embodiments, the antagonist binds to an epitope comprising Tyr69. Alternatively or in addition, the antagonist may preferably bind to an epitope comprising an amino acid adjacent to Tyr69, specifically Glu68 or Gly70. In one embodiment, the antagonist binds to an epitope
10 comprising Glu68, Tyr69 and Gly70.

In a particular embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, His114, Arg115, Ile116, Lys117 and Ala120. In a particular embodiment, the antagonist binds to an epitope
15 comprising or consisting of the amino acids: Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, His114, Arg115, Ile116, Lys117, GLu119, Ala120 and Lys122. In a particular embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, Gln74, Asp98, His114, Arg115, Ile116, Lys117, GLu119, Ala120 and Lys122.
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In certain embodiments, the antagonist binds to an epitope comprising the amino acids: Glu33, Gly59, Arg60 and Lys79. The epitope may additionally comprise the amino acids: Gln74, Gln75 and Glu77.

25 In a particular embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Leu31, Glu33, Gly59, Arg60, Ser78, Lys79, Asn80, Met81, Pro82 and Gln84. In a particular embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Glu33, Gly59, Arg60, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Pro82 and Ser109. In a particular embodiment, the antagonist binds to an epitope comprising or
30 consisting of the amino acids: Glu33, Gly59, Arg60, Trp72, Gln74, Gln75, Val76, Glu77, Lys79, Asn80, Met81, Pro82, Gln84 and Ser109. In a particular embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Glu33, Gly59, Arg60, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Pro82, Phe83, Gln84.

35 In alternative embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Thr42, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, Arg115, Ile116, Lys117, Glu119 and Ala120. In another embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Glu43, Asp49, Glu68, Tyr69, Lys73, Asp98, Asp113, His114, Arg115,

Lys117, Glu119 and Ala120. In a further embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Asp49, Glu68, Tyr69, Lys73, Gln74, Asp98, Asp113, His114, Arg115, Ile116 and Lys117. In a further embodiment, the antagonist binds to an epitope comprising or consisting of the amino acids: Ser2, Leu3, Leu4, Pro5, Pro7, Tyr8, Thr9, Glu10, Ala11, Lys23, Arg25, Met44, Gly86, Gln87, Glu88, Phe89, Glu90, Asn105, Gln125, Thr133, Lys134 and Phe135.

The amino acid positions of galectin-10 are identified with respect to the human protein shown as SEQ ID NO: 141.

In certain embodiments, the antagonist binds to an epitope comprising one or more amino acids from the dimerization interface of galectin-10. The amino acids of galectin-10 that form the dimerization domain may differ from the amino acids that participate in the crystal packing interfaces. However, it is possible for an antagonist that binds to amino acids located in the dimerization interface to also shield the crystal packing interfaces and thereby disrupt crystallization of galectin-10. The amino acids of galectin-10 that form the dimerization interface are typically identified as: Pro5, Pro7, Leu27, Ala28, Cys29, Leu31, Asn32, Glu33, Pro34, Tyr35, Gln37, His41, Glu46, Glu47, Gln55, Arg60, Arg61, Arg67, Trp72, Gln75, Trp127, Arg128 and Asp129. Thus, in certain embodiments, the galectin-10 antagonists of the invention bind to an epitope comprising or consisting of one or more, two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more amino acids selected from the group consisting of: Pro5, Pro7, Leu27, Ala28, Cys29, Leu31, Asn32, Glu33, Pro34, Tyr35, Gln37, His41, Glu46, Glu47, Gln55, Arg60, Arg61, Arg67, Trp72, Gln75, Trp127, Arg128 and Asp129. The amino acid positions of galectin-10 are identified with respect to SEQ ID NO: 141.

In certain embodiments, the galectin-10 antagonists of the invention bind to an epitope of galectin-10 comprising one or more amino acids from the crystal packing interfaces and one or more amino acids from the dimerization interface. The one or more amino acids from the crystal packing interfaces and the one or more amino acids from the dimerization interface may be any of the specific amino acids identified above. In preferred embodiments, the antagonists of the invention bind to an epitope comprising Glu68, Tyr69 and Gly70.

C. Galectin-10 antibodies and antigen binding fragments thereof

In preferred embodiments, the galectin-10 antagonists of the present invention are antibodies or antigen binding fragments thereof. The term "antibody" herein is used in the broadest sense and encompasses, but is not limited to, monoclonal antibodies (including full length monoclonal

antibodies), polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), VHH antibodies, so long as they exhibit the appropriate immunological specificity for the galectin-10 protein. The galectin-10 antibodies and antigen binding fragments described herein may exhibit immunological specificity for any of the galectin-10 epitopes described in section B above.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes) on the antigen, each monoclonal antibody is directed against a single determinant or epitope on the antigen. "Antibody fragments" or “antigen binding fragments” comprise a portion of a full length antibody, generally the antigen binding or variable domain thereof. Antibody fragments are described elsewhere herein and examples of antibody fragments include Fab, Fab', F(ab')2, bi-specific Fab’s, and Fv fragments, diabodies, linear antibodies, single-chain antibody molecules, a single chain variable fragment (scFv) and multispecific antibodies formed from antibody fragments (see Holliger and Hudson, Nature Biotechnol. 23:1126-36 (2005), the contents of which are incorporated herein by reference).

The galectin-10 antibodies and antigen binding fragments described herein are intended for human therapeutic use and therefore, will typically be immunoglobulins of the IgA, IgD, IgE, IgG, IgM type, often of the IgG type, in which case they can belong to any of the four sub-classes IgG1, IgG2a and b, IgG3 or IgG4. In preferred embodiments, the galectin-10 antibodies are IgG antibodies. Particularly preferred are IgG1 antibodies. Monoclonal antibodies are preferred since they are highly specific, being directed against a single antigenic site. In certain preferred embodiments, the galectin-10 antigen binding fragments are Fab fragments or “Fabs”.

The galectin-10 antibodies and antigen binding fragments thereof may exhibit high human homology as defined elsewhere herein. Such antibody molecules having high human homology may include antibodies comprising VH and VL domains of native non-human antibodies which exhibit sufficiently high % sequence identity to human germline sequences. In certain embodiments, the antibodies or antigen binding fragments thereof are humanised or germlined variants of non-human antibodies.

In certain embodiments, the galectin-10 antibodies and antigen binding fragments described herein may be camelid-derived. Camelid-derived antibodies may be heavy-chain only antibodies i.e. VHH antibodies or may be conventional heterotetrameric antibodies. In preferred

embodiments, the galectin-10 antibodies and antigen binding fragments are derived from camelid heterotetrameric antibodies. In further preferred embodiments, the galectin-10 antibodies are VHH antibodies or are derived from VHH antibodies.

5 For example, the galectin-10 antibodies and antigen binding fragments may be selected from immune libraries obtained by a method comprising the step of immunizing a camelid with the target of interest i.e. galectin-10. The camelid may be immunized with the target protein or polypeptide fragment thereof, or with an mRNA molecule or cDNA molecule expressing the protein or a polypeptide fragment thereof. Methods for producing antibodies in camelid species
10 and selecting antibodies against preferred targets from camelid immune libraries are described in, for example, International patent application no. WO2010/001251, incorporated herein by reference.

In certain embodiments, the galectin-10 antibodies and antigen binding fragments may be
15 camelid-derived in that they comprise at least one hypervariable (HV) loop or complementarity determining region obtained from a VH domain or a VL domain of a species in the family Camelidae. In particular, the galectin-10 antibodies and antigen binding fragments may comprise VH and/or VL domains, or CDRs thereof, obtained by active immunisation of outbred camelids, e.g. llamas, with galectin-10.
20

The term "obtained from" in this context implies a structural relationship, in the sense that the HVs or CDRs of the antibodies embody an amino acid sequence (or minor variants thereof) which was originally encoded by a Camelidae immunoglobulin gene. However, this does not necessarily imply a particular relationship in terms of the production process used to prepare the
25 antibodies or antigen binding fragments thereof.

Camelid-derived antibodies or antigen binding fragments thereof may be derived from any camelid species, including *inter alia*, llama, dromedary, alpaca, vicuna, guanaco or camel.

30 Antibody molecules comprising camelid-derived VH and VL domains, or CDRs thereof, are typically recombinantly expressed polypeptides, and may be chimeric polypeptides. The term "chimeric polypeptide" refers to an artificial (non-naturally occurring) polypeptide which is created by juxtaposition of two or more peptide fragments which do not otherwise occur contiguously. Included within this definition are "species" chimeric polypeptides created by juxtaposition of
35 peptide fragments encoded by two or more species, e.g. camelid and human.

In certain embodiments, the entire VH domain and/or the entire VL domain may be obtained from a species in the family Camelidae. The camelid-derived VH domain and/or the camelid-derived

VL domain may then be subject to protein engineering, in which one or more amino acid substitutions, insertions or deletions are introduced into the camelid amino acid sequence. These engineered changes preferably include amino acid substitutions relative to the camelid sequence. Such changes include "humanisation" or "germlining" wherein one or more amino acid residues in a camelid-encoded VH or VL domain are replaced with equivalent residues from a homologous human-encoded VH or VL domain.

Isolated camelid VH and VL domains obtained by active immunisation of a camelid (e.g. llama) with galectin-10 can be used as a basis for engineering galectin-10 antibodies and antigen binding fragments in accordance with the present invention. Starting from intact camelid VH and VL domains, it is possible to engineer one or more amino acid substitutions, insertions or deletions which depart from the starting camelid sequence. In certain embodiments, such substitutions, insertions or deletions may be present in the framework regions of the VH domain and/or the VL domain.

In other embodiments, there are provided "chimeric" antibody molecules comprising camelid-derived VH and VL domains (or engineered variants thereof) and one or more constant domains from a non-camelid antibody, for example human-encoded constant domains (or engineered variants thereof). In such embodiments it is preferred that both the VH domain and the VL domain are obtained from the same species of camelid, for example both VH and VL may be from Lama glama or both VH and VL may be from Lama pacos (prior to introduction of engineered amino acid sequence variation). In such embodiments both the VH and the VL domain may be derived from a single animal, particularly a single animal which has been actively immunised with the antigen of interest.

As an alternative to engineering changes in the primary amino acid sequence of Camelidae VH and/or VL domains, individual camelid-derived hypervariable loops or CDRs, or combinations thereof, can be isolated from camelid VH/VL domains and transferred to an alternative (i.e. non-Camelidae) framework, e.g. a human VH/VL framework, by CDR grafting.

In non-limiting embodiments, the galectin-10 antibodies may comprise CH1 domains and/or CL domains (from the heavy chain and light chain, respectively), the amino acid sequence of which is fully or substantially human. For antibody molecules intended for human therapeutic use, it is typical for the entire constant region of the antibody, or at least a part thereof, to have fully or substantially human amino acid sequence. Therefore, one or more or any combination of the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may be fully or substantially human with respect to its amino acid sequence. The CH1 domain, hinge region, CH2 domain, CH3 domain and/or CL domain (and/or CH4 domain if

present) may be derived from a human antibody, preferably a human IgG antibody, more preferably a human IgG1 antibody of subtype IgG1, IgG2, IgG3 or IgG4.

Advantageously, the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may all have fully or substantially human amino acid sequence. In the context of the constant region of a humanised or chimeric antibody, or an antibody fragment, the term "substantially human" refers to an amino acid sequence identity of at least 90%, or at least 92%, or at least 95%, or at least 97%, or at least 99% with a human constant region. The term “human amino acid sequence” in this context refers to an amino acid sequence which is encoded by a human immunoglobulin gene, which includes germline, rearranged and somatically mutated genes. The invention also contemplates polypeptides comprising constant domains of “human” sequence which have been altered, by one or more amino acid additions, deletions or substitutions with respect to the human sequence, excepting those embodiments where the presence of a “fully human” hinge region is expressly required.

The galectin-10 antibodies may have one or more amino acid substitutions, insertions or deletions within the constant region of the heavy and/or the light chain, particularly within the Fc region. Amino acid substitutions may result in replacement of the substituted amino acid with a different naturally occurring amino acid, or with a non-natural or modified amino acid. Other structural modifications are also permitted, such as for example changes in glycosylation pattern (e.g. by addition or deletion of N- or O-linked glycosylation sites).

The galectin-10 antibodies may be modified within the Fc region to increase binding affinity for the neonatal receptor FcRn. The increased binding affinity may be measurable at acidic pH (for example from about approximately pH 5.5 to approximately pH 6.0). The increased binding affinity may also be measurable at neutral pH (for example from approximately pH 6.9 to approximately pH 7.4). By “increased binding affinity” is meant increased binding affinity to FcRn relative to the unmodified Fc region. Typically the unmodified Fc region will possess the wild-type amino acid sequence of human IgG1, IgG2, IgG3 or IgG4. In such embodiments, the increased FcRn binding affinity of the antibody molecule having the modified Fc region will be measured relative to the binding affinity of wild-type IgG1, IgG2, IgG3 or IgG4 for FcRn.

In certain embodiments, one or more amino acid residues within the Fc region may be substituted with a different amino acid so as to increase binding to FcRn. Several Fc substitutions have been reported that increase FcRn binding and thereby improve antibody pharmacokinetics. Such substitutions are reported in, for example, Zalevsky et al. (2010) *Nat. Biotechnol.* 28(2):157-9; Hinton et al. (2006) *J Immunol.* 176:346-356; Yeung et al. (2009) *J Immunol.* 182:7663-7671; Presta LG. (2008) *Curr. Op. Immunol.* 20:460-470; and Vaccaro et al.

(2005) *Nat. Biotechnol.* 23(10):1283-88, the contents of which are incorporated herein in their entirety.

In certain embodiments, the galectin-10 antibodies comprise a modified human IgG Fc domain comprising or consisting of the amino acid substitutions H433K and N434F, wherein the Fc domain numbering is in accordance with EU numbering. In a further embodiment, the galectin-10 antibodies described herein comprise a modified human IgG Fc domain comprising or consisting of the amino acid substitutions M252Y, S254T, T256E, H433K and N434F, wherein the Fc domain numbering is in accordance with EU numbering.

In certain embodiments, the galectin-10 antibodies comprise a modified human IgG Fc domain consisting of up to 2, up to 3, up to 4, up to 5, up to 6, up to 7, up to 8, up to 9, up to 10, up to 12, up to 15, up to 20 substitutions relative to the corresponding wild-type IgG sequence.

The galectin-10 antibodies may also be modified so as to form immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (e.g., an enzymatically active toxin of bacterial, fungal, plant or animal origin, or fragments thereof), or a radioactive isotope (i.e., a radioconjugate). Fc regions may also be engineered for half-life extension, as described by Chan and Carter (2010) *Nature Reviews: Immunology* 10:301-316, incorporated herein by reference.

In yet another embodiment, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids.

In particular embodiments, the Fc region may be engineered such that there is no effector function. In certain embodiments, the antibody molecules of the invention may have an Fc region derived from naturally-occurring IgG isotypes having reduced effector function, for example IgG4. Fc regions derived from IgG4 may be further modified to increase therapeutic utility, for example by the introduction of modifications that minimise the exchange of arms between IgG4 molecules *in vivo*. Fc regions derived from IgG4 may be modified to include the S228P substitution.

In certain embodiments, the antibody molecules are modified with respect to glycosylation. For example, an aglycoslated antibody can be made (i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for the target antigen. Such carbohydrate modifications can be accomplished by; for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid

substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen.

5 Also envisaged are variant galectin-10 antibodies having an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or a fully or partially de-fucosylated antibody (as described by Natsume *et al.*, Drug Design Development and Therapy, Vol.3, pp7-16, 2009) or an antibody having increased bisecting GlcNac structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC activity of 10 antibodies, producing typically 10-fold enhancement of ADCC relative to an equivalent antibody comprising a "native" human Fc domain. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation enzymatic machinery (as described by Yamane-Ohnuki and Satoh, mAbs 1:3, 230-236, 2009). Examples of non-fucosylated antibodies with enhanced ADCC function are those produced using the 15 Potelligent™ technology of BioWa Inc.

D. Exemplary galectin-10 antibodies

20 The present invention provides exemplary galectin-10 antibodies and antigen binding fragments thereof. These galectin-10 antibodies and antigen binding fragments serve as preferred galectin-10 antagonists in accordance with the invention. The exemplary galectin-10 antibodies and antigen binding fragments of the invention may be defined exclusively with respect to their structural characteristics, as described below.

25

Provided herein is an antibody or antigen binding fragment thereof which binds to galectin-10, wherein the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:

30 (i) HCDR3 comprising or consisting of SEQ ID NO: 3; HCDR2 comprising or consisting of SEQ ID NO: 2; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or consisting of SEQ ID NO: 58; LCDR2 comprising or consisting of SEQ ID NO: 57; LCDR1 comprising or consisting of SEQ ID NO: 56;

(ii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ 35 ID NO: 5; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 61; LCDR2 comprising or consisting of SEQ ID NO: 60; LCDR1 comprising or consisting of SEQ ID NO: 59;

(iii) HCDR3 comprising or consisting of SEQ ID NO: 9; HCDR2 comprising or consisting of SEQ ID NO: 8; HCDR1 comprising or consisting of SEQ ID NO: 7; LCDR3 comprising or consisting of SEQ ID NO: 64; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 62;

5 (iv) HCDR3 comprising or consisting of SEQ ID NO: 12; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 10; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;

(v) HCDR3 comprising or consisting of SEQ ID NO: 15; HCDR2 comprising or consisting of SEQ

10 ID NO: 14; HCDR1 comprising or consisting of SEQ ID NO: 13; LCDR3 comprising or consisting of SEQ ID NO: 70; LCDR2 comprising or consisting of SEQ ID NO: 69; LCDR1 comprising or consisting of SEQ ID NO: 68;

(vi) HCDR3 comprising or consisting of SEQ ID NO: 18; HCDR2 comprising or consisting of SEQ ID NO: 17; HCDR1 comprising or consisting of SEQ ID NO: 16; LCDR3 comprising or consisting

15 of SEQ ID NO: 72; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 71;

(vii) HCDR3 comprising or consisting of SEQ ID NO: 20; HCDR2 comprising or consisting of SEQ ID NO: 19; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 75; LCDR2 comprising or consisting of SEQ ID NO: 74; LCDR1

20 comprising or consisting of SEQ ID NO: 73;

(viii) HCDR3 comprising or consisting of SEQ ID NO: 23; HCDR2 comprising or consisting of SEQ ID NO: 22; HCDR1 comprising or consisting of SEQ ID NO: 21; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;

25 (ix) HCDR3 comprising or consisting of SEQ ID NO: 25; HCDR2 comprising or consisting of SEQ ID NO: 24; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 77; LCDR1 comprising or consisting of SEQ ID NO: 76;

(x) HCDR3 comprising or consisting of SEQ ID NO: 28; HCDR2 comprising or consisting of SEQ

30 ID NO: 27; HCDR1 comprising or consisting of SEQ ID NO: 26; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 79;

(xi) HCDR3 comprising or consisting of SEQ ID NO: 31; HCDR2 comprising or consisting of SEQ ID NO: 30; HCDR1 comprising or consisting of SEQ ID NO: 29; LCDR3 comprising or consisting

35 of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 80;

(xii) HCDR3 comprising or consisting of SEQ ID NO: 33; HCDR2 comprising or consisting of SEQ ID NO: 32; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or

consisting of SEQ ID NO: 84; LCDR2 comprising or consisting of SEQ ID NO: 83; LCDR1 comprising or consisting of SEQ ID NO: 82;

(xiii) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 35; HCDR1 comprising or consisting of SEQ ID NO: 34; LCDR3 comprising or consisting of SEQ ID NO: 87; LCDR2 comprising or consisting of SEQ ID NO: 86; LCDR1 comprising or consisting of SEQ ID NO: 85;

(xiv) HCDR3 comprising or consisting of SEQ ID NO: 38; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 37; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 88;

(xv) HCDR3 comprising or consisting of SEQ ID NO: 41; HCDR2 comprising or consisting of SEQ ID NO: 40; HCDR1 comprising or consisting of SEQ ID NO: 39; LCDR3 comprising or consisting of SEQ ID NO: 91; LCDR2 comprising or consisting of SEQ ID NO: 90; LCDR1 comprising or consisting of SEQ ID NO: 89;

(xvi) HCDR3 comprising or consisting of SEQ ID NO: 43; HCDR2 comprising or consisting of SEQ ID NO: 42; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 92;

(xvii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 44; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 97; LCDR2 comprising or consisting of SEQ ID NO: 96; LCDR1 comprising or consisting of SEQ ID NO: 95;

(xviii) HCDR3 comprising or consisting of SEQ ID NO: 47; HCDR2 comprising or consisting of SEQ ID NO: 46; HCDR1 comprising or consisting of SEQ ID NO: 45; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71;

(xix) HCDR3 comprising or consisting of SEQ ID NO: 50; HCDR2 comprising or consisting of SEQ ID NO: 49; HCDR1 comprising or consisting of SEQ ID NO: 48; LCDR3 comprising or consisting of SEQ ID NO: 96; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 95;

(xx) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 52; HCDR1 comprising or consisting of SEQ ID NO: 51; LCDR3 comprising or consisting of SEQ ID NO: 98; LCDR2 comprising or consisting of SEQ ID NO: 97; LCDR1 comprising or consisting of SEQ ID NO: 80; and

(xxi) HCDR3 comprising or consisting of SEQ ID NO: 55; HCDR2 comprising or consisting of SEQ ID NO: 54; HCDR1 comprising or consisting of SEQ ID NO: 53; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71.

In certain embodiments, there is provided an antibody or antigen binding fragment thereof, which binds galectin-10, said antibody or antigen binding fragment comprising a heavy chain variable domain and a light chain variable domain, wherein
the variable heavy chain CDR3 sequence comprises or consists of SEQ ID NO:3
5 [DRNLGYRLGYPYDY] or sequence variant thereof;
the variable heavy chain CDR2 sequence comprises or consists SEQ ID NO:2
[GISWNGGSTYYAESMKG] or sequence variant thereof;
the variable heavy chain CDR1 sequence comprises or consists of SEQ ID NO:1 [DYAMS] or sequence variant thereof;
10 the variable light chain CDR3 sequence comprises or consists of SEQ ID NO:58
[ASYRSSNNAV] or sequence variant thereof;
the variable light chain CDR2 sequence comprises or consists SEQ ID NO:57 [EVNKRAS] or sequence variant thereof;
the variable light chain CDR1 sequence comprises or consists of SEQ ID NO:56
15 [AGTSSDVGYGNYVS] or sequence variant thereof; and
wherein the sequence variant comprises one, two or three amino acid substitutions (e.g., conservative substitutions, humanising substitutions or affinity variants) in the recited sequence.

In certain embodiments, there is provided an antibody or antigen binding fragment thereof, which
20 binds galectin-10, said antibody or antigen binding fragment comprising a heavy chain variable domain and a light chain variable domain, wherein
the variable heavy chain CDR3 sequence comprises or consists of SEQ ID NO:6
[PGDRLWYYRYDY] or sequence variant thereof;
the variable heavy chain CDR2 sequence comprises or consists SEQ ID NO:5
25 [AINSGGGSTSYADSVKG] or sequence variant thereof;
the variable heavy chain CDR1 sequence comprises or consists of SEQ ID NO:4 [SYAMS] or sequence variant thereof;
the variable light chain CDR3 sequence comprises or consists of SEQ ID NO:61
[ASYRYRNNVV] or sequence variant thereof;
30 the variable light chain CDR2 sequence comprises or consists SEQ ID NO:60 [KVSRRAS] or sequence variant thereof;
the variable light chain CDR1 sequence comprises or consists of SEQ ID NO:59
[AGTSSDIGYGNYVS] or sequence variant thereof; and
wherein the sequence variant comprises one, two or three amino acid substitutions (e.g.,
35 conservative substitutions, humanising substitutions or affinity variants) in the recited sequence.

In certain embodiments, there is provided an antibody or antigen binding fragment thereof, which binds galectin-10, said antibody or antigen binding fragment comprising a heavy chain variable domain and a light chain variable domain, wherein
the variable heavy chain CDR3 sequence comprises or consists of SEQ ID NO:9
5 [YIRGSSWSGWSAYDY] or sequence variant thereof;
the variable heavy chain CDR2 sequence comprises or consists SEQ ID NO:8 [VIASDGSTYYSPSLKS] or sequence variant thereof;
the variable heavy chain CDR1 sequence comprises or consists of SEQ ID NO:7 [TSYYAWS] or sequence variant thereof;
10 the variable light chain CDR3 sequence comprises or consists of SEQ ID NO:64 [QSADSSDNPV] or sequence variant thereof;
the variable light chain CDR2 sequence comprises or consists SEQ ID NO:63 [KDSERPS] or sequence variant thereof;
the variable light chain CDR1 sequence comprises or consists of SEQ ID NO:62
15 [QGGNFGYYYGS] or sequence variant thereof; and
wherein the sequence variant comprises one, two or three amino acid substitutions (e.g., conservative substitutions, humanising substitutions or affinity variants) in the recited sequence.

In certain embodiments, there is provided an antibody or antigen binding fragment thereof, which
20 binds galectin-10, said antibody or antigen binding fragment comprising a heavy chain variable domain and a light chain variable domain, wherein
the variable heavy chain CDR3 sequence comprises or consists of SEQ ID NO:12 [RPNWYRALDA] or sequence variant thereof;
the variable heavy chain CDR2 sequence comprises or consists SEQ ID NO:11
25 [AIAYSGSTYYSPSLKS] or sequence variant thereof;
the variable heavy chain CDR1 sequence comprises or consists of SEQ ID NO:10 [TNSYYWS] or sequence variant thereof;
the variable light chain CDR3 sequence comprises or consists of SEQ ID NO:67 [QSYESSTSPV] or sequence variant thereof;
30 the variable light chain CDR2 sequence comprises or consists SEQ ID NO:66 [GDSNRPS] or sequence variant thereof;
the variable light chain CDR1 sequence comprises or consists of SEQ ID NO:65 [QGANLGRYYGI] or sequence variant thereof; and
wherein the sequence variant comprises one, two or three amino acid substitutions (e.g.,
35 conservative substitutions, humanising substitutions or affinity variants) in the recited sequence.

In certain embodiments, the antibodies and antigen binding fragments that bind to galectin-10 are selected from antibody molecules comprising or consisting of a variable heavy chain domain (VH) and a variable light chain domain (VL) selected from the following:

(i) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 99 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 100 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(ii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 101 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 102 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(iii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 103 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 104 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(iv) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 105 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 106 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(v) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 107 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 108 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(vi) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 109 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 110 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(vii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 111 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 112 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(viii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 113 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(ix) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or

consisting of the amino acid sequence of SEQ ID NO: 116 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(x) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 117 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 118 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xi) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 119 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 120 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 121 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 122 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xiii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 123 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 124 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xiv) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 125 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 126 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xv) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 127 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 128 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xvi) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 129 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 130 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xvii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 131 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 132 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xviii) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 133 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 134 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xix) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 135 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 136 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
5 (xx) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 137 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and
(xxi) a VH comprising or consisting of the amino acid sequence of SEQ ID NO: 139 or an amino
10 acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising or consisting of the amino acid sequence of SEQ ID NO: 140 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

Provided herein is an antibody or antigen binding fragment thereof which binds to galectin-10,
15 wherein the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:
(i) HCDR3 comprising or consisting of SEQ ID NO: 162; HCDR2 comprising or consisting of SEQ ID NO: 161; HCDR1 comprising or consisting of SEQ ID NO: 160; LCDR3 comprising or
20 consisting of SEQ ID NO: 179; LCDR2 comprising or consisting of SEQ ID NO: 178; LCDR1 comprising or consisting of SEQ ID NO: 177;
(ii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 182; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1
25 comprising or consisting of SEQ ID NO: 180;
(iii) HCDR3 comprising or consisting of SEQ ID NO: 168; HCDR2 comprising or consisting of SEQ ID NO: 167; HCDR1 comprising or consisting of SEQ ID NO: 166; LCDR3 comprising or consisting of SEQ ID NO: 185; LCDR2 comprising or consisting of SEQ ID NO: 184; LCDR1 comprising or consisting of SEQ ID NO: 183;
30 (iv) HCDR3 comprising or consisting of SEQ ID NO: 171; HCDR2 comprising or consisting of SEQ ID NO: 170; HCDR1 comprising or consisting of SEQ ID NO: 169; LCDR3 comprising or consisting of SEQ ID NO: 187; LCDR2 comprising or consisting of SEQ ID NO: 186; LCDR1 comprising or consisting of SEQ ID NO: 180;
(v) HCDR3 comprising or consisting of SEQ ID NO: 174; HCDR2 comprising or consisting of
35 SEQ ID NO: 173; HCDR1 comprising or consisting of SEQ ID NO: 172; LCDR3 comprising or consisting of SEQ ID NO: 189; LCDR2 comprising or consisting of SEQ ID NO: 188; LCDR1 comprising or consisting of SEQ ID NO: 180;

(vi) HCDR3 comprising or consisting of SEQ ID NO: 176; HCDR2 comprising or consisting of SEQ ID NO: 175; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 192; LCDR2 comprising or consisting of SEQ ID NO: 191; LCDR1 comprising or consisting of SEQ ID NO: 190; and

5 (vii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 193; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1 comprising or consisting of SEQ ID NO: 180.

10 In certain embodiments, the antibodies and antigen binding fragments that bind to galectin-10 are selected from antibody molecules comprising or consisting of a variable heavy chain domain (VH) and a variable light chain domain (VL) selected from the following:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 194 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

15 sequence of SEQ ID NO: 195 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(ii) a VH comprising the amino acid sequence of SEQ ID NO: 196 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 197 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99%

20 identical thereto;

(iii) a VH comprising the amino acid sequence of SEQ ID NO: 198 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 199 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

25 (iv) a VH comprising the amino acid sequence of SEQ ID NO: 200 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 201 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(v) a VH comprising the amino acid sequence of SEQ ID NO: 202 or an amino acid sequence at

30 least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 203 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(vi) a VH comprising the amino acid sequence of SEQ ID NO: 204 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

35 sequence of SEQ ID NO: 205 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and

(vii) a VH comprising the amino acid sequence of SEQ ID NO: 206 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

sequence of SEQ ID NO: 207 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

For embodiments wherein the domains of the antibodies or antigen binding fragments are defined by a particular percentage sequence identity to a reference sequence, the VH and/or VL domains may retain identical CDR sequences to those present in the reference sequence such that the variation is present only within the framework regions.

Provided herein is an antibody or antigen binding fragment thereof which binds to galectin-10, wherein the antibody is a VHH antibody and wherein the VHH domain comprises the CDR sequences selected from the group consisting of:
(i) CDR3 comprising or consisting of SEQ ID NO: 210; CDR2 comprising or consisting of SEQ ID NO: 209; CDR1 comprising or consisting of SEQ ID NO: 208;
(ii) CDR3 comprising or consisting of SEQ ID NO: 213; CDR2 comprising or consisting of SEQ ID NO: 212; CDR1 comprising or consisting of SEQ ID NO: 211;
(iii) CDR3 comprising or consisting of SEQ ID NO: 216; CDR2 comprising or consisting of SEQ ID NO: 215; CDR1 comprising or consisting of SEQ ID NO: 214;
(iv) CDR3 comprising or consisting of SEQ ID NO: 219; CDR2 comprising or consisting of SEQ ID NO: 218; CDR1 comprising or consisting of SEQ ID NO: 217;
(v) CDR3 comprising or consisting of SEQ ID NO: 222; CDR2 comprising or consisting of SEQ ID NO: 221; CDR1 comprising or consisting of SEQ ID NO: 220;
(vi) CDR3 comprising or consisting of SEQ ID NO: 225; CDR2 comprising or consisting of SEQ ID NO: 224; CDR1 comprising or consisting of SEQ ID NO: 223;
(vii) CDR3 comprising or consisting of SEQ ID NO: 228; CDR2 comprising or consisting of SEQ ID NO: 227; CDR1 comprising or consisting of SEQ ID NO: 226;
(viii) CDR3 comprising or consisting of SEQ ID NO: 231; CDR2 comprising or consisting of SEQ ID NO: 230; CDR1 comprising or consisting of SEQ ID NO: 229;
(ix) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
(x) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xi) CDR3 comprising or consisting of SEQ ID NO: 238; CDR2 comprising or consisting of SEQ ID NO: 237; CDR1 comprising or consisting of SEQ ID NO: 232;
(xii) CDR3 comprising or consisting of SEQ ID NO: 241; CDR2 comprising or consisting of SEQ ID NO: 240; CDR1 comprising or consisting of SEQ ID NO: 239;
(xiii) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;

(xiv) CDR3 comprising or consisting of SEQ ID NO: 244; CDR2 comprising or consisting of SEQ ID NO: 243; CDR1 comprising or consisting of SEQ ID NO: 242;
(xv) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
5 (xvi) CDR3 comprising or consisting of SEQ ID NO: 247; CDR2 comprising or consisting of SEQ ID NO: 246; CDR1 comprising or consisting of SEQ ID NO: 245; and
(xvii) CDR3 comprising or consisting of SEQ ID NO: 249; CDR2 comprising or consisting of SEQ ID NO: 248; CDR1 comprising or consisting of SEQ ID NO: 217.

10 In certain embodiments, the VHH antibodies that bind to galectin-10 comprise a VHH domain comprising or consisting of an selected from antibody molecules comprising or consisting of the amino acid sequence represented by any one of SEQ ID NOs: 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265 or 266, or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

15

For embodiments wherein the VHH domains are defined by a particular percentage sequence identity to a reference sequence, the VHH domain may retain identical CDR sequences to those present in the reference sequence such that the variation is present only within the framework regions.

20

The invention also provides antibodies or antigen binding fragments thereof, which bind to the same epitope as the galectin-10 antibodies exemplified herein.

In certain embodiments, the exemplary galectin-10 antibodies and antigen binding fragments
25 thereof defined as having the CDR sequences recited above or defined as having a particular percentage identity to the specific VH/VL/VHH domain amino acid sequences recited above are humanised, germlined or affinity variants of the antibodies or antigen binding fragments thereof from which the CDR, VH, VL and/or VHH sequences derive.

30 In a preferred embodiment, the exemplary galectin-10 antibody molecules having the CDR sequences recited above exhibit high human homology, for example are humanised or germlined variants of the antibodies or antigen binding fragments thereof from which the CDR sequences derive.

35 In non-limiting embodiments, the exemplary galectin-10 antibodies and antigen binding fragments thereof having the CDR, VH and/or VL sequences described herein may comprise CH1 domains and/or CL domains (from the heavy chain and light chain, respectively), the amino acid sequence of which is fully or substantially human. For antibody molecules intended for

human therapeutic use, it is typical for the entire constant region of the antibody, or at least a part thereof, to have fully or substantially human amino acid sequence. Therefore, one or more or any combination of the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may be fully or substantially human with respect to its amino acid sequence.

Advantageously, the CH1 domain, hinge region, CH2 domain, CH3 domain and CL domain (and CH4 domain if present) may all have fully or substantially human amino acid sequence. In the context of the constant region of a humanised or chimeric antibody, or an antibody fragment, the term "substantially human" refers to an amino acid sequence identity of at least 90%, or at least 92%, or at least 95%, or at least 97%, or at least 99% with a human constant region. The term “human amino acid sequence” in this context refers to an amino acid sequence which is encoded by a human immunoglobulin gene, which includes germline, rearranged and somatically mutated genes. The invention also contemplates polypeptides comprising constant domains of “human” sequence which have been altered, by one or more amino acid additions, deletions or substitutions with respect to the human sequence, excepting those embodiments where the presence of a “fully human” hinge region is expressly required. Any of the exemplary Fc region modifications described herein may be incorporated into the galectin-10 antibodies having the CDR and/or VH/VL domain sequences recited above. In certain embodiments, the galectin-10 antibodies having the CDR and/or VH/VL domain sequences recited above comprise a modified human IgG Fc domain comprising or consisting of the amino acid substitutions H433K and N434F, wherein the Fc domain numbering is in accordance with EU numbering. In certain embodiments, the galectin-10 antibodies having the CDR and/or VH/VL domain sequences recited above comprise a modified human IgG Fc domain comprising or consisting of the amino acid substitutions M252Y, S254T, T256E, H433K and N434F.

Unless otherwise stated in the present application, % sequence identity between two amino acid sequences may be determined by comparing these two sequences aligned in an optimum manner and in which the amino acid sequence to be compared can comprise additions or deletions with respect to the reference sequence for an optimum alignment between these two sequences. The percentage of identity is calculated by determining the number of identical positions for which the amino acid residue is identical between the two sequences, dividing this number of identical positions by the total number of positions in the comparison window and multiplying the result obtained by 100 in order to obtain the percentage of identity between these two sequences. For example, it is possible to use the BLAST program, "BLAST 2 sequences" (Tatusova et al, "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", FEMS Microbiol Lett. 174:247-250) available on the site http://www.ncbi.nlm.nih.gov/ gorf/bl2.html, the parameters used being those given by default (in

particular for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the matrix chosen being, for example, the matrix "BLOSUM 62" proposed by the program), the percentage of identity between the two sequences to be compared being calculated directly by the program.

5

E. Polynucleotides encoding galectin-10 antibodies

The invention also provides polynucleotide molecules encoding the galectin-10 antibodies of the invention or fragments thereof. Polynucleotide molecules encoding the full-length galectin-10
10 antibodies are provided, together with polynucleotide molecules encoding fragments, for example the VH, VL and/or VHH domains of the galectin-10 antibodies described herein. Also provided are expression vectors containing said nucleotide sequences of the invention operably linked to regulatory sequences which permit expression of the antibodies or fragments thereof in a host cell or cell-free expression system, and a host cell or cell-free expression system containing this
15 expression vector.

Polynucleotide molecules encoding galectin-10 antibodies of the invention include, for example, recombinant DNA molecules. The terms "nucleic acid", “polynucleotide” or a "polynucleotide molecule" as used herein interchangeably and refer to any DNA or RNA molecule, either single-
20 or double-stranded and, if single-stranded, the molecule of its complementary sequence. In discussing nucleic acid molecules, a sequence or structure of a particular nucleic acid molecule may be described herein according to the normal convention of providing the sequence in the 5' to 3' direction. In some embodiments of the invention, nucleic acids or polynucleotides are "isolated." This term, when applied to a nucleic acid molecule, refers to a nucleic acid molecule
25 that is separated from sequences with which it is immediately contiguous in the naturally occurring genome of the organism in which it originated. For example, an "isolated nucleic acid" may comprise a DNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a prokaryotic or eukaryotic cell or non-human host organism. When applied to RNA, the term "isolated polynucleotide" refers primarily to an RNA molecule
30 encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been purified/separated from other nucleic acids with which it would be associated in its natural state (i.e., in cells or tissues). An isolated polynucleotide (either DNA or RNA) may further represent a molecule produced directly by biological or synthetic means and separated from other components present during its production.

35

For recombinant production of a galectin-10 antibody according to the invention, a recombinant polynucleotide encoding it or recombinant polynucleotides encoding the different chains or domains may be prepared (using standard molecular biology techniques) and inserted into a

replicable vector for expression in a chosen host cell, or a cell-free expression system. Suitable host cells may be prokaryote, yeast, or higher eukaryote cells, specifically mammalian cells. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen. Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); mouse myeloma cells SP2/0-AG14 (ATCC CRL 1581; ATCC CRL 8287) or NS0 (HPA culture collections no. 85110503); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2), as well as DSM's PERC-6 cell line. Expression vectors suitable for use in each of these host cells are also generally known in the art.

It should be noted that the term "host cell" generally refers to a cultured cell line. Whole human beings into which an expression vector encoding an antigen binding polypeptide according to the invention has been introduced are explicitly excluded from the definition of a “host cell”.

F. Antibody production

In a further aspect, the invention also provides a method of producing antibodies of the invention which comprises culturing a host cell (or cell free expression system) containing polynucleotide (e.g. an expression vector) encoding the antibody under conditions which permit expression of the antibody, and recovering the expressed antibody. This recombinant expression process can be used for large scale production of antibodies, including galectin-10 antibodies according to the invention, including monoclonal antibodies intended for human therapeutic use. Suitable vectors, cell lines and production processes for large scale manufacture of recombinant antibodies suitable for *in vivo* therapeutic use are generally available in the art and will be well known to the skilled person.

G. Pharmaceutical compositions

The scope of the invention includes pharmaceutical compositions, containing one or a combination of galectin-10 antibodies or antigen binding fragments thereof, formulated with one or more pharmaceutically acceptable carriers or excipients. Such compositions may include one

or a combination of (e.g., two or more different) galectin-10 antibodies. Techniques for formulating monoclonal antibodies for human therapeutic use are well known in the art and are reviewed, for example, in Wang *et al.*, Journal of Pharmaceutical Sciences, Vol.96, pp1-26, 2007, the contents of which are incorporated herein in their entirety.

5

Pharmaceutically acceptable excipients that may be used to formulate the compositions include, but are not limited to: ion exchangers, alumina, aluminum stearate, lecithin, serum proteins, such as human serum albumin, buffer substances such as phosphates, glycine, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes,
10 such as protamine sulfate, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances (for example sodium carboxymethylcellulose), polyethylene glycol, polyacrylates, waxes, polyethylene- polyoxypropylene- block polymers, polyethylene glycol and wool fat.

15

In certain embodiments, the compositions are formulated for administration to a subject via any suitable route of administration including but not limited to intramuscular, intravenous, intradermal, intraperitoneal injection, subcutaneous, epidural, nasal, oral, rectal, topical, inhalational, buccal (*e.g.*, sublingual), and transdermal administration.

20

H. Methods of treatment

The galectin-10 antagonists, particularly the galectin-10 antibodies and antigen binding fragments described herein, may be used in methods of treatment. Thus, provided herein is a
25 galectin-10 antagonist in accordance with the first aspect of the invention for use as a medicament. Alternatively, provided herein is a galectin-10 antagonist in accordance with the first aspect of the invention for use in a method of treatment. In preferred embodiments, the invention provides galectin-10 antibodies and antigen binding fragments as described elsewhere herein for use as medicaments. Alternatively, the invention provides galectin-10 antibodies and
30 antigen binding fragments as described elsewhere herein for use in a method of treatment. The galectin-10 antagonists, including the galectin-10 antibodies and antigen binding fragments thereof, for use as medicaments are typically formulated as pharmaceutical compositions. Importantly, all embodiments described above in relation to the galectin-10 antagonists, particularly the galectin-10 antibodies and antigen binding fragments thereof, are equally
35 applicable to the methods described herein.

The present invention also provides methods of treating a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of a galectin-10

antagonist in accordance with the first aspect of the invention. In preferred embodiments, the galectin-10 antagonist is a galectin-10 antibody or antigen binding fragment thereof as described elsewhere herein. In such methods of treatment, the galectin-10 antagonists, including the galectin-10 antibodies and antigen binding fragments thereof, are typically formulated as pharmaceutical compositions. As used herein, the term “therapeutically effective amount” is intended to mean the quantity or dose of galectin-10 antagonist, e.g. antibody, that is sufficient to produce a therapeutic effect, for example, the quantity or dose of antagonist required to eradicate or at least alleviate the symptoms associated with a disease or condition. An appropriate amount or dose can be determined by a physician, as appropriate. For example, the dose can be adjusted based on factors such as the size or weight of a subject to be treated, the age of the subject to be treated, the general physical condition of the subject to be treated, the condition to be treated, and the route of administration.

For clinical use, in certain embodiments, the galectin-10 antagonist is a galectin-10 antibody or antigen binding fragment thereof as described elsewhere herein and it is administered to a subject as one or more doses of about 0.1 mg/kg body weight to about 20 mg/kg body weight. In certain embodiments, the galectin-10 antagonist is a galectin-10 antibody or antigen binding fragment thereof as described elsewhere herein and it is administered to a subject in a dose of about 0.1 mg/kg body weight to about 10 mg/kg body weight. In certain embodiments, the galectin-10 antagonist is a galectin-10 antibody or antigen binding fragment thereof as described elsewhere herein and it is administered to a subject in a dose of about 0.5 mg/kg body weight to about 10 mg/kg body weight. In certain embodiments, the galectin-10 antagonist is a galectin-10 antibody or antigen binding fragment thereof as described elsewhere herein and it is administered to a subject in a dose of about 1 mg/kg body weight to about 10 mg/kg body weight.

The galectin-10 antagonists, particularly the galectin-10 antibodies and antigen binding fragments thereof, are useful in therapeutic methods, for the reason that they can disrupt galectin-10 crystallization. As explained elsewhere herein, the galectin-10 antagonists of the present invention bind to an epitope of galectin-10 thereby shielding a crystal packing interface and consequently disrupting the crystallization of galectin-10. In certain embodiments, the galectin-10 antagonists inhibit the crystallization of galectin-10. In certain embodiments, the galectin-10 antagonists promote dissolution of crystalline galectin-10.

The galectin-10 antagonists, including the galectin-10 antibodies and antigen binding fragments thereof, may be for use in preventing or treating diseases or conditions associated with the presence or formation of galectin-10 crystals or CLCs. Provided herein are methods of preventing or treating a disease or condition associated with the presence or formation of galectin-10 crystals or CLCs in a patient or subject in need thereof by administering an effective

amount of a galectin-10 antagonist as described herein, particularly a galectin-10 antibody or antigen binding fragment thereof.

As used herein, a method of “preventing” a disease or condition means preventing the onset of the disease, preventing the worsening of symptoms, preventing the progression of the disease or condition or reducing the risk of a subject developing the disease or condition. As used herein, a method of “treating” a disease or condition means curing a disease or condition and/or alleviating or eradicating the symptoms associated with the disease or condition such that the patient’s suffering is reduced.

For patients having diseases or conditions characterised by the presence of galectin-10 crystals, the methods of treatment will typically involve the administration of a galectin-10 antagonist, preferably a galectin-10 antibody or antigen binding fragment thereof, capable of dissolving the galectin-10 crystals located in the patient’s tissues. For patients identified as “at risk” of developing a disease or condition characterised by the formation of galectin-10 crystals, the methods of prevention may involve the administration of a galectin-10 antagonist, preferably a galectin-10 antibody or antigen binding fragment thereof, capable of inhibiting the crystallization of galectin-10.

Galectin-10 crystals or CLCs have been observed in patients having a range of diseases and conditions. It follows that the galectin-10 antagonists described herein may be used to prevent or treat a disease or condition selected from the group consisting of: asthma; chronic rhinosinusitis; celiac disease; helminth infection; gastrointestinal eosinophilic inflammation; cystic fibrosis (CF); allergic bronchopulmonary aspergillosis (ABPA); Churg-Straus vasculitis; chronic eosinophilic pneumonia; and acute myeloid leukemia. In preferred embodiments, galectin-10 antibodies or antigen binding fragments thereof are used to prevent or treat a disease or condition selected from the group consisting of: asthma; chronic rhinosinusitis; celiac disease; helminth infection; gastrointestinal eosinophilic inflammation; cystic fibrosis (CF); allergic bronchopulmonary aspergillosis (ABPA); Churg-Straus vasculitis; chronic eosinophilic pneumonia; and acute myeloid leukemia.

As noted above, galectin-10 crystals or CLCs are particularly associated with diseases or conditions characterised by eosinophilic inflammation. In preferred embodiments, the galectin-10 antagonists described herein, preferably galectin-10 antibodies or antigen binding fragments thereof described herein, are used to treat disorders or conditions associated with eosinophilic inflammation.

In particularly preferred embodiments, the galectin-10 antagonists described herein, preferably galectin-10 antibodies or antigen binding fragments thereof described herein, are used to prevent or treat asthma.

5 The results presented herein highlight the important role of CLCs in inducing an innate immune response and inducing airway inflammation *in vivo*. These effects were successfully reversed by exemplary galectin-10 antibodies described herein. The inflammation observed in the mouse model described herein was found to be independent of the NLRP3 inflammasome complex. These results indicate for the first time a causative role for CLCs in inflammatory responses
10 mediated via a pathway independent of the NLRP3 inflammasome i.e. the inflammatory complex previously implicated in CLC pathology. It follows, that the methods of the present invention may be used to treat inflammatory conditions or disorders, particularly inflammatory conditions or disorders of the airways. The therapeutic effect may be mediated independently of the NLRP3 inflammasome complex.

15

The present invention also provides use of a galectin-10 antagonist for the detection of galectin-10 in a sample obtained from a patient. In certain embodiments, a galectin-10 antibody or antigen binding fragment in accordance with the invention is used to detect galectin-10 in a sample obtained from a patient. The antagonists, antibodies or antigen binding fragments
20 thereof are typically used to detect crystalline galectin-10. As noted above, galectin-10 crystals or CLC crystals have been observed in patients having a number of different diseases and conditions. It follows, that the patient sample may be isolated from a subject having or suspected of having any one of the following diseases or conditions: asthma, chronic rhinosinusitis, celiac disease, helminth infection, gastrointestinal eosinophilic inflammation, cystic fibrosis (CF),
25 allergic bronchopulmonary aspergillosis (ABPA), Churg-Straus vasculitis, chronic eosinophilic pneumonia, or acute myeloid leukemia. The detection of crystalline galectin-10 in the patient sample may be used to diagnose the disease or condition in the subject from which the sample was obtained. The sample may be any suitable patient sample, for example any fluid or tissue in which CLCs are observed in a disease state. In certain embodiments, the sample is a tissue
30 sample obtained from a polyp, for example a nasal polyp. In certain embodiments, the sample is a mucus sample. In such embodiments, the detection of crystalline galectin-10 in the mucus sample using the antagonists of the invention may be used to detect or diagnose chronic rhinosinusitis. In preferred embodiments, the patient sample is a sputum sample. In such embodiments, the detection of crystalline galectin-10 in the sputum sample using the antagonists
35 of the invention may be used to detect or diagnose asthma.

I. Kits

Any of the galectin-10 antagonists, antibodies or antigen binding fragments described herein can be packaged as a kit and optionally include instructions for use.

5

EXAMPLES

The invention will be further understood with reference to the following non-limiting examples.

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Example 1. Production of recombinant Charcot-Leyden crystals (CLCs)

Previous studies on CLCs have been performed on crystals obtained by auto-crystallization of protein rich lysates of primary human blood eosinophils or leukemic cell lines, which has led to co-purification of contaminating proteins like lysophospholipase (Ackerman et al. (1980); Weller
15 et al. (1984) and Archer et al. (1965)).

To generate large amounts of pure CLC crystals for use in functional studies *in vivo*, human galectin-10, carrying a TEV-cleavable N-terminal His-tag, was produced in *E. coli* and purified by a combination of immobilized affinity chromatography and size-exclusion chromatography (**Fig.
20 1A**). A synthetic codon-optimized DNA sequence encoding human galectin-10 (residues 1 -142, Uniprot Q05315) was cloned into the NcoI/XhoI sites of the pET28a bacterial expression vector (Novagen, cat#69864-3) with an His-tag and two protease cleavage sites, enterokinase (DDDDK) and TEV protease (ENLYFQG), at the N-terminus (MASTTHHHHHHDTDIPTTGGGSRPDDDD-KENLYFQGHM). pET28a-galectin-10 was
25 transformed in BL21(DE3) cells using kanamycin (25 µg/mL) as a selection marker. Expression cultures were grown at 28° C in Luria-Bertani medium, containing kanamycin (25 µg/mL). Expression of galectin-10 was induced at a culture OD_{600} of 0.6, by the addition of isopropyl-β-D-thiogalactopyranoside (ITPG) to a final concentration of 1 mM, after which the culture was allowed to grow overnight. The bacteria were harvested by centrifugation (6,000g for 20 min at 4°
30 C) and the cellular paste was stored at -80° C. The bacterial pellet was thawed and resuspended in lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl pH 7.4). The cells were lysed by sonication on a Branson sonifier (total run time of 4 min with 30 s pulses at 30% output interspersed with 30 s of down time). Cell debris was removed by centrifugation at 4° C (20,000 g for 30 min). The supernatant was clarified by filtration using a 0.22 µm bottle top filter and loaded onto a Ni
35 Sepharose column equilibrated with 50 mM NaH_2PO_4, 300 mM NaCl pH 7.4. Next the column was washed with loading buffer supplemented with 20 mM imidazole and 0.1 % empigen detergent, followed by washing with loading buffer supplemented with 20 mM imidazole. Next, the protein was eluted using loading buffer supplemented with 50 mM and 500 mM imidazole.

The 50 mM and 500 mM elution peaks were pooled and concentrated and injected onto a HiLoad 16/600 Superdex 200 pg column using PBS pH 7.4 as running buffer. The elution fractions corresponding to galectin-10 were pooled and stored at -80° C. Endotoxin-levels were determined with an Endosafe-PTS system (Charles River) as lower than 5 EU mg^{-1} recombinant
5 protein. The His-tagged galectin-10 protein was soluble up to 30 mg/ml.

To analyse molecular mass and oligomeric state of His-tagged galectin-10, SEC-MALLS was performed (**Fig 1B**). Protein samples (100 µL) were injected onto a Superdex 200 Increase 10/300 GL column (GE Healthcare), with PBS pH 7.4 as running buffer at 0.5 ml min^{-1}, coupled
10 to an online UV-detector (Shimadzu), a multi-angle light scattering miniDAWN TREOS instrument (Wyatt) and a Optilab T-rEX refractometer (Wyatt) at 25 °C. A refractive index increment (dn/dc) value of 0.185 ml g^{-1} was used for protein concentration and molecular mass determination. Data were analyzed using the ASTRA6 software (Wyatt). Correction for band broadening was applied using parameters derived from BSA injected under identical running
15 conditions. SEC-MALLS analysis showed that His-tagged galectin-10 is a dimer in solution (**Fig. 1B**). The determined molecular weight was 40 ± 0.8 kDa, which closely matches the theoretical weight for tagged dimeric galectin-10 of 41.2 kDa.

To form recombinant galectin-10 crystals, N-terminally tagged galectin-10 (at a concentration
20 between 2 to 4.5 mg/ml) was incubated with in-house produced TEV protease (Kapust et al., 2001). The pRK793 plasmid encoding His-tagged TEV was a kind gift from David Waugh (Addgene plasmid #8827). Following overnight digestion, the protein solution was agitated by inverting it 5 times after which the solution turned cloudy in about 30 minutes due to the spontaneously formation of needle-shaped CLC crystals (**Fig. 1C**). Following TEV cleavage,
25 recombinant galectin-10 autocrystallized in PBS buffer and was only soluble up to a concentration of 0.2 mg/ml. Following TEV-digestion three residues are left at the N-terminus (GHM) of recombinant galectin-10. The crystals closely resembled the various macroscopic shapes originally described by Charcot and von Leyden (**Fig. 1D**).

30 Fluorescently-labelled forms of galectin-10 crystals were also produced. Since galectin-10 contains two solvent exposed cysteine-residues (Cys29 and Cys57), the thiol-reactive fluorescent dye 5-iodoacetamidofluorescein (5-IAF) was used to fluorescently label tagged galectin-10. 5-IAF was solubilized in 100% dimethylformamide to a concentration of 100 mM. The pH of the galectin-10 protein solution (~5 mg/mL) was adjusted to pH 8.5 by adding 100 mM
35 Tris pH 8.5 (using a 1 M Tris pH 8.5 stock solution). Next, a 10-fold molar excess of 5-IAF to galectin-10 (monomer) was added to the protein solution and the labeling reaction was kept in the dark at room temperature for 2 hours. For galectin-10 carrying an N-terminal His-tag a molar extinction coefficient of 21430 cm^{-1} M^{-1} was used. Next, the excess of non-reacted 5-IAF was

quenched by adding 5 mM DTT (using a 1 M DTT). The excess of 5-IAF was then removed by running the sample on a 50 mL HiTrap desalting column (GE Healthcare) using PBS as running buffer. Next, 5-IAF labeled galectin-10 was concentrated and injected on HiLoad 16/600 Superdex 200 pg column. The fractions of the elution peak were then pooled and stored at -80 °C. The endotoxin-levels were determined with an Endosafe-PTS system (Charles River) as lower than 5 EU mg^{-1} recombinant protein. To form fluorescent galectin-10 crystals the N-terminal His-tag of 5-IAF labeled galectin-10 was removed by overnight incubation as described above. These fluorescent crystals had the myriad shapes originally described by Charcot (**Fig. 1E**).

Example 2. Characterisation of *in vivo* grown CLCs

To date, there has been no description of the crystal lattice structure of *in vivo* grown CLC. Therefore, crystals were isolated from the sticky mucus of CRSwNP patients. Airway mucosal tissue and/or secretions were collected from patients undergoing endoscopic sinus surgery for chronic rhinosinusitis with nasal polyps (CRSwNP) (**Fig. 2A**). Nasal polyposis was diagnosed on the basis of symptoms, clinical examination, nasal endoscopy, and sinus computed tomography scan according to the European Position Paper on Rhinosinusitis and Nasal Polyps guidelines. All patients refrained from using oral and/or topical corticosteroids at least 4 weeks before surgery. The study and collection of samples were approved by the ethics committee of the Ghent University Hospital and an informed consent was obtained from all patients prior to enrollment in the study. Mucus “sticky allergic mucin type” obtained from patients was stored overnight at 4°C in RPMI 1640 (Sigma-Aldrich, Bornem, Belgium) containing antibiotics (50 IU/mL penicillin and 50 mg/mL streptomycin; Invitrogen), and 0.1% BSA (Sigma).

To reveal the presence and identity of CLC crystals, immunofluorescence staining was performed for galectin-10. The collected allergic mucin was fixed with 4% paraformaldehyde and embedded in paraffin. Tissue slides (5 µm) of the embedded mucin were cut, deparaffinized using xyleen (3x 10 minutes) and rehydrated by stepwise immersion in decreasing ethanol concentrations (100%, 90%, 60%, 30%, 0% ethanol, 2 minutes/step). After rehydration, the slides were immersed in PBS for 5 minutes and subsequently incubated for 1h with 0.05% trypsin-EDTA (Life Technologies) at 37°C in a moist chamber. After washing with PBS (Life Technologies) for 10 minutes, the slides were incubated for 1h with blocking buffer (7.5% BSA (Sigma Aldrich) in PBS) in a moist chamber at RT. Subsequently, the slides were incubated overnight at 4°C with an anti-human galectin-10 antibody (Clone EPR11197, Abcam, 1/200 dilution in blocking buffer). The next day, the slides were washed with PBS for 10 minutes and incubated with a FITC labeled secondary goat anti-rabbit antibody (A11034 Life Technologies, 1/400 dilution). After washing for 10 minutes with PBS, the slides were mounted with Vectashield containing DAPI. The slides were stored in the dark and analyzed with a confocal laser-scanning

microscope (Leica) the next day. This revealed the presence of large amounts of needle shaped crystals immunoreactive for galectin-10 (**Fig. 2B**).

To purify crystals for *ex vivo* crystallography, the medium was discarded and 1 g of the mucus was cut thoroughly in 10 ml RPMI 1640 (Sigma-Aldrich, Bornem, Belgium) containing antibiotics (50 IU/mL penicillin and 50 mg/mL streptomycin; Invitrogen), 0.1% BSA (Sigma) and 1 mg/ml Collagen type 2 (Worthington). The mucus was further homogenized using a GentleMACS Dissociator (Myltenyi Biotec) and subsequently incubated at 37°C for 45 minutes under continuous rotation. After incubation, the partly dissolved mucus was homogenized with the GentleMACS Dissociator (Myltenyi Biotec) and centrifuged at 400g for 7 minutes at RT. After centrifugation the supernatant was discarded and the pellet was dissolved in 3 ml PBS (Life technologies) containing 50 IU/ml penicillin and 50 mg/ml streptomycin (Life technologies). 3 ml of the isolated fluid was mixed with 6 ml Ficoll-Paque (GE Healthcare) and centrifuged at 250g for 10 minutes. After removal of the supernatant and most of the Ficoll layer, 2.8 ml PBS with antibiotics was added to the remaining fluid (200 µl) at the bottom of the tube. This precipitation process was repeated 5 more times. The final fluid containing the crystals at the bottom of the tube was resuspended in 2 ml PBS with antibiotics and centrifuged at 200g for 5 minutes. Most of the supernatant was removed and 800 µl of PBS with antibiotics was added to the crystals in the remaining 200 µl of fluid at the bottom of the tube. Single crystals were harvested from the solution using mounted cryoloops (**Fig. 2C**). Before flash-freezing in liquid nitrogen the crystals were cryoprotected by a brief soak in PBS supplemented with 35% (v/v) glycerol. Diffraction experiments at 100 K were conducted on beamline P14 of PetraIII (DESY, Hamburg, Germany). All data were integrated and scaled using the XDS suite (Kabsch, 2010). Molecular replacement (MR) was performed with Phaser (McCoy et al., 2007) using search models based on the structure of galectin-10 (PDB 1LCL). Model (re)building was performed in COOT (Emsley et al., 2010) and individual coordinate and ADP refinement was performed in PHENIX (Adams et al., 2010) and autoBuster (Bricogne et al., 2017). Model and map validation tools in COOT and the PHENIX suite were used throughout the work flow to guide improvement and validate the quality of crystallographic models. Using this methodology, the structure of a human CLC crystal isolated from a patient suffering from nasal polyps was determined using single crystal X-ray diffraction using synchrotron radiation (to 2.2 Å resolution) (**Fig. 2D, 2E, Table 17**). The crystal structure obtained was compared with that of recombinant galectin-10 crystals (1.4 Å resolution), and a published structure of galectin-10 crystals obtained by eosinophil lysis and crystallization *in vitro* (pdb 1LCL, 1.8 A) (Leonidas et al., 1995). The resulting analysis showed that all three galectin-10 crystal forms belong to space group $P\,6_522$ with similar unit cell parameters (Table 17). Moreover, the atomic structures for recombinant galectin-10 produced in *E. coli* and for galectin-10 obtained from a human eosinophilic cell line (pdb 1LCL) can be considered virtually identical as the structure of galectin-10 in human CLC crystals (RMSD < 0.2 Å) (**Fig. 2F**).

Example 3 Production of a non-autocrystallizing galectin-10 variant, galectin-10-Tyr69Glu

In order to produce a non-autocrystallizing variant of galectin-10, the crystal packing interactions (**Fig. 3A** and **Fig. 3B**) in the reported structure for galectin-10 (PDB 1LCL) were analysed. Residue Tyr69 which engages in crystal packing interactions with a neighbouring galectin-10-
5 Tyr69 residue in the crystal lattice was selected as a potential key residue for autocrystallization (**Fig. 3A**). Accordingly, a variant of galectin-10 carrying the Tyr69Glu (Y69E) substitution was recombinantly produced following an identical protocol as for wild-type galectin-10. To produce a potentially non-autocrystallizing galectin-10 variant, residue Tyr69 was mutated to a glutamate residue using Quickchange site-directed mutagenesis (Agilent). For the mutagenesis PCR the
10 following forward and reverse primers were used, FP: GATGAACTCTCGTGAAGAAGGTGCATGGAAACAG (SEQ ID NO: 154) and RP: CTGTTTCCATGCACCTTCTTCACGAGAGTTCATC (SEQ ID NO: 155). The pET28a-galectin-10 plasmid was used as a template. The resulting plasmid, pET28a-galectin-10-Y69E was used to transform BL21(DE3) cells. Protein production and purification were identical as compared to
15 wild-type galectin-10. Following TEV-mediated removal of the N-terminal tag of galectin-10-Y69E, His-tagged TEV was removed by running the digestion mixture on a Ni-sepharose column using PBS as running buffer. Next, the column flow-through, containing galectin-10-Y69E, was concentrated and injected onto HiLoad 16/600 Superdex 200 pg column. The SEC elution fractions corresponding to galectin-10-Y69E were pooled and stored at -80° C. Endotoxin-levels
20 were determined with an Endosafe-PTS system (Charles River) as lower than 5 EU mg^{-1} recombinant protein. It was found that following TEV treatment, the Y69E galectin-10 mutein did not autocrystallize as compared to wild type galectin-10 (**Fig. 3C**). According to similar methodology, several other crystallization resistant muteins were made based on predicted importance in the crystal packing interface (**Fig. 3C**). SEC-MALLS analysis also showed that the
25 Y69E variant is a dimer in solution. The determined molecular weight was 32.6 kDa, which closely matches the theoretical molecular weight for TEV-cleaved dimeric galectin-10-Tyr69Glu (33.2 kDa).

A crystal structure of the non-autocrystallizing galectin-10-Y69E mutein was also obtained. For
30 this, galectin-10-Y69E was concentrated to 6 – 7 mg/mL before crystallization experiments. Sitting-drop nanoliter-scale vapour diffusion crystallization experiments were set up at 293 K using a Mosquito crystallization robot (TTP Labtech) and commercially available sparse-matrix screens (Molecular Dimensions, Hampton research). Crystals of the mutant galectin-10-Y69E mutein appeared after 24 hrs in condition D12 of the PEG/Ion screen (Hampton Research – 0.2
35 M ammonium citrate pH 5.1, 20% PEG_{3350}). Before flash-freezing into liquid nitrogen crystals of galectin-10-Y69E were cryoprotected by briefly soaking the crystals in mother liquor supplement with 25% PEG 400.

Analysis of galectin-10-Tyr69Glu by X-ray crystallography showed that the non-autocrystallizing variant adopts a virtually identical structure as the crystallographic galectin-10 dimer in pdb 1LCL (RMSD < 0.3 Å) (**Fig. 3D** and **F, Table 17**).

5 The structure of the soluble mutein in solution was also studied using small-angle X-ray scattering in solution (SAXS). For this, SAXS data were measured on the SWING beam line at the SOLEIL Synchrotron (Gif-sur-Yvette, France). 50 µl of galectin-10-Tyr69Glu was injected onto an Agilent 4.6 x 300 mm Bio SEC-3 column with 300 Å pore size and HBS pH 7.4 as running buffer at a flow speed of 0.3 ml min^{-1} at 15 °C. X-ray scattering data were collected in 10 continuous flow mode with 1s exposure time per frame. Data were recorded within a momentum transfer range of $0.0066\ Å^{-1} < q < 0.609\ Å^{-1}$, with $q = 4\pi sin\theta/\lambda$. Raw data were radially averaged and buffer subtracted using Foxtrot v3.3.4 (developed at Synchrotron SOLEIL and provided by Xenocs, Sassenage, France). The quality of the data was analyzed with Foxtrot by checking the stability of the radius of gyration over the length of the elution peak and by scaling 15 all curves to the most intense scattering profile. The final scattering curve was obtained by averaging the unscaled, buffer-subtracted scattering profiles from frames 255 – 268, which correspond to the top of the elution peak. Structural parameters were determined with the ATSAS suite version 2.8.3 (Franke et al., 2017). Molecular weight estimates were calculated using DATMW by methods based on the Porod volume (Petoukhov et al., 2012), the volume of 20 correlation (Rambo et al., 2013) and the apparent volume (Fischer et al., 2010). The theoretical SAXS profile for dimeric galectin-10-Tyr69Glu was calculated from the determined X-ray structure and fitted to the experimental data using the FoXS server (Schneidman-Duhovny et al., 2016). The error-weighted residual difference plot was calculated as $\Delta/\sigma = [I_{exp}(q) - cI_{mod}(q)]/\sigma(q)$ versus q (Trewhella et al., 2017). SAXS analysis revealed that the dimeric 25 assembly obtained by X-ray crystallography corresponds to the in-solution structure (**Fig. 3E**).

Example 4. CLCs induce an innate immune response *in vivo*

To probe whether galectin-10 crystals promoted lung inflammation *in vivo*, naïve C57Bl/6 mice (Janvier) received an intra-tracheal injection of crystalline galectin-10 or control soluble galectin-30 10-Tyr69Glu mutein. For this, mice were anesthetized with isoflurane (2 l/min, 2-3%; 05260-05, Abbott Laboratories) and then injected intratrachally (i.t.) with 100 µg galectin-10 crystals or control soluble galectin-10-Tyr69Glu mutein (in 80 µl PBS). After 6 and 24h, mice were euthanized by CO_2 inhalation and lungs were collected. In order to obtain single-cell suspensions, lungs were first cut with a scissor and then digested at 37°C for 30 min in RPMI-35 1640 (Thermo Fisher Scientific) containing Liberase™ (1:50; 05 401 127 001, Sigma-Aldrich) and DNase I (1:1000; 04 536 282 001, Sigma-Aldrich). The obtained suspension was filtered through a 70 µm filter and depleted of red blood cells by RBC lysis buffer (0.15 M NH_4Cl, 1 mM $KHCO_3$, 0.1 mM Na_2EDTA in MilliQ H_2O). Single-cell suspensions were stained for flow

cytometry. The following antibodies were used: anti-CD3e FITC (145-2C11) (35-0031-U500, Tonbo Biosciences), anti-CD19 FITC (1D3) (35-0193-U500, Tonbo Biosciences), anti-CD11c FITC (HL3) (553801, BD Biosciences), anti-Siglec-F PE (552126, E50-2440) (BD Biosciences), anti-CD127 PE-CF594 (SB/199) (562419, BD Biosciences), anti-CD25 PE-Cy7 (PC61.5) (25-0251-82, ThermoFisher Scientific), anti-CD11b BD Horizon V450 (M1/70) (560455, BD Biosciences), anti-CD45 BV605 (30-F11) (563053, BD Biosciences), anti-CD90.2 APC (52-2.1) (17-0902-82, ThermoFisher Scientific), anti-Ly6G AF700 (1A8) (561236, BD Biosciences), and anti-CD117 APC-eFluor780 (2B8) (47-1171-82, ThermoFisher Scientific). Viable cells were discriminated by the use of eBioscience™ Fixable Viability Dye eFluor™506 (ThermoFisher Scientific). To block unspecific antibody binding Fc Block 2.4.G2 (1:600, Bioceros) was used. Cell surface markers were stained for 30 min at 4 °C in the dark. 123count eBeads™ Counting Beads (ThermoFisher Scientific) were added to each sample. Settings were done using UltraComp eBeads™ Compensation Beads (ThermoFisher Scientific). Data were collected on a BD LSRFortessa and were analyzed with FlowJo software (Tree Star Incorporation).

After 6h there was a strong influx of neutrophils in the airways of mice receiving galectin-10 crystals but not those receiving soluble galectin-10-Y69E mutein or control PBS solution (**Fig. 4A**). After 24h there was a strong influx of monocytes in the airways of mice receiving galectin-10 crystals but not those receiving soluble galectin-10-Y69E mutein or control PBS solution (**Fig 4A**).

The production of pro-inflammatory cytokines in mice receiving galectin-10 crystals was also measured (**Fig. 4B and C**). The production of the chemokine CCL-2 was also measured (**Fig. 4C**). Mice were euthanized by an overdose of pentobarbital and bronchoalveolar lavage (BAL) was performed by injecting 1 ml of PBS containing 0.01 mM EDTA. Subsequently, BAL was spun down (400 g, 5 min, 4°C) and supernatant was stored at -20°C. Lysates of lung tissue were also produced. For evaluation of the cytokine secretion, the Ready-Set-Go ELISA kit from ThermoFisher Scientific was used. A flatbottom 96 half area well plate (Greiner) was coated with 50 µl per well of capture antibody diluted in 1x coating buffer (00-0000-53, ThermoFisher Scientific) and incubated overnight at 4°C. The following antibodies were used: anti-mouse IL-1β capture antibody (1:250; 14-7012-68A); anti-mouse IL-6 capture antibody (1:250; 14-7061-68); anti-mouse TNFα capture antibody (1:250; 14-7423-68), using the instructions of the commercial provider ThermoFisher Scientific . 50 µl per well of the samples, the standard (mouse IL-1β standard (39-8012-60); mouse IL-6 standard (39-8061-60); mouse TNFα standard (39-8321-60), all from ThermoFisher Scientific) and a blank was added in duplo. After incubation and washing, 50 µl per well of detection antibody (biotinylated anti-mouse IL-1β detecting antibody (1:250; 13-7112-68A); biotinylated anti-mouse IL-6 detecting antibody (1:250; 13-7062-68A); biotinylated anti-mouse TNFα detecting antibody (1:250; 13-7341-68A) all from ThermoFisher Scientific)

diluted in 1x assay diluent was added, followed by another incubation of 1h at room temperature. Subsequently, the wells were washed and streptavidin-HRP reagent (1:250; 00-4100-94, ThermoFisher Scientific) diluted in assay diluent was added. After an incubation of 30 min at room temperature, the wells were washed and TMB substrate solution (00-4201-56,
5 ThermoFisher Scientific) was added. The reaction was stopped by 2.5 N H_2SO_4, and absorbance was read at 450 nm with a Perkin Elmer Multilabel counter and data were collected with Wallac 1420 Manager software. For evaluation of CCL-2 levels, the mouse CCL2 (MCP-1) ELISA Ready-SET-Go!™ kit from eBioscience was used in accordance with manufacturer's instructions (cat no. 50-112-5204).
10

Injection of galectin-10 crystals was accompanied by production of IL-6 and TNF-α at 6h post injection, whereas no induction of IL-1β was observed in the BAL samples (not shown). Injection of control soluble galectin-10-Y69E mutein or PBS did not lead to cytokine production (**Fig. 4B**).

15 Injection of galectin-10 crystals was also accompanied by production of large quantities of IL-1β and CCL-2 as measured in lung tissue (**Fig. 4C**).

Example 5. The innate immune response induced by CLCs is not dependent on the Nlrp3 inflammasome *in vivo*

20 Many inorganic and organic crystals have the potential to elicit IL-1β secretion from inflammatory cells through triggering of the Nlrp3 inflammasome, leading to ASC adaptor recruitment, ASC spec formation, and caspase 1 activation for pro-IL-1β processing (Kool et al., 2011). A recent paper posted on $BioR_x$Iv (bioRxiv 252957; doi: https://doi.org/10.1101/252957) reported that Charcot-Leyden crystals purified from a human eosinophilic cell line had the potential to trigger
25 the NLRP3 inflammasome *in vitro*. It was however not reported whether crystal induced inflammation was dependent on NLRP3 *in vivo*, which would pinpoint the inflammasome as a therapeutic target. Inflammation was studied at 24 h as in Example 4. When galectin-10 crystals were injected in the airways of *Nlrp3*$^{-/-}$, or *Casp1/11*$^{-/-}$ mice, there was no reduction in crystal-induced cellular influx compared with wild type littermate C57Bl/6 control mice (**Fig. 5**).
30 Therefore, inflammasome inhibition is unlikely to be successful in inhibiting CLC-induced inflammation *in vivo*.

Example 6. The innate immune response induced by CLCs is independent of *Tlr4*

Recombinant galectin-10 crystals are produced from galectin-10 protein produced in E coli, that
35 has bacterial endotoxin in its cell wall. As endotoxin can trigger an innate immune response in the lungs, it was important to check the potential importance of contamination of galectin-10 crystals with endotoxin to induction of airway inflammation. For this reason, the immune response to galectin-10 crystals in *Tlr4*$^{-/-}$ mice that lack the receptor for endotoxin was also

studied. Inflammation was studied as in Example 4. In these mice, there was no reduction in crystal-induced neutrophilic airway inflammation 24h after injection compared with wild type littermate C57Bl/6 mice (**Fig. 6**).

5 **Example 7. Galectin-10 crystals simulate airway inflammation and IgE synthesis in humanized mice receiving PBMCs from human asthmatics**

Galectin-10 is a neoantigen to the mouse immune system, as mice do not carry the LGALS10 gene coding for galectin-10. Therefore, experiments were set up in humanized mice carrying the immune system of a human house dust mite (HDM) allergic asthmatic donor. To collect PBMCs,
10 50 ml of blood from a house dust mite allergic patient were collected in EDTA-coated tubes. The blood was diluted in RPMI 1640 (v/v) and layered over 12 ml of Ficoll. After centrifugation (1200g, 20 min, room temperature), PBMCs were harvested and washed in PBS. Cells were counted using trypan blue to exclude dead cells. PBMCs were resuspended in PBS at a concentration of $15x10^6$ cells/ml. On day 0, NOD *Rag*$^{-/-}$ $\gamma c^{-/-}$ (NRG) mice were reconstituted by
15 intraperitoneal injection of $3x10^6$ PBMCs. On days 1-4 and 7-9, all mice were injected intratracheally with 20 µg of HDM extract (Greer) diluted in 50 µl of PBS. In experiments addressing the pro-inflammatory effects of galectin-10 crystals, on days 1, 3, 7 and 9 NRG mice were treated with the following regimens (**Fig. 7A**): Regimen 1, PBS control 30 µl; Regimen 2, 100 µg of recombinant galectin-10 crystals (1 µl of the stock) diluted in 30 µl of PBS; Regimen 3,
20 100 µg of recombinant galectin-10-Tyr69Glu mutein (1 µl of the stock) diluted in 30 µl of PBS.

On day 27, all mice were challenged one final time intratracheally with 20 µg of HDM extract (Greer) diluted in 80 µl of PBS. All mice were sacrificed on day 28 using an overdose of pentobarbital injected intraperitoneally. Mice were bled through the iliac vein. Blood was collected in dry tubes. These tubes were centrifuged (5000 rpm for 10 minutes) to obtain serum.
25 To obtain single lung cell suspensions, the left lung was collected, minced using iridectomy scissors, homogenized in PBS over a 100 µm mesh, washed by adding an excess of PBS, and centrifuged at 400g for 7 minutes. Pellets were resuspended in PBS and stored on ice until further use (flow cytometry).

30 The upper and lower lobes of the right lung were fixed in 4% PFA before being embedded in paraffin for histology. The middle lobe of the right lung was embedded in OCT and frozen at -80 degrees until further use (qRT-PCR and immunofluorescence).

To detect human cells, single cell suspensions from the left lung of mice were incubated for 20
35 minutes at 4 degrees with APC-labeled anti-human CD45. Dead cells were stained using the Aqua Live/Dead fixable dead cell stain kit (BD). After washing the cells in PBS, 15000 counting beads were added to each sample. Cells were then analyzed by flow cytometry on a Fortessa

(BD). None of the Abs used cross-react with murine tissues.

Human IgE concentrations were measured in the serum of NRG mice using a Human IgE uncoated ELISA kit (ThermoFischer). Briefly, ELISA plates were coated overnight at 4 degrees with anti-human IgE antibodies in coating buffer. After washing with an excess of PBS-0.05% Tween 20, ELISA plates were blocked for 2 hours at room temperature with the blocking buffer provided by the manufacturer. After washing, the IgE standards (1:2 serial dilution) as well as the sera from NRG mice were added to the plates (dilution 1:5 in blocking buffer), and incubated for 2 hours. The detection antibody was added for 1 hour at room temperature. The presence of human IgE was revealed by adding TMB substrate in all wells. Plates were read at 450 nm on a spectrophotometer.

After 28 days, the degree of human CD45+ cell influx in the airways (**Fig. 7B**), and human IgE synthesis (**Fig. 7C**) was considerably higher in mice receiving 4 injections of crystalline galectin-10 compared with those receiving galectin-10-Tyr69Glu mutein or those receiving PBS during the HDM challenge period, showing that galectin-10 crystals boost human cellular influx in the lungs and IgE synthesis in an asthma model of immunodeficient mice reconstituted with the immune system of a HDM allergic donor.

Example 8. Production of galectin-10 antibodies

A. Immunization of llamas

Two llamas (*llama glama*), named Ynigo and Montoyo, were protein immunized intramuscularly with the galectin-10 crystals (1mg/dose/llama). Protein immunization was started on day 0 and galectin-10 crystals were administered every 14 days for a total of three injections (total five weeks). Five days after the last immunization, 400 mL of blood from the immunized llamas was collected to isolate the PBMCs and allow for RNA extraction.

In order to determine the immune response of the two immunized llamas, an enzyme-linked immunosorbent assay (ELISA) set-up was used. For the ELISA, a homogeneous, non-crystallized form of galectin-10 was used.

To carry out the ELISA, a Maxisorp plate was coated with galectin-10 (100 µg/mL) and blocked with casein. Serial dilutions of llama serum pre- and post-immunization were added to the wells of the plate. Then llama IgG1 bound to coated galectin-10 was detected with a mouse anti-llama CH1 specific antibody (10D12), and detection was realized with an anti-mouse IgG-HRP (DAMPO). Finally, after the addition of TMB, the reaction was stopped with 0.5M H_2SO_4 and absorbance was measured at 450 nm (Tecan Sunrise, Magellan software). Both immunized

llamas showed a strong immune response against galectin-10, even though only 3 injections were carried out.

B. Library construction (scFv)

5 scFv libraries were constructed as follows. mRNA was purified from PBMCs isolated from the blood of the immunized llamas. The mRNA was reverse transcribed with random hexamer primers to obtain cDNA. For construction of heavy and light chain libraries, a two-step PCR was carried out. First, non-tagged primers were used directly on the cDNA to amplify the VH-CH1, VL-CL and Vk-Ck regions. The PCR product was then purified and used in a second PCR with 10 the tagged scFv primers to amplify the VH, VL and Vk and these were cloned separately in the phagemid vector to create the “Lambda” and “Kappa” llama scFv libraries, respectively. The scFv fusion protein consisted of the VH and VL sequences coupled by a $(G_4S)_3$ linker (glycine and serine residues) for a size around 25 kDa. The choice to make scFv libraries was based on the fact that scFv fragments are better expressed as protein III fusions at the tip of the phage than 15 Fabs. This results in a better yield and diversity of the phage library during the phage display selection. However, scFv fragments tend to form aggregates, which can result in apparent better affinities, due to the avidity effect. The antibody fragments in scFv format can be secreted into the periplasmic space of E. coli bacteria by induction with IPTG.

20 Enrichment of the phage expressing specific galectin-10 scFv fragments was performed by three rounds of selection on immobilized galectin-10.

The initial selection of the appropriate scFv clones specific for galectin-10 was carried out by a biopanning approach. Briefly, galectin-10-HIS was immobilized on Maxisorp ELISA plates, then 25 the scFv phages library (Input) was added. Unbound phages were removed via multiple washing steps. Finally, the bound phages were eluted with Trypsin, and E. coli infection was performed in order to amplify the selected phages. This process resulted in the enrichment of the phage population expressing scFv with high affinity anti-galectin-10. At the end of the round of selection, the number of eluted phages was estimated by titration of infected E. coli, spotted 30 (from 10^{-1} to 10^{-6}) on Petri dishes containing solid LB medium with ampicillin and glucose. The first round of selection of the Lambda and Kappa library from both llamas resulted in a minor enrichment of specific anti-galectin-10 phages. These second and third rounds of selection resulted in an enrichment of phages expressing scFv with probably a higher affinity for galectin-10.

35

From the output of round 2 (Library Kappa and Lambda versus 5 µg/mL of galectin-10) and round 3 (Library Kappa versus 0.2 µg/mL and Lambda library versus 0.02 µg/mL), single clones were generated and resulted in the creation of six Master plates. From these Master plates,

periplasmic master plates (PMP) were produced. For this purpose, single clones from the Master plates were first amplified in 96 well format (deep well), and production of the scFv was induced by an overnight incubation with IPTG. The next day, the bacteria were lysed by two cycles of freeze/thaw (-80°C and -20°C). After centrifugation, the supernatant (periplasmic extract) was collected and transferred into separate 96 wells plate in order to be tested for binding capacity (ELISA and Biacore).

C. Screening of the scFv periplasmic extracts by ELISA

In order to test the binding capacity of the scFvs to galectin-10, an ELISA binding assay was established. Briefly, a maxisorp plate was coated with soluble galectin-10 (1 µg/mL), then blocked with Casein, before being incubated with the periplasmic extract (dilution 1/5 in PBS) containing the scFv-Myc. Detection of the binder was carried out with an anti-Myc-HRP antibody. Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. A significant number of galectin-10 scFv binders were isolated after the second round of selection (45-87% of binders). For both llamas, the Lambda library showed higher numbers of galectin-10 binders than the kappa library. The third round of selection resulted in an increase in the number of scFv clones with high binding capacity to galectin-10. The scFv clones generated from the lambda library showed 74-93% binders to galectin-10, whereas the scFv generated from the kappa library showed 15-20% binders.

D. Sequencing and reformatting of scFv clones

Selected scFv clones that showed binding to galectin-10 were sequenced. Based on their CDR1-2-3, VH and VL sequences, each clone was classified as belonging to a particular family. This process resulted in the determination of 65 VH families, 13 VKappa families and 23 VLambda families. Twelve clones shown in Table 3 below were selected for further characterization.

Table 3 scFv clones binding to galectin-10

Clone name	Isolated from selection round	Gal10 concentration during selection (ug/ml)	PERI-ELISA binding (OD values)	VH Family nb	Lambda Family nb	Kappa Family nb
1A12	2	5	2,134	32	4	
2B11	2	0,5	3,793	57	1	
2C07	2	0,5	3,626	7	1	
2E11	2	0,5	3,511	23	23	
3A03	3	0,2	3,4	65		2
4B10	3	0,02	2,435	17	4	
4G05	3	0,2	3,803	18	4	
4H10	3	0,02	3,484	59	17	
5012	3	0,02	2,415	64		3
6A11	3	0,02	2,346	24	23	

6F05	3	0,2	3,827	26	17	
6F011	3	0,02	3,757	53	23	

The CDR, VH and VL sequences of these clones are shown in Tables 14, 15 and 16 below.

The 12 scFv clones in Table 3 were re-cloned as scFv-human Fc fusion molecules. For this purpose, the DNA of each selected scFv clone was first digested with restriction enzymes (AscI/SfiI). After extraction of the DNA from agarose gel, ligation of the DNA was performed into pre-digested vector containing the CH2-CH3 constant domains of the human IgG1 (pUPEX50: pScFv-Fc fusion vector). The transformation of each ligated product was performed using Top10 bacteria by heat shock and transfer onto LB-agarose plates with Ampicillin. After one night of incubation, ligated products showed high numbers of single bacterial colonies whereas no colonies were observed for the negative controls (empty vectors). Per scFv clone, four to eight colonies were picked and sent for sequencing. The clones that showed the proper insert (VH/VL) were selected and amplified in order to purify the DNA sequence (MidiPrep).
Production into mammalian cells was then initiated. Each DNA of the scFv-human Fc clone was transfected into HEK293E cells via the polyethylenimine (PEI). After 6 days, scFv-human Fc molecules were purified from the cell supernatant using the protein-A sepharose beads. Finally, SDS-PAGE analysis was carried out to assess the purity and the integrity of the scFv-human Fc molecules (~100 kDa).

E. Characterization of the scFv-human Fc panel

ELISA and SPR with a T3000 Biacore were used to assess the binding properties of the scFv-human Fc panel.

(i) ELISA analysis

In a similar set-up to that used during the initial screening, the relative binding properties of the 12 scFv-human Fc clones were analyzed by ELISA. Briefly, a maxisorp plate was coated with galectin-10-His at 0.2 µg/mL and blocked with Casein, before being incubated with a serial dilution of the scFv-human Fc fusion molecules. After several washing steps, detection of the bound scFv-human Fc was carried out with an anti-human Fc-HRP antibody. Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. Finally, the raw data (OD values) were plotted on GraphPad Prism 7.01. The EC50 values of each compound, calculated with a non-linear regression (log(agonist) vs. response Variable slope (four parameters)). The results are shown in Table 4 below.

Table 4 ELISA binding characteristics of the lead panel of scFv-human Fc antibodies

Clone name (ScFv-hFc)	Bmax (OD values)	EC50 (nM)
1A12	0,409	ambiguous
2B11	2,432	0,08
2C07	2,887	0,05
2E11	1,425	0,14
3A03	1,81	0,06
4B10	1,28	0,08
4G05	3,034	0,09
4H10	1,301	0,2
5E12	1,84	0,25
6A11	1,595	0,01
6F05	2,895	0,02
6F11	1,565	0,48
Isotype control	0,018	/

- Clones 2C07, 6F05, 4G05 and 2B11 showed the best relative binding capacity with EC50 values between 0.02-0.09 nM.
5
- Clone 6F11 and 5E12 showed the lowest binding capacity (EC50 values between 0.25-0.48 nM).
- One clone (1A12) showed weak binding to galectin-10, with an ambiguous fit.

In addition, the whole panel, showed similar binding capacity to coated galectin-10 and galectin-10-His tagged.

10

(ii) SPR analysis

In order to determine the binding properties (on-rate/off-rate) of the scFv-human Fc panel, their binding capacity to galectin-10 was analyzed on Biacore T3000. For this purpose, a capture approach was set-up. A CM5 Chip was coated with polyclonal anti-human Fc at 8000 RU, then a
15 fixed concentration of the scFv-human Fc panel (1.5 µg/mL), diluted in HBS-EP pH7.4, were captured to reach a binding signal around 150 RU. Finally, a serial dilution of galectin-10-His (serial dilution, 1 over 2 from 5 µg/mL, 6 points of dilution) diluted in HBS-EP pH7.4 was injected. Raw data were analyzed via BIA evaluation software with a blank subtraction (4-3). The kd / KD and Rmax of each scFv-human Fc for galectin-10-His was determined using the Fit Kinetics
20 simultaneous ka/kd / Binding with mass transfer / Local Rmax on BIA evaluation software. The results are shown in Table 5 below.

Table 5 Characterisation of the binding properties of the panel of scFv-human Fc antibodies on Biacore T3000

Clone name (ScFv-hFc)	Rmax	kd 1E-04 (1/s)	KD (nM)
1A12	12,4	31,5	11,7
2B11	37,3	180	0,875
2C07	41,3	2,59	0,677
2E11	21,6	111	1,16
3A03	18,3	111	1,56
4B10	20,6	128	1,94
4G05	23,6	437	0,911
4H10	19,6	27,7	1,55
5E12	6,67	17,7	4,73
6A11	35,6	1240	0,711
6F05	38	1,17	0,918
6F11	28,84	172	2,96
Isotype control	6,06	/	/

- The clones 6F05 and 2C07 showed clearly the best affinity (0.9 and 0.6 nM,
5 respectively), with off-rates of 1.17E-04 and 2.59 E-04 s^{-1}, respectively.
- However, the other 10 clones showed fast off-rate with affinities in a nanomolar range (>17nM).

F. Screening of the scFv clones by Bio-Layer Interferometry (BLI) technology (Octet)

10 In addition to the sequencing and characterization of clones described in sections D and E above, 272 scFv clones from the Master plates 1-6, that showed a clear binding in ELISA (screening) were selected and their binding capacity to galectin-10-His was analyzed on BLI, using the Octet RED96. The BLI is a label-free technology for measuring biomolecular interactions. It is an optical analytical technique that analyzes the interference pattern of white
15 light reflected from two surfaces: a layer of immobilized protein on the biosensor tip, and an internal reference layer. Any change in the number of molecules bound to the biosensor tip causes a shift in the interference pattern that can be measured in real-time.

Briefly, galectin-10-His tagged diluted in Kinetic Buffer was captured on Anti-Penta His 1K sensor
20 tips until an immobilization level of 1 nm was reached. Then, diluted periplasmic extracts (1/10) were applied and association/dissociation to immobilized galectin-10-His was measured using the ForteBio Data analysis 9.0 software (subtraction of the reference Tips, 1.1 binding model). During the screening, only the dissociation (off-rate) of the scFv can be determined since the effective concentration of the scFv is unknown and can vary a lot from clone to clone. The results
25 confirmed that most of the selected clones within the scFv panel showed fast off-rates. However,

some clones, mainly from the lambda library (round 2 and round 3 of selection) showed slow off-rate (dissociation) to captured galectin-10. Therefore, taking into consideration the ELISA and BLI data, a new panel of lead scFvs was selected.

5 Based on the ELISA data and BLI data, a second panel of lead scFv clones was taken forward for further characterisation. The characteristics of these scFv clones is shown in the table below.

Table 6 Second panel of lead scFv clones

10

Clone name	Isolated from selection round	Concentration of galectin-10 used for selection	ELISA binding (OD values)	BLI binding (off rate kdis(1/s))	VH Family	Lambda Family
1C08	2	5	3,7	8,2E-03	59	17
1C09	2	5	3,5	1,4E-03	58	4
1D011	2	5	3,3	5,2E-03	62	17
2C07	2	0,5	3,6	6,7E-03	7	1
2F09	2	0,5	3,6	9,9E-03	57	23
4E08	3	0,2	3,6	9,4E-03	56	1
6A08	3	0,02	3,5	8,2E-03	26	14
6B06	3	0,2	3,7	5,8E-03	53	23
6E10	3	0,02	3,7	1,6E-02	35	23
6F06	3	0,2	3,8	5,7E-03	14	1

The CDR, VH and VL sequences of these scFv clones is shown in Tables 14-16 below.

G. Reformatting of selected scFv clones into a mouse IgG1 backbone

15 The selected leads shown in Table 6 above were re-cloned into a mouse IgG1 backbone for further characterization. For this purpose, the VH and the VL of each clone were PCR amplified using specific primers, isolated by electrophoresis, purified and digested with restriction enzymes (BsmBi). After digestion and clean-up, ligation of the DNA (VH or VL) was performed into BsmBi pre-digested vectors containing the constant domains of the mouse lambda light chain 20 (pUPEX116.35) or of the mouse IgG1 heavy chain (CH1-CH2-CH3, pUPEX116.33). The transformation of each ligated product was carried out using Top10 bacteria by heat shock and transfer onto agarose plates with Ampicillin (resistance gene of the vectors). After one night of incubation, ligated products showed high numbers of single bacterial colonies whereas no colonies were observed for the negative controls (empty vectors). Per clone (HC and LC), four to 25 eight colonies were picked and sent for sequencing. The clones that showed the proper insert were selected and amplified in order to purify the DNA sequence (MidiPrep).

The production of the 10 mouse IgG1 was done by transfection with a ratio of 1 heavy chain for 3 light chains incorporated in HEK293E cells via the polyethylenimine (PEI). After 6 days, mouse 30 monoclonal antibodies were purified from the cell supernant using the protein-A sepharose

beads. Finally, SDS-PAGE analysis was carried out to assed the purity and the integrity of the antibodies (150 kDa).

H. Characterization of the binding properties of the galectin-10 mouse IgG1 panel

5 Several assays were performed to evaluate the binding and functional properties of the galectin-10 mouse IgG1 panel. Both ELISA and SPR with a T3000 Biacore were used to determine the binding capacity of the lead panel.

(i) ELISA analysis

10 In a similar set-up to that used during the characterization of the scFv-human Fc molecules, the relative binding affinities of the 10 mouse IgG1 antibodies were analyzed by ELISA. A maxisorp plate was coated overnight with 0.2 µg/mL of galectin-10-His. Then a serial dilution of each clone (from 100 nM, dilution 1/4, 12 points of dilutions) was incubated on coated galectin-10. After several washing steps, detection of the bound mouse IgG1 was carried out with an anti-mouse 15 Fc-HRP antibody. Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. A mouse IgG1 isotype control was used as negative control. A polyclonal anti-galectin-10 antibody was used as positive control for the coating of galectin-10. The raw data (OD values) were plotted on GraphPad Prism 7.01. The EC50 values for each antibody, calculated with a non-linear regression (log(agonist) vs. response Variable slope (four 20 parameters)), are reported in the table below.

The new panel of galectin-10 mouse IgG1 antibodies showed a relative binding capacity between 3.22 nM to 0.04 nM against coated galectin-10. The clones 2F09, 6A05, 6B06 and 2C07 showed the best relative binding affinity (0.05 – 0.08 nM).

25

Table 7 Characterization of the binding properties of the mouse IgG1 panel by ELISA

Constructs (mouse IgG1)	Bmax	EC50 (nM)
1C09	2,297	0,13
1D011	1,498	0,1
2C07	2,947	0,08
2F09	3,293	0,05
4E08	2,063	3,22
6A05	1,744	0,07
6A08	3,196	0,15
6B06	3,278	0,08
6E10	2,398	0,17
6F06	2,926	0,08
Isotype control	0,042	/

(ii) SPR analysis

The binding capacity of the mouse IgG1 antibodies to galectin-10 was analyzed on Biacore T3000. For this purpose, a capture approach was set-up. A CM5 Chip was coated with polyclonal anti-mouse Fc at 8000 RU, then a fixed concentration of the mouse IgG1 antibody panel (1.5 µg/mL), diluted in HBS-EP pH7.4, was captured to reach a binding signal around 150 RU. Finally, a serial dilution of galectin-10-His (serial dilution, 1 over 2 from 5 µg/mL) diluted in HBS-EP pH7.4 was injected. Raw data were analyzed via BIA evaluation software with a blank subtraction (4-3). The kd / KD and Rmax of each mAb to galectin-10-His was determined using the Fit Kinetics simultaneous ka/kd / Binding with mass transfer / Local Rmax on BIA evaluation software.

Table 8 Characterization of the binding properties of the mouse IgG1 panel by Biacore T3000

Constructs (mouse IgG1)	KD (nM)	kd 1E-04 (1/s)
1C09	0,5	52,9
1D11	1	52,9
2C07	0,5	3,6
2F09	0,3	3,4
4E08	0,8	8,5
6A05	0,6	48,3
6A08	1	6,7
6B06	0,3	30,6
6E10	0,5	8,2
6F06	0,7	48,7

In this set-up, the 10 clones showed an affinity in a nanomolar up to sub-nanomolar range, with an off-rate between 3.4-53 1^{E-04}(1/s). In line with the ELISA binding data, the clones 2F09 and 2C07 were found on the top of the panel. The off-rate measured during the characterization of the mouse panel was not in line with the off-rate measured during the screening. This is mainly explained by the difference in the assay set-up used during the screening (BLI, galectin-10-His captured by anti-His sensor tips) and the characterization (SPR, mouse IgG1 captured by polyclonal anti-mouse Fc Chip).

I. Epitope mapping of the galectin-10 mouse IgG1 panel

In order to identify the galectin-10 binding sites of the different clones, an epitope mapping method (TANDEM), using the BLI technology, was established. Briefly, the galectin-10-His was captured on anti-HIS 1K sensor tips, before incubation with an excess of one antibody (called the

"saturating" antibody) then directly transferred to a solution containing the "competitor" antibody at a suboptimal concentration. If the "saturating" and the competitor antibody bind to the same binding sites, no binding of "competitor" will be detected (expressed in nm shift). If they do not share the epitope, the "competitor" antibody will be able to bind in presence of the "saturating"
5 antibody. An isotype control was used as a negative control for "saturating" antibody, where all the clones used as "competitor" antibodies showed a clear binding to galectin-10.

Table 9 Epitope mapping by competition assay

	Antibody 2 (competitor) at 1 ug/mL (binding signal in nm)										
Antibody 1 (Saturating Ab) (10 ug/mL)	1C09	1D011	2F09	4E08	6A05	2C07	6A08	6B06	6E010	6F06	Buffer
1C09	0,03	0,01	0,04	-0,02	0,00	0,00	0,00	0,01	0,03	0,00	-0,02
1D011	0,10	0,06	0,12	0,03	0,04	0,04	0,05	0,07	0,10	0,06	0,02
2F09	0,02	0,02	0,03	0,01	0,01	-0,01	0,00	0,00	0,00	0,00	0,01
4E08	0,08	0,00	0,10	-0,08	-0,03	-0,05	-0,05	0,03	0,10	0,02	0,01
6A05	0,09	0,06	0,12	0,03	0,05	0,04	0,05	0,06	0,09	0,05	0,01
6A08	0,04	0,01	0,07	-0,03	0,00	-0,01	-0,04	0,02	0,04	0,00	0,01
6E010	0,02	-0,01	0,03	-0,05	-0,03	-0,04	-0,05	-0,02	0,00	-0,03	-0,21
2C07	0,03	0,02	0,05	0,00	0,01	0,03	0,05	0,04	0,06	0,03	0,02
6B06	0,02	0,00	0,03	0,00	0,00	0,04	0,05	0,03	0,04	0,03	0,03
6F06	0,04	0,03	0,06	0,01	0,02	0,04	0,05	0,05	0,07	0,04	-0,05
Isotype	1,21	1,18	1,14	0,97	1,02	0,73	1,04	0,94	1,34	1,15	-0,05
Buffer	/	/	/	/	/	0,80	1,08	0,98	1,38	1,22	/

10 The results showed that the 10 clones tested compete with each other suggesting that they bind to the same binding site/epitope on galectin-10-His.

A similar approach was used for the first panel of scFv-human Fc fusion. All bound on the same epitope on galectin-10 (data not shown) i.e. the same epitope as the mIgG1 anti-galectin-10.

15

Example 9. Characterization of galectin-10 antibodies for ability to affect crystallization *in vitro*

The effects of the lead galectin-10 scFv-human Fc molecules and the lead galectin-10 mouse IgG antibodies on the formation of the recombinant CLCs described in Example 1 was tested.
20 Because galectin-10 autocrystallization is so reproducible *in vitro*, a Mosquito crystallization robot (TTP Labtech) was used to screen anti-galectin-10 antibodies for their potential to block galectin-10 autocrystallization. Using this approach, clones that inhibited galectin-10 crystallization were screened. For the crystal inhibition assay, soluble TEV-cleaved wild-type recombinant galectin-10 in PBS was equilibrated overnight against a solution of 50% PEG 3350 in the presence of
25 anti-galectin antibodies or an irrelevant antibody. To evaluate the inhibition of crystal formation, 250 nL of soluble TEV-cleaved wild-type recombinant galectin-10 in PBS at a concentration of 0.4 – 0.7 mg/mL was mixed with 100 nL anti-galectin-10 antibody or an irrelevant antibody. Next,

the protein mixture was equilibrated against 40 microliter of 50% (v/v) PEG 3350 contained in the reservoir well of a 96-well crystallization plate. Due to the action of the PEG, the amount of water within the drop decreased and therefore the concentration of galectin-10 increased, until it reached the threshold where the galectin-10 crystallizes to form CLCs. Following overnight incubation the presence or absence of CLC crystals was evaluated using a stereomicroscope. The presence of crystal was determined as 100% crystal formation, few crystals observed equal 50% crystal formation and no crystals observed after the incubation time was set as 0% crystal formation. This experiment was not qualitative and only aimed to rank the potency of the different galectin-10 clones to block the CLC formation. Due to the experimental set-up, and the high concentration of anti-galectin-10 molecules needed, only selected clones from the scFv-human Fc and the mouse IgG1 anti-galectin-10 panel were tested.

CLC crystals consistently appeared in control conditions but were absent in conditions containing anti-galectin-10 antibodies. This experimental setup is exemplified in **Fig. 8A** and effectiveness of several clones summarized in **Fig. 8B**.

The results show that all of the clones tested (17 in total) were able to block the CLC formation. Among the scFv-human Fc panel, the clones 4B10, 2E11 and 6F5 showed the best efficacy to block the CLC apparition. The clone 1D11 showed the best potency to block the formation of the CLC among the mouse IgG1 panel. Clone 6A05 showed no CLC formation, even at the lowest ratio galectin-10/mouse IgG1 anti-galectin-10 and the reason for this is unclear. However, all the negative controls (galectin-10 incubated with irrelevant scFv-human Fc or mouse IgG1, buffer or BSA (2mg/mL)) showed 100% CLC formation after 2 days of incubation.

Example 10 Galectin-10 antibodies can solubilize pre-existing CLCs *in vitro*

Using an analogous experimental setup the antibody-mediated solubilization of already formed recombinant galectin-10 crystals was evaluated using the lead galectin-10 scFv-human Fc molecules and the lead galectin-10 mouse IgG antibodies. In this assay, antibodies were added to the crystals that had formed following overnight equilibration against the PEG3350 solution (250 nL of soluble TEV-cleaved wild-type recombinant galectin-10 in PBS against 50% PEG 3350 overnight). Crystal solubilization was observed in function of time after addition of antibodies. 100 nL of anti-galectin-10 or control antibody was added and the solubilization of galectin-10 crystals was observed under a steromicroscope. Each condition was performed in 12 replicas. As a negative control, an irrelevant scFv-human Fc and mouse IgG1 were included, as well as buffer only.

Table 10 shows that most of the tested galectin-10 antibody clones were able to dissolve pre-existing CLCs. This dissolution happened in less than 2 hours after incubation with the anti-galectin-10 molecules.

Clone name	**Format**	**100% Solubilization of CLC (Conc µM)**	**Clone name**	**Format**	**100% Solubilization of CLC (Conc µM)**
1A12	ScFv-human Fc	n.t	1C09	mouse IgG1	10,48
2B11	ScFv-human Fc	5,04	1D11	mouse IgG1	7,86
2C07	ScFv-human Fc	6,86	2C07	mouse IgG1	>2,2
2E11	ScFv-human Fc	4,36	2F09	mouse IgG1	10,67
3A03	ScFv-human Fc	n.t	4E08	mouse IgG1	>10
4B10	ScFv-human Fc	6,14	6A05	mouse IgG1	10,1
4G05	ScFv-human Fc	4,36	6A08	mouse IgG1	>10
4H10	ScFv-human Fc	6	6B06	mouse IgG1	>10
5E12	ScFv-human Fc	n.t	6E10	mouse IgG1	10,48
6A11	ScFv-human Fc	n.t	6F06	mouse IgG1	7,57
6F05	ScFv-human Fc	4	6F06	mouse IgG1	7,57
6F11	ScFv-human Fc	n.t	Isotype	mouse IgG1	No solubilization
6F11	ScFv-human Fc	n.t			
Isotype	ScFv-human Fc	No solubilization			

Example 11 Further characterization of the crystal dissolving properties of selected galectin-10 antibodies

The four clones shown in the table below were selected for further analysis.

Table 11 Summary of characteristics of 6F05, 1C09, 1D11 and 4E08

		ELISA binding	Biacore (capture approach)		Potency in inhibition of the CLC formation and solubilization	
Clone name	Format	EC50 (nM)	KD (nM)	kd 1E-04 (1/s)	100% Inhibition of CLC formation (Conc (µM)	100% Solubilization of CLC (Conc µM)
6F05	scFv-human Fc	0,02	0,9	1,2	1,00	4,00
1C09	mouse IgG1	0,13	0,5	52,9	0,82	10,48
1D11	mouse	0,1	1	52,9	0,34	7,86

	IgG1					
4E08	mouse IgG1	3,22	0,8	8,5	1,11	>10

A. Time-lapse solubilization of CLCs by 6F05, 1C09, 1D11 and 4E08

To better document and characterize the solubilization process of CLC crystals by anti-galectin-10 antibodies, time-lapse experiments on a spinning disk confocal microscope were conducted.
5 2.5 µL of autocrystallized CLC solution (at 0.7 mg/mL) in PBS was spotted in a well of a chamber microscope slide with glass bottom (Ibidi). Crystal solubilization was then initiated and followed over time by the addition of 2 µL of anti-galectin-10 antibodies at a concentration of 7 mg/mL. To prevent evaporation the chamber was sealed with vacuum grease and a glass cover slide.
The CLC dissolution induced by 6F05, 1C09, 1D11 and 4E08 was monitored. Briefly, the solution
10 containing CLCs was spotted in a µ-slide wells plate, before being incubated with a fixed concentration (8mg/mL) of one of the four lead anti-galectin-10 antibodies. For each well, imaging positions were defined, and each position was imaged every 3-5 minutes. These experiments showed that several anti-galectin antibodies can solubilize recombinant CLC crystals in 1 to 2 hours, whereas irrelevant control isotype antibodies cannot (**Fig. 9A**).
15 Furthermore, these high-resolution time lapse experiments show that the CLC crystals diminish in size almost exclusively along their longest axis.

Clone 1D11 showed the best capacity to dissolve the pre-existing CLCs (**Fig. 9B**), where full dissolution was reached after 1 hour. The clones 6F05 and 4E08 showed similar dissolution
20 capacity, with more than 90% CLC dissolution after 90 minutes of incubation. The mouse isotype control showed no effect on the CLCs; however, due to the displacement of the CLCs on the field, the software mis-interpreted this as a diminution of the space occupied by the CLCs.

B. Characterisation of the binding properties of 6F05, 1C09, 1D11 and 4E08 in Fab format

25 The four clones of interest were reformatted as Fab fragments. As a first step the VH and the VL of each clone were PCR amplified using specific primers, purified by electrophoresis, digested with restriction enzymes (BsmBi) and ligated in the pre-digested vectors containing the human constant domains: the human lambda constant domain for the VL (pUPEX116.9) or the CH1 constant domain for the VH (pUPEX86, including part of the hinge region). The transformation of
30 each ligated products was done into Top10 bacteria by heat shock and transfered on agarose plate with Ampicillin (resistance gene of the vectors). After one night of incubation, ligated products showed high number of single colonies whereas no colony was observed for the negative controls (empty vectors). Per clones (VH and VL), four to eight colonies were picked and sent for sequencing. The clones that showed the proper insert were selected and amplified
35 in order to purify the DNA sequence (MidiPrep). The production of the 4 lead clones was initiated in mammalian cells. Transfection was performed with a ratio 1 heavy chain to 1 light

chain incorporated in HEK293E cells via the polyethylenimine (PEI). After 10 days of production, human Fab were purified using the Capture Select IgG-CH1 sepharose beads. Finally, SDS-PAGE analysis was carried out to assess the conformation, the purity and the integrity of the Fab molecules (55 kDa).

5

The binding properties of the leads in Fab format was evaluated in ELISA binding and BLI binding, using an OctetRed96 in accordance with the protocols described above. The ELISA binding data (see Table 12 below), showed that the 4 leads could be separated into 2 groups based on their binding capacity to coated galectin-10-His. The clones 1C09 and 1D11 showed

10 the best relative binding capacity, with an EC50 value between 1.6-1.9 nM, whereas the clones 4E08 and 6F5 showed lower binding potency with an affinity between 25.6-26.7 nM.

Table 12 Characterization of the binding properties of the 4 lead anti-galectin-10 antibodies (Fab format) by ELISA

Constructs (Fab)	Bmax	EC50 (nM)
1C09	1,0	1,9
1D11	0,9	1,6
4E08	0,7	25,6
6F05	0,8	26,7

15

The binding data, obtained with the BLI technology showed similar results, with the clones 1C09 and 1D11 having the best binding capacity (KD between 10-13 nM, kdis between 3-3.8 $^{E-03}$ (1/s)), and the clones 4E08 and 6F05 showing weaker binding (KD between 147-188 nM, kdis between 25-35 $^{E-03}$ (1/s)) – see Table 13.

20

Table 13 Characterization of the binding properties of the 4 lead anti-galectin-10 antibodies (Fab format) by BLI technology

Constructs (Fab)	KD (nM)	kd 1E-04 (1/s)
1C09	13,4	3,83
1D11	10,7	2,99
4E08	147	24,8
6F05	188	35,6

25 **<u>Example 12. Crystal structure of galectin-10 Fab fragments in complex with galectin-10</u>**

The crystal structure of different Fab fragments in complex with galectin-10 was obtained, revealing how galectin-10 antibodies could dissolve existing crystals. For structural studies Fab

fragments of selected antibodies (1D11, 6F5, 4E8, 1C9) were produced in HEK293 cells. Recombinant His-tagged galectin-10 at 1 mg/mL was digested with TEV at room temperature overnight using a TEV:galectin-10 ratio of 1:100. Next, purified Fab was added to digested galectin-10 in 1.25 molar excess. Next, the protein mixture was injected on a HiLoad 16/600
5 Superdex 200 pg column running on HBS buffer to isolate the galectin-10:Fab complex. Fractions corresponding to the galectin-10:Fab complex were pooled and stored at -80°C until further use. Galectin-10:Fab complexes were concentrated to 6 – 7 mg/mL before crystallization experiments. Sitting-drop nanoliter-scale vapour diffusion crystallization experiments were set up at 293 K using a Mosquito crystallization robot (TTP Labtech) and commercially available sparse-
10 matrix screens (Molecular Dimensions, Hampton research).

Crystals of galectin-10 complexed with Fab 1D11 grew overnight in condition B7 of the ProPlex Screen (Molecular Dimensions – 0.2 M ammonium acetate, 0.1 M sodium acetate pH 4.0, 15% PEG_{4000}). Galectin-10 complexed with Fab 6F5 crystallized within 24 hrs in condition G7 of the
15 BCS Eco Screen (Molecular Dimensions) (0.04 M $CaCl_2$, 0.04 M Na-formate, 0.1 M PIPES pH 7.0, 8% PEG Smear High). After 2 weeks crystals appeared of galectin-10 in complex with Fab 4E8 in condition B7 of the PEG/Ion screen (Hampton Research) (0.2 M ammonium nitrate, 20% PEG3350).

20 Before flash-freezing into liquid nitrogen crystals of the galectin-10:Fab complexes were cryoprotected by briefly soaking the crystals in mother liquor supplement with 25% PEG 400. Diffraction experiments at 100 K were conducted on beamlines Proxima 2A of the SOLEIL synchrotron (Gif-sur-Yvette, France) and ID23-2 of the ESRF (Grenoble, France). All data were integrated and scaled using the XDS suite (Kabsch, 2010). Molecular replacement (MR) was
25 performed with Phaser (McCoy et al., 2007) using search models based on the structure of galectin-10 (PDB 1LCL) and a high-resolution mouse Fab structure (PDB 5X4G). Model (re)building was performed in COOT (Emsley et al., 2010) and individual coordinate and ADP refinement was performed in PHENIX (Adams et al., 2010) and autoBuster (Bricogne et al., 2017). Model and map validation tools in COOT and the PHENIX suite were used throughout the
30 work flow to guide improvement and validate the quality of crystallographic models.

The structure of three different galectin-10:Fab complexes was determined (for antibodies 6F5, 1D11, and 4E8) (**Fig. 10A-C, Table 17**). These structures show that the different antibodies target an epitope on galectin-10 around residue Tyr69 (**Fig. 10 D-F**), which was found to be key
35 residue for the autocrystallizing behavior of galectin-10 (**Fig. 3A and C**).

Example 13. Galectin-10 antibodies solubilise patient grown galectin-10 crystals in allergic mucin *ex vivo*

The potential of selected clones to solubilize crystals contained in allergic mucin obtained from patients with CRSwNP was studied. These crystals were therefore in a native mucus environment, and grown *in vivo*. Time-lapse experiments for the antibody-mediated solubilization of human CLCs in mucus were conducted as follows. 4 µL of CLC-containing mucus isolated from a patient was spotted in a well of a chamber microscope slide with glass bottom (Ibidi). Next, 4 µL of anti-galectin-10 or control antibody (at 7 mg/mL) was added to the spotted mucus. To prevent evaporation, the chamber was sealed with vacuum grease and a glass cover slide. The solubilization of human CLC was followed over time using a spinning disk confocal microscope. Microscopic data was analyzed using Fiji. These time lapse experiments are shown in **Fig. 11**. The human CLCs undergo a similar solubilization process as recombinant galectin-10 crystals, but solubilisation took longer. It was however complete by 18h incubation of the allergic mucin with crystal dissolving antibodies.

Example 14. Galectin-10 antibodies inhibit the airway inflammation induced by CLCs

Crystal dissolving antibodies were administered to humanized NRG mice reconstituted with PBMCs of an HDM allergic asthmatic individual (Perros et al. 2009). Since mice do not produce galectin-10 crystals and the PBMC fraction does not contain human eosinophils (the source of endogenous galectin-10 in humans), galectin-10 crystals were administered to the airways of mice together with HDM challenge. It is exceedingly difficult to adoptively transfer viable human eosinophils to mice which is why the galectin-10 crystals had to be administered intratracheally at the time of the HDM allergen exposure. When CLCs were observed in the mice at day 28 of the protocol, they were always associated with Periodic Acid Schiff (PAS)-positive mucus inside the airways, just as seen in patients, adding validity to the adoptive transfer approach.

On day 0, NOD *Rag*$^{-/-}$ γc$^{-/-}$ (NRG) mice were reconstituted by intraperitoneal injection of 3x10^{6} PBMCs. On days 1-4 and 7-9, all mice were injected intratracheally with 20 µg of house dust mite (HDM) extract (Greer) diluted in 50 µl of PBS. The use of humanized mice avoided any confounding effects of murine galectin-10 IgG1 antibodies that would inevitably be induced over the time frame of the 28-day protocol.

In experiments addressing the pro-inflammatory effects of galectin-10 crystals, on days 1,3,7 and 9 NRG mice were treated with the following regimens: Regimen 1, 200 µg of isotype control antibodies intratracheally (diluted in 30 µl of PBS); Regimen 2, 10 µg of recombinant galectin-10 crystals (1 µl of the stock) + 200 µg of isotype control antibodies intratracheally (diluted in 30 µl of PBS); Regimen 3, 10 µg of recombinant galectin-10 crystals (1 µl of the stock) + 200 µg of 1D11 antibodies intratracheally (diluted in 30 µl of PBS) (**Fig. 12A**).

From day 11 onwards, mice received i.t. injections of 200 µg of isotype antibody or 200 µg of 1D11 antibody three times per week until the day of sectioning. On day 27, all mice were challenged one final time intratracheally with 20 µg of house dust mite extract (Greer) diluted in 80 µl of PBS. All mice were sacrificed on day 28

5 After 28 days, the degree of lung inflammation (**Fig. 12B**) was considerably higher in mice receiving galectin-10 crystals + isotype versus isotype alone during the HDM challenge period. Treatment with 1D11 antibody reversed the enhancing effect of galectin-10 crystals on lung inflammation. Furthermore, an investigator blinded morphometric analysis (using QuPath image analysis software for pathology) was performed to assess the degree of cell influx in a perimeter 10 of 500 µm from the basement membrane of the airways. This analysis revealed a markedly increased influx of inflammatory cells around the airways (**Fig. 12E**).

The degree of IgE synthesis (**Fig. 12C**) was assessed by ELISA (see example 7). After 28 days, the serum concentration of IgE (**Fig. 12C**) was considerably higher in mice receiving galectin-10 15 crystals + isotype versus isotype alone during the HDM challenge period. Treatment with 1D11 antibody reversed the enhancing effect of galectin-10 crystals on IgE concentration.

The presence of goblet cell metaplasia, measured using mucin MUC5AC mRNA levels was also assessed. For this, frozen lung tissue was collected in an eppendorf tube and 1 ml of Tripure was added. Tissue was homogenized using a tissue homogenizer. To extract RNA, 200 µl of 20 chloroform was added to the tubes containing the homogenized lung. After an incubation period of 5 minutes, tubes were centrifuged at 12000g for 15 minutes. The upper transparent phase was collected in an RNase-free eppendorf, and was mixed with 500 µl of Isopropanol and 1 µl of Glycogen for 10 minutes. The tubes were centrifuged at 12000g for 5 minutes. The supernatant was discarded, and the pellet containing the purified RNA was washed in 75% ethanol 25 (centrifugation at 7500g for 5 minutes). The pellet was air dried for 10 minutes at room temperature and was resuspended in 20 µl of RNase-free water. The tubes were placed for 10 minutes at 60°C. The concentration of RNA was determined in each sample using a Nanodrop instrument. 1 µg of RNA was used to make cDNA using the sensifast cDNA sysnthesis kit (Bioline). The leftover RNA was frozen at -80°C. The cDNA was diluted 10 times in water, and 30 frozen until further use. To perform real time PCR, the following mastermix was used for each well of the PCR plate: 10 µl of Sensifast SYBR No-Rox mix, 4,75 µl Water, 5 µl of cDNA. 0.125 µl of forward primer and 0.125 µl of reverse primer (taken from a 100 µM stock) were added to each PCR reaction. Primers used were the following:murine Muc5ac (Fwd: CTCCGTCTTAGTCAATAACCACC (SEQ ID NO: 156); Rev: GGAACTCGTTGGATTTTGGACTG 35 (SEQ ID NO: 157)); Murine GAPDH as housekeeping (Fwd: ACAAAATGGTGAAGGTCGGTG (SEQ ID NO: 158); Rev: TGGCAACAATCTCCACTTTGC (SEQ ID NO: 159)).

After 28 days, the mRNA concentration of Muc5AC (**Fig. 12D**) was considerably higher in mice receiving galectin-10 crystals + isotype versus isotype alone during the HDM challenge period. Treatment with 1D11 antibody reversed the enhancing effect of galectin-10 crystals on MUC5AC mRNA concentration. More detailed histological analysis revealed markedly enhanced goblet cell metaplasia and mucus production, as visualized by enhanced PAS staining of lung epithelial cells that had the typical granule-rich aspect of goblet cells.

Bronchial hyperreactivity (BHR) was also assessed in the mouse model since BHR is an essential feature of asthma. The effect of CLC administration on responsiveness of mechanically ventilated mice to the inhaled bronchoconstrictor methacholine was assessed using the FlexiVent invasive measurement of dynamic resistance (Hammad et al. 2007). In brief, mice were anesthetized with urethane and paralyzed using D-tubocurarine, tracheotomized and intubated with an 18G catheter, followed by mechanical ventilation by a Flexivent apparatus. Increasing concentrations of methacholine (0-200 µg/ml) were aerosolized via the catheter. Dynamic resistance (rrs) was recorded after a standardized inhalation maneuver given every 10 seconds for 2 minutes after methacholine administration. Addition of CLCs to HDM boosted the degree of bronchoconstriction compared with HDM alone and treatment with 1D11 completely neutralized this effect (see **Fig. 12F**).

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Table 14: Heavy chain CDR sequences of scFv antibodies binding to galectin-10

scFv clone	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3	SEQ ID NO.
1D11	DYAMS	1	GISWNGGSTYYAESMKG	2	DRNLGYRLGYPYDY	3
6F05	SYAMS	4	AINSGGGSTSYADSVKG	5	PGDRLWYYRYDY	6
4E08	TSYYAWS	7	VIASDGSTYYSPSLKS	8	YIRGSSWSGWSAYDY	9
1C09	TNSYYWS	10	AIAYSGSTYYSPSLKS	11	RPNWYRALDA	12
3A03	VYAMS	13	DINTSGDSTTYADSVKG	14	RYTQE	15
1A12	SYYMS	16	AINSGGGSTYYADSVKG	17	NGGIWSFGS	18
2E11	SYAMS	4	PINSGSDSASYVDSVKG	19	ARTSVVAGGMDY	20
4G05	RYWMI	21	SIYNDGGNTYYADSVKG	22	LKAAYYGMDY	23
2C07	SYAMS	4	NINSGGGSTGYADSVKG	24	YLRTYYPNAAFGMDY	25
4B10	NYWMY	26	AIDVGGGTTDYAGSVKG	27	GGSYYGGMDY	28
6A11	AYAMN	29	GVNSGGGLTSYGESVKG	30	SKRGAVVAGTGDDY	31
4H10	DYAMS	1	AISWNGGSTYYAESMKG	32	DLSASGSYYHTFGS	33
6F11	TGPYSWS	34	YIGYSGSTYYSPSLKS	35	SRSSPTTFGMDY	36
2B11	TNYYYWS	37	AIAYSGSTYYSPSLKS	11	APYGISREYDY	38
5E12	NYPMT	39	AINGGGDIPYYADSVKG	40	QKWGYDPRRTDFEF	41
6A05	SYAMS	4	AINSGGGWTSYVDSVKG	42	YSGPELNTQYGMDY	43

6A08	SYAMS	4	AINRGGGSTYYADSVKG	44	PGDRLWYYRYDY	6
2F09	TNYFYWS	45	AIAYSGRTYYSPSLKS	46	GPKGLASYYDY	47
6F06	RYSMS	48	TINSGGGSTSYVDSVKG	49	SQGGISFSTQYGMDY	50
6B06	TNYYSWS	51	YIAYSGSTSYSPSLKS	52	SRSSPTTFGMDY	36
6E10	SYWMY	53	AINTGGGSTYYADSVKG	54	AGSGVAGTGYDY	55

Table 15: Light chain CDR sequences of scFv antibodies binding to galectin-10

scFv clone	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3	SEQ ID NO.
1D11	AGTSSDVGYGNYVS	56	EVNKRAS	57	ASYRSSNNAV	58
6F05	AGTSSDIGYGNYVS	59	KVSRRAS	60	ASYRYRNNVV	61
4E08	QGGNFGYYYGS	62	KDSERPS	63	QSADSSDNPV	64
1C09	QGANLGRYYGI	65	GDSNRPS	66	QSYESSTSPV	67
3A03	KPGRTLVHTDGRTYLY	68	QVSNRGS	69	AQATYYPLT	70
1A12	QGGNFGYYYVS	71	GDSNRPS	66	LSYESSDYPV	72
2E11	QGGKFGSYYVS	73	KDNERPS	74	QSGSSSDNIV	75
4G05	QGANLGRYYGI	65	GDSNRPS	66	QSYESSTSPV	67
2C07	QGGNFGRYYAS	76	RDSERPS	77	QSGRSSDNAV	78
4B10	QGAKLGRYYGI	79	GDSNRPS	66	QSYESSTSPV	67

6A11	QGGNFGRYYVS	80	KDSERPS	63	QSGSSSDNAV	81
4H10	AGTSSDVGYGDYVS	82	KVKTRAS	83	ASYKNGGTAV	84
6F11	QGGDFGRYYVA	85	QDSERPS	86	QSGISSDNIV	87
2B11	QGGKFGRYYAS	88	KDSERPS	63	QSGRSSDNAV	78
5E12	KSSQSVRIESNHKTYLN	89	DASSRES	90	QQAYAAPT	91
6A05	QGGNFGSYYAS	92	RDSGRPS	93	QSGSSSDNTV	94
6A08	GLSSGSVTSSNYPG	95	NTNSRYS	96	ALNRVRGTYRV	97
2F09	QGGNFGYYYVS	71	RDSGRPS	93	QSGSSSDNTV	94
6F06	QGGNFGRYYAN	95	KDSERPS	63	QSGSVSDNAV	96
6B06	QGGNFGRYYVS	80	QDSERPT	97	QSGTSSDNIV	98
6E10	QGGNFGYYYVS	71	RDSGRPS	93	QSGSSSDNAV	81

Table 16: VH and VL sequences of scFv antibodies binding to galectin-10

scFv clone	VH	SEQ ID NO.	VL	SEQ ID NO.
1D11	QVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSGISWNGGSTYYAESMKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCAKDRNLGYRLGYPYDYWGQGTQVTVSS	99	QSVLTQPPSVSGSPGETVTISCAGTSSDVGYGNYVSWYQQLPGMAPRLLIYEVNKRASGITDRFSGSKSGNTASLTISGLQSEDEGDYYCASYRSSNNAVFGGGTHLTVL	100
6F05	QLQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINSGGGSTSYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATPGDRLWYYRYDYWGQGTQVTVSS	101	QAGLTQPPSVSGTLGKAVTISCAGTSSDIGYGNYVSWYQQLPGTAPKLLIYKVSRRASGVPDRFSGSKSGNTASLSISGLQSEDEADYYCASYRYRNNVVFGGGTHLTVL	102
4E08	QVQRQESGPGLVKPSQTLSLTCTVSGGSITTSYYAWSWIRQPPGKGLEWMGVIASDGSTYYSPSLKSRTSISRDTSKNQFSLQLSSVTPEDTAVYYCALYIRGSSWSGWSAYDYWGQGTQVTVSS	103	QPVLNQPSAVSVSLGQTARITCQGGNFGYYYGSWYQQKPGQAPVLVIYKDSERPSGIPERFSGSSSGGTATLTISRAQAEDEADYYCQSADSSDNPVFGGGTHLTVL	104
1C09	QVQLVESGPGLVKPSQTLSLTCTVSGGSITTNSYYWSWIRQPPGKGLEWMGAIAYSGSTYYSPSLKSRTSISRDTSKNQFTLHLSSVTPEDTAVYYCARRPNWYRALDAWGQGTLVTVSS	105	NFMLTQPSAVSVSLGQTARITCQGANLGRYYGIWYQQKPGQAPVQVIYGDSNRPSGIPERFSGSSSGGTATLTISGAQAEDEADYYCQSYESSTSPVFGGGTHLTVL	106
3A03	QVQLVESGGGLVQPGGSLRLSCATSGFTFSVYAMSWVRQAPGKGLEWVADINTSGDSTTYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCANRYTQERGQGTQVTVSS	107	DVVLTQTPGSLSVVPGEAASISCKPGRTLVHTDGRTYLYWLQQKPGQRPQLLIYQVSNRGSGVPDRFTGSGSGTDFTLKISGVKAEDAGVYYCAQATYYPLTFGSGTRLEIK	108
1A12	QVQLVESGGGLVQPGGSLRVSCAASGFTFSSYYMSWVRQAPGKGLEWVSAINSGGGS	109	SSALTQPSAISVSLGQTARITCQGGNFGYYYVSWYQQKPGQAPVQVIYGDSNRPSGIPERFSGSSSGGT	110

	TYYADSVKGRFTISRDNAKNTLYLQMNSLKSEDTAVYYCVQNGGIWSFGSWGQGTQVTVSS		ATLTISGAQAEDEADYYCLSYESSDYPVFGGGTHLTVL	
2E11	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSPINSGSDSASYVDSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCAKARTSVVAGGMDYWGKGTLVTVSS	111	QSALTQPSAVSVSLGQTARITCQGGKFGSYYVSWYQQKPGQAPVMVIYKDNERPSGIPERFSGSSSGSTSTLTISGAQAEDEGTYYCQSGSSSDNIVFGGGTELTVF	112
4G05	QLQVVESGGGLVQTGGSLTLSCTTSGFTFSRYWMIWVRQAPGKGLEWVSSIYNDGGNTYYADSVKGRFTISRDNSENTLYLQMNSLKSEDTAVYYCAKLKAAYYGMDYWGKGTLVTVSS	113	SSALTQPSAVSVSLGQTARITCQGANLGRYYGIWYQQKPGQAPVQVIYGDSNRPSGIPERFSGSSSGGTATLTISGAQAEDEADYYCQSYESSTSPVFGGGTHLTVL	114
2C07	ELQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSNINSGGGSTGYADSVKGRFTISRDNAKNTLYLQMNSLKPEDMAVYYCAKYLRTYYPNAAFGMDYWGKGTLVTVSS	115	QAVLTQPSAVSVSLGQTARITCQGGNFGRYYASWYQQKPGQAPVLVIYRDSERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGRSSDNAVFGGGTHLTGL	116
4B10	QLQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMYWVRQAPGKGLEWVSAIDVGGGTTDYAGSVKGRFTISRDNTKSTVYLQMNTLKPEDTALYYCLRGGSYYGGMDYWGKGTLVTVSS	117	QSALTQPSAVSVSLGQTARITCQGAKLGRYYGIWYQQKPGQAPVQVIYGDSNRPSGIPERFSGSSSGGTATLTISGAQAEDEADYYCQSYESSTSPVFGGGTHLTVL	118
6A11	ELQLVESGGGLVQPGGSLRLSCSASGFTFGAYAMNWVRQAPGKGLEWVSGVNSGGGLTSYGESVKGRFTISRDNAKNTLYLQMNRLNPDDTAVYYCAKSKRGAVVAGTGDDYWGQGTQVTVSS	119	SSALTQPSAVSVSLGQTARITCQGGNFGRYYVSWYQQKPGQAPVLVIYKDSERPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDNAVFGGGTHLTVL	120

4H10	QVQLVESGGGLVQPGGSLRLSCAASGFTFDDYAMSWVRQAPGKGLEWVSAISWNGGSTYYAESMKGRFTISRDNAKNTLYLQMNSLKSEDAAVYYCAKDLSASGSYYHTFGSWGQGTQVTVSS	121	QAGLTQPPSVAGTLGKTVTISCAGTSSDVGYGDYVSWYQHIPGTAPKLLIYKVKTRASGIPDRFSGSKSGNTASLTISGLQSGDESDYYCASYKNGGTAVFGGGTHLTVL	122
6F11	QVQRQESGPGLVKPSQTLSVTCTVSGGSITTGPYSWSWIRQPPGKGLEWIGYIGYSGSTYYSPSLKSRTSISRDTSNNQFSLQLSSVTPEDTAVYYCARSRSSPTTFGMDYWGKGTLVTVSS	123	SSALTQPSAVSVSLGQTARITCQGGDFGRYYVAWYTQKPGQAPVLVIYQDSERPSGIPERFSGSSSGDTATLTISGAQAEDEAEYYCQSGISSDNIVFGGGTHLTVL	124
2B11	QVQLQESGPGLVKPSQTLSLTCTVSGGSITTNYYYWSWIRQPPGKGLEWMGAIAYSGSTYYSPSLKSRTSISRDTSKNQFTLQLSSVTPEDTAVYYCARAPYGISREYDYWGQGTQVTVSS	125	HSAVTQPSAVSVSLGQTARITCQGGKFGRYYASWYQQKPGQAPVLVIYKDSERPSGIPERFSGSSSGDTATLSISGAQAEDEADYFCQSGRSSDNAVFGGGTHLTVL	126
5E12	QLQLVESGGGLVQPGGSLRLSCVASGFTFRNYPMTWVRQAPGKGPEWVSAINGGGDIPYYADSVKGRFTISRDNAKNTVYLQMDSLKPEDTAMYYCAKQKWGYDPRRTDFEFRGQGTQVTVSS	127	ETVPTQSPSSVTASVGEKVTITCKSSQSVRIESNHKTYLNWYQQRPGRGPRLLIYDASSRESGIPDRFSGSGSTSDFTLTISSVQPEDAAVYYCQQAYAAPTFGQGTKLEIK	128
6A05	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAINSGGGWTSYVDSVKGRFTISRDNAKNTLYLQMDSLKPEDTAVYYCTKYSGPELNTQYGMDYWGKGTLVTVSS	129	NFMLTQPSAVSVSLGQTARITCQGGNFGSYYASWYQQKPGQAPVLVIYRDSGRPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDNTVFGGGTHLTVL	130
6A08	QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGPEWVSAINRGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDAAVYYCATPGDRLWYYRYDYWGQGAQVTVSS	131	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPGWYQQTPGQAPRLLIYNTNSRYSGVPNRFSGSISGNKAALIISGAQPEDEADYHCALNRVRGTYRVFGGGTHLTVL	132

2F09	QVQVQESGPGLVKPSQTLSLTCTVSGGSITTNYFYWSWIRQSPGKGLEWIGAIAYSGRTYYSPSLKSRTSISRDTSNNQFTLQLSSVTPEDTAVYYCARGPKGLASYYDYWGQGTQVTVSS	133	SSALTQPSAVSVSLGQTARITCQGGNFGYYYVSWYQQKPGQAPVLVIYRDSGRPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDNTVFGGGTHLTVL	134
6F06	QVQLVESGGGLVQPGGSLRLSCAASEFTFSRYSMSWVRQAPGKGLEWVSTINSGGGSTSYVDSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCTKSQGGISFSTQYGMDYWGKGTLVTVSS	135	NFMLTQPSAVSVSLGQTARITCQGGNFGRYYANWYQQKPGQAPVLVIYKDSERPSGIPERFSGSSSGDTATLTISGAQAEDESDYYCQSGSVSDNAVFGGGTHLAVL	136
6B06	QVQRQESGPGLVKPSQTLSLTCTVSGGSITTNYYSWSWIRQPPGKGLEWIGYIAYSGSTSYSPSLKSRTSISRDTSNNQFSLQLSSVTPEDTAVYYCARSRSSPTTFGMDYWGKGTLVTVSS	137	SSALTQPSAVSVSLGQTARITCQGGNFGRYYVSWYTQKPGQAPVLVIYQDSERPTGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGTSSDNIVFGGGTHLTVL	138
6E10	QLQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMYWVRQAPGKGLEWVSAINTGGGSTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARAGSGVAGTGYDYWGQGTQVTVSS	139	QPVLNQPSAVSVSLGQTARITCQGGNFGYYYVSWYQQKPGQAPVLVIYRDSGRPSGIPERFSGSSSGDTATLTISGAQAEDEADYYCQSGSSSDNAVFGGGTHLTVL	140

Table 17 X-ray data and refinement statistics

	Recombinant Gal10	***Ex vivo* CLC crystal**	**Recombinant Gal10-Tyr69Glu**	**Recombinant Gal10:Fab-1D11**	**Recombinant Gal10:Fab-6F5**	**Recombinant Gal10:Fab-4E8**
Beam line	P14 (PetraIII)	P14 (PetraIII)	PXIII (SLS)	ID23-2 (ESRF)	ID23-2 (ESRF)	Proxima 2A (SOLEIL)
Ligands	glycerol	glycerol	PG4, PG6, PGE	PG4	Ca^{2+}, PGE	/
Crystallization conditions	PBS	PBS	0.2 M ammonium sulphate pH 5.1 20 % PEG_{3350}	0.2 M ammonium acetate 0.1 M sodium acetate pH 4.0 15% PEG_{4000}	0.04 M CaCl2, 0.04 M sodium formate, 0.1 M PIPES pH 7.0 8% PEG Smear High	0.2 M ammonium nitrate 20% PEG_{3350}
cryo	35% glycerol	35% glycerol	25 % PEG_{400}	25% PEG_{400}	25% PEG_{400}	20% PEG_{400}
Data collection						
wavelength	0.9763	0.9763	0.99998	0.87313	0.87313	0.980058
Space group	P 6522	P 6522	P 21	P 21 21 21	C 2 2 21	P 21
Cell dimensions						
a, *b*, *c* (Å)	48.88, 48.88, 258.64	48.86, 48.86, 257.88	72.82, 93.30, 93.07	61.63, 89.22, 249.27	59.01, 146.19, 185.70	41.33, 150.45, 94.50
α, β, γ (°)	90.00, 90.00, 120.00	90.00, 90.00, 120.00	90.00, 108.94, 90.00	90.00, 90.00, 90.00	90.00, 90.00, 90.00	90.00, 96.31, 90.00
Resolution (Å)	50.00-1.34 (1.42-1.34)	50.00-2.22 (2.35-2.22)	50.00-2.10 (2.23-2.10)	50.00-1.90 (2.02-1.90)	50.00-1.91 (2.02-1.91)	50.00-3.39 (3.60-3.39)
R_{meas}	7.6 (71.5)	30.7 (194.6)	15.4 (227.8)	23.4 (137.0)	23.6 (215.1)	21.9 (107.0)
$I / \sigma I$	25.64 (5.81)	7.85 (1.29)	9.33 (0.70)	6.72 (1.30)	7.67 (1.00)	5.09 (1.05)
Completeness (%)	92.4 (68.3)	99.9 (99.1)	99.0 (98.6)	99.1 (94.6)	99.7 (98.3)	98.4 (98.1)
Redundancy	17.9 (14.65)	11.86 (12.20)	6.90 (6.74)	6.40 (5.99)	12.85 (11.44)	3.22 (3.33)
Wilson B factor	10.13	23.36	39.68	20.09	25.08	81.87
Refinement						
Resolution (Å)	43.10-1.38	43.06-2.30	47.30-2.10	46.97-1.90	47.24-1.91	46.97-3.39
No. reflections	37 604	10 056	68 227	107 597	63 022	15 645
R_{work} / R_{free}	16.81 / 17.94	18.26 / 23.54	17.24 / 20.43	17.47 / 22.34	16.61 / 20.11	31.74 / 34.44
No. atoms						

Protein	1248	1122	6766	8812	4452	8344
Ligand/ion	6	6	112	13	11	/
Water	209	129	648	1457	835	/
B-factors						
Protein	15.04	28.68	50.77	26.14	38.06	133.84
Ligand/ion	25.31	53.67	70.35	65.21	71.99	/
Water	36.26	45.50	60.13	40.34	52.48	/
R.m.s. deviations						
Bond lengths (Å)	0.005	0.010	0.010	0.010	0.010	0.008
Bond angles (°)	0.91	1.14	1.12	1.14	1.09	1.16

Example 15. Production of additional galectin-10 antibodies

Additional galectin-10 antibodies were selected by bio-panning against human and cynomolgus galectin-10 and screened by ELISA and BLI for binding to galectin-10 (human and cynomolgus).

5 The sequences of the three cynomolgus galectin-10 isoforms (WGS, REF and YRT) are shown below together with the human sequence.

```
   Human: MSLLPVPYTEAASLSTGSTVTIKGRPLACFLNEPYLQVDFHTEMKEESDIVFHFQVCFGRRVVMNSREY
   WGS:   MSLLSVPHTESVSLSTGSTVTIKGRPLVCFLNEPHLQVDFHTEMKEDSDIAFHFQVYFGNRVVMNSREF
10 REF:   MSLLSVPHTESVSLSTGSTVTIKGRPLVCFFNEPHLQVDFHTEMKEDSDIAFHFQVYFGNRVVMNSREF
   YRT:   MSLLSVPHTESVSLSTGSTVTIEARPLVCFFNEPHLQVDFHTEMKEDSDIAFHFQVYFGNRVVMNSREY

   GAWKQQVESKNMPFQDGQEFELSISVLPDKYQVMVNGQSSYTFDHRIKPEAVKMVQVWRDISLTKFNVSYLKR
    KIWKEEVESKNMPFQDGQEFELSILVLEDKYQVMVNGQAYYNFNHRIPVSSVKMVQVWRDISLTKFNVSN---
15  KIWKEEVESKNMPFQDGQEFELSILVLEDKYQVMVNGQAYYNFNHRIPVSSVKMVQVWRDISLTKFNVSN---
   RTWKEEVESKNMPFQDGQEFELRILVLEDKYQVMVNGQAYYNFNHRIPVSSVKMVQVWRDISLTKFNVSN---
```

(WGS: SEQ ID NO: 267; REF: SEQ ID NO: 268; YRT: SEQ ID NO: 269)

20 Sequences analysis of the human and the 3 cynomolgus isoforms revealed the following:

- The isoforms of cynomolgus galectin-10 show 78 – 81% identity to human galectin-10
- The WGS and the REF isoform differ by a single amino acid at position 31 (F/L)
- The YRT isoform shows 6 different amino acids compared to the REF isoform at position 23 (K/E), 24 (G/R), 69 (F/Y), 70 (K/R) , 71 (I/T) and 92 (S/R).
- 25 Only the YRT isoform has a Tyrosine residue at position 69, as in the human.

Eleven scFv clones that showed binding to galectin-10 were sequenced and reformatted as human Fc fusion molecules, as described in Example 8.

30 A. Screening of the scFv by ELISA and Bio-Layer Interferometry (BLI) technology

(i) ELISA analysis

The binding capacity of the scFv (periplasmic extract) to human galectin-10 and the 2 available isoforms of cynomolgus galectin-10 (WGS and REF) was analyzed on ELISA binding. Briefly, a
35 maxisorp plate was coated overnight with 1 µg/mL of human galectin-10-His or cynomolgus WGS or REF isoform galectin-10-His, then blocked with PBS 1% Casein, before being incubated with the periplasmic extract (dilution 1/5 in PBS 0.1%Casein) containing the scFv-Myc tagged. Detection of the binder was carried out with an anti-Myc-HRP antibody (Bethyl, Catalog A190-105P). Then TMB substrate was added and the reaction was stopped with 0.5M H_2SO_4.
40 Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. Finally, the raw data (OD values) were plotted using GraphPad Prism 7.01 and are shown in **Fig. 13**. The

number of binders, defined by an OD value higher than 0.5, per MP plate and per library was determined. A blank control and negative control (scFv periplasmic extract binding to irrelevant target) were included and showed, as expected, no binding.

5 The results showed that a significant number of scFv binding to human or cynomolgus galectin-10 were isolated after the last round of selection. However, significant difference in the enrichment of human cynomolgus binders (REF and WGS isoforms) was observed between master plates, with 10,6-87% of human binders and 0-92,6 % of cynomolgus WGS & REF isoforms binders. For both llamas, the Lambda library showed clearly higher percentage of scFv 10 binding to human or cynomolgus galectin-10 than the kappa library. It was particularly the case for the library 1K that showed 0-4% of cynomolgus binders (WGS & REF isoforms). In opposition the library 1L and 2L showed 76-92.6 % human cynomolgus binders, indicating that the lambda library may be the mean provider of human cynomolgus cross reactive binders. Master plates generated from the condition where acidic pH elution was applied (MP07 and MP08) also 15 showed relatively high percentage of human and cynomolgus binders with 0-60.9% of binders for the Kappa libraries and 76.1-89.1% of binders for the Lambda libraries. As expected and due to their extremely close homology a general trend scFv showed similar binding to the WGS and the REF isoform of the cynomolgus galectin-10-His

20 **Table 18 ELISA binding screening of the scFv periplasmic extracts from MP generated after selection.**

MP number	Library	Galectin-10-His	Hits	% Hits
MP07	1K	human	24	52,2
		cynomolgus_WGS	1	2,2
		cynomolgus_REF	0	0,0
	1L	human	41	89,1
		cynomolgus_WGS	41	89,1
		cynomolgus_REF	41	89,1
MP08	2K	human	5	10,9
		cynomolgus_WGS	28	60,9
		cynomolgus_REF	28	60,9
	2L	human	35	76,1
		cynomolgus_WGS	36	78,3
		cynomolgus_REF	38	82,6
MP09	1K	human	58	61,7
		cynomolgus_WGS	0	0,0
		cynomolgus_REF	4	4,3
MP10	1L	human	82	87,2
		cynomolgus_WGS	87	92,6
		cynomolgus_REF	87	92,6
MP11	2L	human	76	80,9
		cynomolgus_WGS	80	85,1
		cynomolgus_REF	79	84,0
MP12	2K	human	10	10,6

		cynomolgus_WGS	59	62,8
		cynomolgus_REF	59	62,8

(ii) BLI Analysis

From the six Master plates generated during selection, 321 clones that showed human and cynomolgus binding during the ELISA screening campaign were picked up and their binding capacity was tested on BLI, using the Octet RED96. Briefly, human and cynomolgus WGS or REF isoforms of galectin-10-His tagged were diluted in Kinetic buffer (PBS 0.01%BSA 0.002%Tween 20) at 200 µg/mL before being captured on Anti-Penta His 1K sensor tips (ForteBio, Cat#18-5120) until an immobilization level of 1 nm was reached. Then, diluted periplasmic extracts (1/5 in Kinetic buffer), containing the scFv clone, were applied and association/dissociation to immobilized galectin-10-His was measured using the ForteBio Data analysis 9.0 software (subtraction of the reference Tips, 1.1 binding model). During the screening, only the dissociation (off-rate) of the scFv could be determined since the effective concentration of the scFv was unknown and can vary a lot from clone to clone. The results confirmed that most of the selected scFv clones show human cynomolgus cross-reactivity (see **Fig. 14**).

B. Characterization of the scFv-human Fc panel

ELISA and SPR with a T3000 Biacore were used to assess the binding properties of the scFv-human Fc panel.

(i) ELISA analysis

In a similar set-up to that used during the initial screening, the relative binding properties of the 11 new scFv-human Fc clones were analyzed by ELISA. Briefly, a maxisorp plate was coated overnight with human or cynomolgus WGS or YRT isoforms of galectin-10-His at 0.2µg/mL and blocked with PSB 1% Casein, before being incubated with a serial dilution of the scFv-human Fc fusion molecules (from 100 nM, dilution 1/5, 8 points of dilutions). After several washing steps, detection of the bound scFv-human Fc was carried out with an anti-human Fc-HRP antibody (Jackson ImmunoResearch, Catalog 109-035-008). Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. Finally, the raw data (OD values) were plotted on GraphPad Prism 7.01. The EC50 values of each clone, calculated with a non-linear regression (log(agonist) vs. response Variable slope (four parameters)) are reported in Table 19 below. As a positive control, clone 6F05 from the previous galectin-10 antibody panel was included.

Table 19 ELISA binding characteristics of the panel of scFv-human Fc galectin-10 antibodies

	humanGal10-His (0.2 µg/mL)		cynoGal10-His_WGS (0.2 µg/mL)		cynoGal10-His_YRT (0.2 µg/mL)	
Clone (scFv-human Fc)	Bmax	EC50 (nM)	Bmax	EC50 (nM)	Bmax	EC50 (nM)
7B07	0,6	1,11	0,6	0,82	0,0	No binding
7C05	1,0	0,67	1,5	ambiguous	2,1	2,53
7D10	1,5	ambiguous	2,3	ambiguous	2,3	ambiguous
7E09	2,0	1,48	2,9	1,66	2,3	1,64
8H11	2,3	0,18	3,1	0,13	3,1	0,10
10A06	1,2	0,08	2,5	0,10	3,0	0,04
10B02	2,2	0,09	3,3	0,05	3,2	0,05
10D02	1,9	0,56	3,1	1,12	2,0	1,40
10H06	1,9	0,63	2,9	0,90	2,4	0,96
11F02	1,5	0,77	2,5	ambiguous	2,5	ambiguous
11F12	1,8	0,56	3,1	0,23	3,0	0,24
6F05	2,4	0,02	0,3	ambiguous	0,3	ambiguous

5 The binding of the scFv-human Fc panel to coated human and cynomolgus galectin-10 showed a relative binding capacity ranging from 0.08-1.48 nM on human and 0.04-2.53 nM on cynomolgus WGS and YRT isoforms of galectin-10. The following observations were made:

- The positive control clone 6F05 showed the best relative binding capacity of the scFv panel against the human target (0.02 nM EC50 value and 2.4 OD value as Bmax).
10 However, this clone showed weak binding to both cynomolgus isoforms (ambiguous fitting and Bmax equal to 0.3 OD value).
- Clones 10A06, 10B02 and 8H11 showed the best relative binding capacity, with EC50 values between 0.08-0.18 nM against human galectin-10 and 0.04-0.13 nM against both isoforms of cynomolgus galectin-10.
15
- With the exception of clone 7C05, the rest of the scFv-human Fc panel showed similar binding profiles on the 2 isoforms of cynomolgus galectin-10.

(ii) SPR analysis

The binding properties of the scFv-human Fc panel to galectin-10 were analyzed on the Biacore
20 T3000. For this purpose, a CM5 Chip was coated with polyclonal anti-human Fc at 8000 RU, then a fixed concentration of the scFv-human Fc panel (1.5 µg/mL) were captured to reach a binding signal around 150 RU. Finally, a serial dilution of human or cynomolgus WGS isoform of galectin-10-His (serial dilution, 1 over 2 from 5 µg/mL, 6 points of dilution) was injected. Raw data were analyzed via BIA evaluation software with a blank subtraction (4-3). Finally, the kd /
25 KD and Rmax of each scFv-human Fc to galectin-10-His was determined using the Fit Kinetics

simultaneous ka/kd / Binding with mass transfer / Local Rmax on BIA evaluation software. The results are shown in the table below.

Table 20 Characterisation of the binding properties of the panel of scFv-human Fc galectin-10 antibodies on Biacore T3000

	Biacore binding (capture approach) of scFv-human Fc to							
	humanGal10-His				cynoGal10-His_WGS			
Name	ka (1/Ms) E05	kd (1/s) E-04	Rmax	KD (nM)	ka (1/Ms) E05	kd (1/s) E-04	Rmax	KD (nM)
007B07	15,0	12,4	90,0	0,8	500,0	766,0	37,0	1,5
007C05	9,9	12,4	31,0	1,3	3,8	1,4	10,0	0,4
007D10	7,9	22,2	28,0	2,8	1,8	5,8	35,0	3,2
007E09	5,4	19,0	19,0	3,5	4,9	17,4	14,0	3,6
008H11	4,53	8,2	55	1,8	17,5	16,1	41	0,9
010A06	9,1	2,5	42,0	0,3	6,6	3,5	27,0	0,5
010B02	5,0	8,1	39,0	1,6	7,7	8,0	52,0	1,0
010D02	3,4	6,6	39,0	2,0	4,5	12,2	24,0	2,7
010H06	3,2	5,6	40,0	1,8	3,4	26,2	15,0	7,8
011F02	3,7	20,3	22,0	5,4	2,4	18,5	21,0	7,9
011F12	3,2	7,2	40,0	2,3	75,4	112,0	35,0	1,5

In line with the ELISA binding data reported above, the panel of scFv-human Fc fusions showed diverse binding properties to both human and cynomolgus WGS isoform of galectin-10-His. The following observations were made.

- The scFv-human Fc panel showed an affinity between 0.3-5.4 nM on human galectin-10-His and 0.5-7.9 nM on cynomolgus WGS isoform of galectin-10-His.
- The clones 10A06, 7B07 and 7C05 showed the highest affinity (0.3 nM up to 1.3 nM, respectively), with off-rates of 2.5^{E-04} s^{-1} up to 12.4^{E-04} s^{-1} on human galectin-10-His. Clone 7B07 showed the fastest on-rate (15^{E05} 1/Ms) and Rmax (90 RU) of this panel.
- The scFv-human Fc panel showed a 2-fold lower Rmax on cynomolgus galectin-10-His compared to human target.
- The clones 7C05, 10A06, 8H11 showed the highest affinity (0.4 nM up to 0.9 nM, respectively), with off-rates of 1.4^{E-04} s^{-1} up to 8^{E-04} s^{-1} on cynomolgus WGS isoform of galectin-10-His. Clone 7B07 showed the fastest on-rate (500^{E05} 1/Ms) and off-rate (766^{E-04}) of the panel.

C. Reformatting of selected scFv clones into a mouse IgG1 backbone

Seven selected leads, as shown in the table below, were re-cloned into a mouse IgG1 backbone for further characterization.

Table 21 Panel of scFv clones reformatted into a mouse IgG1 backbone

			bELISA hGal10	bELISA cynoGal10-WGS	bELISA cynoGal10-YRT	Biacore on human Gal10			Biacore on cyno Gal10 WGS		
Clone ScFv-hFc	VH Family	VL Family	EC50 (nM)	EC50 (nM)	EC50 (nM)	ka (1/Ms) E05	kd (1/s) E-04	KD (nM)	ka (1/Ms) E05	kd (1/s) E-04	KD (nM)
007B07	2	9	1,11	0,82	No binding	15,0	12,4	0,8	500,0	766,0	1,5
008H11	7	10	0,18	0,13	0,10	4,53	8,2	1,8	17,5	16,1	0,9
010A06	12	34	0,08	0,10	0,04	9,1	2,5	0,3	6,6	3,5	0,5
010B02	5	26	0,09	0,05	0,05	5,0	8,1	1,6	7,7	8,0	1,0
010D02	6	17*	0,56	1,12	1,40	3,4	6,6	2,0	4,5	12,2	2,7
011F02	7b	33	0,77	10,41	ambiguous	3,7	20,3	5,4	2,4	18,5	7,9
011F12	7	16	0,56	0,23	0,24	3,2	7,2	2,3	75,4	112,0	1,5

The CDR, VH and VL sequences of these seven antibodies are shown in Tables 32, 33 and 34
5 below.

For the reformatting, the VH and the VL regions of each clone were PCR amplified and cloned in frame into an expression vector encoding the mouse IgG1 constant domains.

10 The production of the mouse IgG1 anti-galectin-10 was carried out by transfection with a ratio of 1 heavy chain for 3 light chains incorporated in HEK293E cells via the polyethylenimine (PEI). After 6 days of production, mouse monoclonal antibodies were purified from the cell supernatant using the protein-A sepharose beads. Finally, SDS-PAGE analysis was carried out to assess the purity and the integrity of the antibodies (MW equal to 150 kDa).

15

Example 16. Characterization of the galectin-10 antibodies in mouse IgG1 format

A. Characterization of the binding properties of the galectin-10 mouse IgG1 panel

(i) ELISA analysis

20 In a similar set-up to that used during the characterization of the scFv-human Fc molecules, the relative binding properties of the 7 mouse IgG1 antibodies were analyzed by ELISA. A maxisorp plate was coated overnight with 0.5 µg/mL of human or cynomolgus WGS or YRT isoform of galectin-10-His. Then a serial dilution of each clone (from 100 nM, dilution 1/4, 12 points of dilutions) was incubated on coated galectin-10. After several washing steps, detection of the
25 bound mouse IgG1 was carried out with an anti-mouse Fc-HRP antibody (DAMPO, Jackson ImmunoResearch, Catalog 715-035-150). Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. The raw data (OD values) were plotted on GraphPad Prism 7.01. The EC50 values for each antibody, calculated with a non-linear regression (log(agonist)

vs. response Variable slope (four parameters)), are shown in the table below. Antibody 1D11 from the previous panel was included for comparison.

Table 22 Characterization of the binding properties of the mouse IgG1 panel by ELISA

	humanGal10-His (0.5 µg/mL)		cynoGal10-His_WGS (0.5 µg/mL)		cynoGal10-His_YRT (0.5 µg/mL)	
Clones (mouse IgG1)	OD values	EC50 (nM)	OD values	EC50 (nM)	OD values	EC50 (nM)
1D11	2,0	0,10	0,05	n.d	0,03	n.d
7B07	0,4	0,57	3,22	0,06	0,01	n.d
8H11	1,5	1,46	3,61	0,03	2,97	0,30
10A06	0,3	0,63	3,69	0,03	3,38	0,06
10B02	1,4	2,28	3,70	0,04	3,36	0,14
10D02	2,7	3,55	3,51	0,14	2,63	ambiguous
11F02	0,9	3,08	3,29	0,60	1,70	ambiguous
11F12	2,7	4,83	3,69	0,05	3,35	0,21

5

The following observations were made.

- The new panel of mouse IgG1 anti-galectin-10 antibodies showed a relative binding capacity in ELISA between 0.57 nM to 4.83 nM against human galectin-10, 0.03 nM to 0.60 nM against cynomolgus WGS isoform and 0.06 nM to 0.30 nM against cynomolgus
10 YRT isoform, respectively.
- Clone 1D11 showed the best relative binding capacity against human galectin-10 (0.10 nM EC50 value) but no binding to the cynomolgus galectin-10.
- Against human galectin-10, clones 7B07, 10A06 and 8H11 showed the best relative binding capacity with an EC50 value between 0.57 nM and 1.46 nM. Against the WGS
15 isoform of galectin-10, clones 8H11, 10A06 and 10B02 were found to be the best binders, with EC50 values between 0.03 nM and 0.04 nM, 18-53-fold more potent compared to the human target.
- Finally, clones 10A06, 10B02 and 11F12 showed the best relative binding capacity against the YRT isoform of the cynomolgus galectin-10-His, with EC50 values between
20 0.06 nM and 0.21 nM.

(ii) SPR analysis

The binding capacity of the mouse IgG1 antibody panel to galectin-10 was analysed on Biacore T3000. For this purpose, a capture approach was used, where a fixed concentration (1.5 µg/mL)
25 of the mouse IgG1 clone was captured by polyclonal anti-mouse Fc antibodies immobilized on CM5 Chip to reach a binding signal around 150 RU. Then a serial dilution of human and cynomolgus WGS isoform of galectin-10-His (serial dilution, 1 over 2 from 5 µg/mL) diluted in HBS-EP pH7.4 was injected. Raw data were analysed via BIA evaluation software with a blank subtraction. The kd / KD and Rmax of each mAbs to galectin-10-His was determined using the

Fit Kinetics simultaneous ka/kd / Binding with mass transfer / Local Rmax on BIA evaluation software.

Table 23 Characterization of the binding properties of the mouse IgG1 panel by Biacore T3000

	Biacore binding (capture approach) of mouse IgG1 to:							
	humanGal10-His				cynoGal10-His_WGS			
Clone (mouse IgG1)	ka (1/Ms) E05	kd (1/s) E-04	Rmax	KD (nM)	ka (1/Ms) E05	kd (1/s) E-04	Rmax	KD (nM)
1D11	127	93,1	68	0,7	n.d	n.d	7	n.d
7B07	27,8	39	65	1,4	454	660	24	1,5
8H11	23,1	19,7	50	0,9	1,7	7,54	30	4,4
10A06	117	101	39	0,9	129	147	21	1,1
10B02	14,2	20,5	37	1,5	4,94	4,2	40	0,9
10D02	5,47	8,65	37	1,6	4,77	9,12	21	1,9
11F02	22,7	48,9	24	2,2	3,94	19,8	17	5,0
11F12	30,4	22,4	37	0,7	4,94	5,94	30	1,2

The following observations were made.

- In this set-up, the 7 human cynomolgus cross reactive clones showed an affinity in a nanomolar up to sub-nanomolar range against human and cynomolgus galectin-10 (WGS isoform), with an off rate ranging between $4.2 \times 10^{-4}\ s^{-1}$ and $660 \times 10^{-4}\ s^{-1}$.
- For both human and cynomolgus galectin-10-His, clones 8H11, 10A06 and 11F12 showed the best binding capacity with an affinity between 0.7 nM and 0.9 nM for the human target and between 0.9 nM and 1.2 nM for the cynomolgus antigen (WGS isoform).
- Clone 1D11 was found to be one of the best binders to human galectin-10 (affinity equal to 0.7 nM) but showed no / low binding to the cynomolgus antigen (Rmax equal to 7 RU).

B. Dissolution of recombinant human CLC crystals *in vitro*

The dissolution of CLCs induced by the anti-galectin-10 mouse IgG1 clones was monitored using a spinning disk confocal microscope. Briefly, the solution containing human CLCs was spotted in a µ-slide wells plate before being incubated with a fixed concentration (50µM) of the mouse IgG1 anti-galectin-10 antibodies. Finally, for each well, imaging positions were defined, and each position was imaged every 3-5 minutes for a total of 120 minutes. Finally, based on software, the surface occupied by the CLC was determined, and the initial area covered by the CLC at the beginning of the experiment was defined as 1. As a negative control, a mouse IgG1 isotype control was included. The overall size of the CLC were measured over time and plotted on GraphPad Prism 7.01 (see **Fig. 15**). The EC50 and EC90 values of each mouse IgG1 clone were

calculated with a non-linear regression (log(agonist) vs. response Variable slope (four parameters)) and are reported in the table below.

Table 24 Dissolution of galectin-10 crystals *in vitro*

Concentration (µM)	Clones	Format	50% dissolution (min)	90% dissolution (min)
50	1D11	mIgG1	30,1	56,7
	7B07		13,2	44,4
	8H11		22,4	43,5
	10A06		44,5	86,6
	10B02		25,1	47,4
	10D02		26,5	58,6
	11F02		25,5	57,5
	11F12		21,3	42,6

5

Example 17. Characterization of the galectin-10 antibodies in Fab format

A. Generation of the Fab clones and bELISA data

In order to determine the exact epitope of the seven galectin-10 mouse IgG1 antibodies on
10 galectin-10, a crystallography study was initiated. For this purpose, the seven anti-galectin-10 antibodies characterised in Example 16 above were produced as Fabs.

For this, the VH and the VL of each clone were PCR amplified using specific primers, purified by electrophoresis, digested with restriction enzymes (BsmBi) and ligated in the pre-digested
15 vectors containing the human constant domains: the human lambda constant domain for the VL (pUPEX116.9) or the CH1 constant domain for the VH (pUPEX86, including part of the hinge region). The transformation of each of the ligated products was carried out into Top10 bacteria by heat shock and the cells were transferred onto agarose plates with Ampicillin (resistance gene of the vectors). After one night of incubation, ligated products showed high numbers of single
20 colonies whereas no colonies were observed for the negative controls (empty vectors). Per clones (VH and VL), four to eight colonies were picked and sent for sequencing. The clones that showed the correct insert were selected and amplified in order to purify the DNA sequence (MidiPrep).

25 The production of the seven Fab lead clones was initiated in mammalian cells. Transfection was performed with a ratio of 1 heavy chain for 3 light chains incorporated in HEK293E cells via the polyethylenimine (PEI). After 10 days of production, human Fab were purified from the cell supernatant using the Capture Select IgG-CH1 sepharose beads. Finally, SDS-PAGE analysis was carried out to assess the purity and integrity of the Fab molecules (MW ~55 kDa).

The binding capacity of the lead anti-galectin-10 Fabs was tested by binding ELISA. Briefly, a maxisorp plate was coated overnight with 1 µg/mL of human galectin-10-His. Then a serial dilution of each clone (from 4 µM, dilution 1/4, 12 points of dilutions) was incubated on coated galectin-10. After several washing steps, detection of the bound Fab was carried out with an anti-human IgG Fab specific-peroxidase. Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. The results are shown in the table below.

Table 25 Characterization of the binding properties of the galectin-10 Fabs

	bELISA on galectin-10-His coated at 1µg/mL	
	Bmax	**EC50 (nM)**
1D11	1,59	1,4
7B07	0,65	24,4
8H11	0,95	24,3
10A06	0,36	89,5
10B02	0,74	61,2
10D02	1,03	16,1
11F02	0,46	81,4
11F12	1,375	22,4

The ELISA binding data showed that the Fabs could be separated into 3 groups based on their binding capacity to coated human galectin-10-His:

- Group 1: Clones 8H11, 10D02 and 11F12 were found as the best binders with the highest relative binding capacity (16-24 nM) and Bmax (0.74-1.3 OD values).
- Group 2: Clone 7B07 showed a relatively good binding capacity with an EC50 value equal to 24 nM and max binding capacity equal to 0.65 OD values, but showed a slow hill slope, suggesting another binding interaction with galectin-10.
- Group 3: The less potent binders with the lowest relative binding capacity (61-90 nM) and Bmax (0.36-0.74 OD values), included clones 10B02, 10A06 and 11F02.
- With a relative binding capacity equal to 1,4 nM, the clone 1D11 showed the best binding capacity to human galectin-10, consistent with the full mAb data.

B. Crystal structure of galectin-10 Fab fragments in complex with galectin-10

The crystal structure of different Fab fragments in complex with galectin-10 was obtained, as described in Example 12. The results are shown in **Fig. 16**. These structures show that clones 8H11, 1D11, 4E8, 6F5, 10D2 and 11F12 bind to tyrosine 69 (Y69), or an epitope in close proximity of Y69, whereas 7B7 binds to the opposite side of the galectin-10 dimer.

The residues of galectin-10 involved in the binding of the galectin-10 Fabs to the galectin-10 dimer are shown in Table 26 below.

5 **Table 26 Residues of galectin-10 that make a direct interaction with the CDRs of the galectin-10 Fabs**

clone	Galectin-10 residues interacting with	
	VH	VL
1D11	42, 49, 68, 69, 73, 115-117, 119-120	69, 70, 71 and 73
6F5	43, 49, 68, 69, 114-117, 119-120	73, 98, 113-115 and 117
4E8	74, 113-115	49, 68, 69,73, 98, 115-117
8H11	33, 59, 60, 78-82 and 109	60, 74, 75, 77 and 79
11F12	33, 59, 60, 72, 79-82, 84 and 109	74, 75, 76, 77 and 79
10B02	33, 59, 60, 77-84	74, 75, 76, 77 and 79
10D02	31, 33, 59, 60, 78-82 and 84	79
7B7	2-5, 7-11, 25, 44, 88, 123, 125, 133 and 135	2, 23, 25, 86-90, 105 and 134

The galectin-10 Fab CDR residues involved in binding to the galectin-10 dimer are shown in Table 27 below.

10

Table 27 CDR residues of the Fabs that make contact with residues of the galectin-10 dimer in the crystal structure

Clone		CDR residues Fab1	Galectin-10 residues (Monomer A)	CDR residues Fab2	Galectin-10 residues (Monomer B)
1D11	**VH**	CDR1 - 31 CDR2 - 53 CDR3 - 102-107	42, 49, 68, 69, 73, 115-117, 119-120	CDR1 - 31 CDR2 - 53 CDR3 - 102-107	42, 49, 68, 69, 73, 115-117, 119-120
	VL	CDR1 - 31 and 34 CDR3 - 93 and 97	69, 70, 71, 73	CDR1 - 31 and 34 CDR3 - 93, 96 and 97	69, 70, 71, 73
6F5	**VH**	CDR1 - 2, 27, 31 and 32 CDR3 - 98, 99, 102-105, 107, 110	43, 49, 68, 69, 114-117, 119-120	CDR1 - 2, 27, 31 and 32 CDR3 - 98, 99, 102-105, 107, 110	43, 49, 68, 69, 114-117, 119-120
	VL	CDR1 - 33 and 34 CDR2 - 51, 52, 55 and 58	73, 98, 113-115 and 117	CDR1 - 33 and 34 CDR2 - 51, 52, 55 and 58	73, 98, 113-115 and 117
4E8	**VH**	CDR2 - 60 CDR3 - 105, 107 and 109	74, 113-115	CDR2 - 60 CDR3 - 104, 105, 107 and 109	74, 113-115

	VL	CDR1 - 26, 28-31 CDR2 - 49 CDR3 - 91-94	49, 68, 69, 73, 98, 115-117	CDR1 - 26, 28-31 CDR3 - 91-94	49, 68, 69, 73, 98, 115-117
7B07	**VH**	CDR1 - 30 and 31 CDR2 - 53-57 CDR3 - 100, 102-105	2, 3, 25, 88 and 133	CDR1 - 30 and 31 CDR2 - 53-57 CDR3 - 100, 102-104	4, 5, 7-11, 44, 123, 125, 135
	VL	CDR1 - 26, 28-31 CDR2 - 48, 50, 51 CDR3 - 65, 91-93	2, 23, 25, 86-90, 105 and 134	CDR1 - 26, 28-31 CDR2 - 48, 50, 51 CDR3 - 65, 91-93	2, 23, 25, 86-90, 105, 134
8H11	**VH**	CDR1 - 31-33 CDR2 - 52-54, 56, 57 and 59 CDR3 - 99-101	33, 59, 60, 78-82 and 109	CDR1 - 30-33 CDR2 - 52-54, 56, 57 and 59 CDR3 - 100 and 101	33, 59, 60, 78-82 and 109
	VL	CDR1 - 31, 32 and 34 CDR3 - 93 and 97	60, 74, 75, 77 and 79	CDR1 - 31, 32 and 34 CDR3 - 93 and 97	60, 74, 75, 77 and 79
11F12	**VH**	CDR1 - 30-33 CDR2 - 52-54, 56 and 57 CDR3 - 99-101	33, 59, 60, 72, 79-82, 84 and 109	CDR1 - 30-33 CDR2 - 52-54, 57 CDR3 - 99-101	33, 59, 60, 78-82 and 109
	VL	CDR1 32 and 34 CDR3 97	74, 75, 76, 77 and 79	CDR1 - 32 and 34 CDR3 - 97	74, 75, 77 and 79,
10B02	**VH**	CDR1 - 31-33 CDR2 - 52-54, 56, 57 and 59 CDR3 - 99-102	33, 59, 60, 70, 72, 77-82, 84	CDR1 - 31 and 33 CDR2 - 53-54, 56, 57 and 59 CDR3 - 99-102	33, 59, 60, 70, 72, 77-82, 84
	VL	CDR1 - 31, 32 and 34 CDR3 - 93 and 97	74, 75, 76, 77 and 79	CDR1 -30, 31, 32 and 34 CDR3 - 93 and 97	74, 75, 76, 77 and 79
10D02	**VH**	CDR1 - 28, 30-33 CDR2 - 53-57 CDR3 - 99-101	31, 33, 59, 60, 70, 72, 78-82, 84,	CDR1 - 28, 30-33 CDR2 - 52-54, 56 and 57 CDR3 - 99-103	31, 33, 59, 60, 70, 72, 78-82, 84,
	VL	CDR3 - 97	79	CDR3 - 97	2, 79

Example 18. Comparison of CLC dissolution by galectin-10 mAbs and Fab fragments

The ability of the galectin-10 Fabs to solubilize pre-existing recombinant human galectin-10 crystals *in vitro* was compared with the galectin-10 mAbs. The protocol was as described in Example 16 above.

5

The results are shown in **Fig. 17** and also in the table below. In some cases, the Fab fragments were more effective at dissolving the CLCs than the corresponding mAbs.

Table 28 CLC dissolution mediated by galectin-10 mAbs and Fabs

Concentration (µM)	Clones	Format	50% crystal dissolution (min)	90% crystal dissolution (min)
50	7B07	mIgG1	31,76	93,02
	8H11		68,29	>120
	10A06		>120	>120
	10B02		73,66	>120
	11F12		65,43	>120
	1D11		100	>120
50	7B07	hFab	15,09	54,14
	8H11		48,53	>120
	10A06		49,15	>120
	10B02		102,2	>120
	10D02		59,67	>120
	11F02		77,86	>120
	11F12		61,96	>120
	1D11		33,42	97,85

10

Example 19. Production of VHH antibodies that bind to galectin-10

Two llamas were immunized with recombinant human galectin-10. Messenger RNA (mRNA) was purified from the PBMCs isolated from the blood of the immunized llamas. The mRNA was reverse transcribed with random hexamer primers to obtain cDNA. Tagged primers were used
15 directly on the cDNA to PCR amplify the VHH region. The PCR product was then purified, digested and cloned in the phagemid vector (VHH-Myc-His tagged) to create a VHH library.

To select VHH clones with the appropriate binding capacity to both human and cynomolgus galectin-10, a bio-panning approach was used. For this purpose, the first round of selection was
20 carried out against human galectin-10-His, followed by a second round of enrichment against human or cynomolgus WGS or YRT isoforms (His tag). Briefly, human or cynomolgus (WGS or YRT isoform) galectin-10-His was immobilized on Maxisorp ELISA plate, then the VHH phage

library (Input) was added. Unbound phages were removed via multiple washing steps. Finally, E. coli infection was carried out in order to amplify the selected phages. This process resulted in the enrichment of the phage population expressing VHH with high affinity against human and cynomolgus galectin-10.

From the eluted phages of round 2, versus 0.5 and 0.05 µg/mL of human, cynomolgus WGS isoform and cynomolgus YRT isoform of galectin-10, single clones were generated. Periplasmic extracts containing the VHH clone anti-human and cynomolgus galectin-10 were screened for binding capacity on ELISA and BioLayer Interferometry (BLI).

The binding capacity of the VHH (periplasmic extract) to human and cynomolgus (WGS and YRT isoforms) galectin-10 was analysed by ELISA. Briefly, a maxisorp plate was coated overnight with 1 µg/mL of human galectin-10-His or cynomolgus WGS or YRT isoform galectin-10-His, then blocked with PBS 1% Casein, before being incubated with the periplasmic extract (dilution 1/5 in PBS 0.1%Casein) containing the VHH-Myc tagged. Detection of the binders was carried out with an anti-Myc-HRP antibody (Bethyl, Catalog A190-105P). TMB substrate was then added and the reaction was stopped with 0.5M H_2SO_4. Absorbance was measured at 450 nm (reference at 620 nm) with Tecan instrument. Finally, the raw data (OD values) were plotted using GraphPad Prism 7.01 and are shown in **Fig. 18**. The number of binders, defined by an OD value higher than 0.5, per MP plate was determined. A blank control and negative control (VHH periplasmic extract binding to irrelevant target) were included and showed, as expected, no binding.

Table 29 Characterization of the VHH periplasmic extracts by ELISA screening

MP number	Antigen	Hits	% Hits
MP13	human Gal10HIS	51	53,1
	cyno Gal10HIS_WGS	57	59,4
	cyno Gal10HIS_YRT	67	69,8
MP14	human Gal10HIS	14	14,6
	cyno Gal10HIS_WGS	13	13,5
	cyno Gal10HIS_YRT	34	35,4

In addition to the binding ELISA, BLI was used to screen for galectin-10-binding clones.

From the two Master plates generated during selection, 130 clones that showed human and cynomolgus binding during the ELISA screening campaign were picked and their binding

capacity was tested on BLI, using the Octet RED96. Briefly, human and cynomolgus WGS or YRT isoforms of galectin-10-His tagged were diluted in Kinetic buffer (PBS 0.01%BSA 0.002%Tween 20) at 200 µg/mL before being captured on Anti-Penta His 1K sensor tips (ForteBio, Cat#18-5120) until an immobilization level of 1 nm was reached. Then, diluted periplasmic extracts (1/5 in Kinetic buffer) containing the VHH clones were applied and association/dissociation to immobilized galectin-10-His was measured using the ForteBio Data analysis 9.0 software (subtraction of the reference Tips (loaded up to 1 nm with irrelevant His tagged protein, 1.1 binding model). During the screening, only the dissociation (off-rate) of the VHH could be determined since the effective concentration of the VHH was unknown and can vary a lot from clone to clone. The results are shown in **Fig. 19** and confirm that most of the selected VHH clones showed human cynomolgus cross reactivity.

Selected VHH clones that showed binding to galectin-10 were sent for sequencing. Based on their CDR1-2-3 VHH sequences, each clone was classified into a family. This process resulted in the determination of 44 families. Based on their screening binding data (ELISA and BLI) and their sequences, 15 VHH clones were selected for further characterization. Production of purified VHH was initiated in *E.coli*. For this purpose, selected VHH were first grown in 2TY medium with low amount of glucose and production of the VHH was induced by an overnight incubation with IPTG (1mM). The next day, the bacteria pellets were lysed by two cycles of freeze/thaw (-80°C and -20°C). After centrifugation, VHH-His tagged were purified from the cell supernatant using the Talon Metal affinity Resin (BD Catalog 635504). Finally, SDS-PAGE analysis was done to assess the purity and the integrity of the VHH molecules (MW~15 kDa).

The CDR and VHH domain sequences of the 15 clones selected for further characterization are shown in Tables 35 and 36 below.

Example 20. Characterization of VHH antibodies that bind to galectin-10

A. Binding of VHH clones to galectin-10 as measured by ELISA

The binding capacity of the 15 VHH clones was analysed by ELISA. Briefly, a maxisorp plate was coated overnight with human or cynomolgus WGS or YRT isoforms galectin-10-His at 1µg/mL and blocked with PSB 1% Casein, before being incubated with a serial dilution of the VHH (from 4 µM, dilution 1/3, 12 points of dilutions). After several washing steps, detection of the bound VHH was carried out with an anti-Myc-HRP antibody (Bethyl, Catalog A190-105P). Absorbance was measured at 450 nM (reference at 620 nm) with Tecan instrument. Finally, the raw data (OD values) were plotted on GraphPad Prism 7.01. The EC50 values of each compound, calculated with a non-linear regression (log(agonist) vs. response Variable slope (four parameters)) was

reported in the table. As a positive control, clone 1D06 from the previous human specific panel was included. The results are shown in the table below.

Table 30 Characterization of the galectin-10 VHH clones by ELISA

5

	bELISA of VHH on coated Gal10-HIS at 1 µg/mL					
	Human		Cyno_WGS		Cyno_YRT	
Clones (VHH)	**Bmax (OD values)**	**EC50 (nM)**	**Bmax (OD values)**	**EC50 (nM)**	**Bmax (OD values)**	**EC50 (nM)**
13A05	1,8	69,7	2,7	77,8	2,9	66,3
13B06	1,0	ambiguous	1,4	Ambiguous	2,0	ambiguous
13C03	2,0	30,6	0,1	/	0,1	/
13C04	1,1	ambiguous	2,7	369,1	2,5	ambiguous
13C06	2,8	27,5	2,9	10,3	2,9	16,2
13C07	1,3	106,7	2,9	7,1	2,8	33,2
13C10	1,0	ambiguous	2,3	Ambiguous	2,7	185,8
13D12	0,8	ambiguous	1,3	Ambiguous	2,4	118,9
13E07	1,1	ambiguous	2,3	371,0	2,4	ambiguous
13E09	1,8	105,7	2,3	438,4	3,0	66,9
13G12	1,6	141,0	2,9	11,8	3,1	3,1
13H07	1,5	179,9	2,7	15,6	3,1	1,3
14E02	0,7	ambiguous	1,8	Ambiguous	2,1	ambiguous
14E10	0,5	ambiguous	1,4	Ambiguous	2,7	15,8
14F10	0,5	ambiguous	2,6	60,4	3,0	3,0
1D06	1,2	90,7	0,0	/	0,0	/

The following observations were made:

- Clones 13C06 and 13A05 were the best human cynomolgus cross reactive binders, with a relative binding capacity equal to 27-69.7 nM EC50 values on human galectin-10 and 10.3-77.8 nM EC50 values on WGS isoform and 16.2-66.3 nM EC50 values on YRT isoform of cynomolgus galectin-10.
- Clones 13C07, 13E09, 13G12 and 13H07 showed a moderate binding capacity to human galectin-10, with an EC50 values between 105-180 nM, but a strong binding capacity to the cynomolgus galectin-10, with EC50 values between 7.1-438 nM on WGS isoform and 1.3-33 nM on YRT isoform.
- Clone 13C03 showed a strong binding to the human galectin-10, with a relative binding capacity equal to 30 nM but a weak binding to the cynomolgus isoforms (Bmax 0.1 OD value).
- Clone 1D06, isolated from the previous scFv library selected exclusively against human galectin-10, showed an EC50 value equal to 90 nM but no cynomolgus cross reactivity.

B. VHH clones dissolve human recombinant CLCs *in vitro*

The CLC dissolution induced by the VHH clone 1D06 was monitored using a spinning disk confocal microscope. Briefly, the solution containing human CLCs was spotted in a µ-slide wells plate, before being incubated with a fixed concentration (50µM or 100 µM) of the VHH antibody 1D06. Finally, for each well, imaging positions were defined, and each position was imaged every 3-5 minutes for a total of 120 minutes. Finally, based on software, the surface occupied by the CLC was determined, and the initial area covered by the CLC at the beginning of the experiment was defined as 1. As a negative control a VHH isotype control was included. The overall size of the CLC were measured over time and plotted on GraphPad Prism 7.01 (see **Fig.20**). The EC50 and EC90 values of the VHH clone were calculated with a non-linear regression (log(agonist) vs. response Variable slope (four parameters)) and reported in the table below.

Table 31 Dissolution of CLCs by the VHH clone 1D06

Concentration (µM)	Clones	Format	50% dissolution (min)	90% dissolution (min)
50	1D06	VHH	20,1	13,6
100			7,2	43,96

Table 32: Heavy chain CDR sequences of scFv antibodies binding to galectin-10

scFv clone	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3	SEQ ID NO.
7B07	SYDMS	160	AIKNGGGITYYADSVKG	161	THGIGTLGFGS	162
8H11	TNYMN	163	GITSGGGRTYYADSVKG	164	TDHAWLDA	165
10A06	NYDMS	166	DINSGGGSTYYADSVKG	167	GYTGYYY	168
10B02	YHYMN	169	GISAGGGRTYYADSVKG	170	VHGITNDY	171
10D02	SYYMS	172	GIVTGGGRTHYTDSVKG	173	VNGVVTNYDY	174
11F02	TNYMN	163	GITSHGARTYYADSVKG	175	TDHASLDA	176
11F12	TNYMN	163	GITSGGGRTYYADSVKG	164	TDHAWLDA	165

Table 33: Light chain CDR sequences of scFv antibodies binding to galectin-10

scFv clone	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3	SEQ ID NO.
7B07	DGNNIGSKSAQ	177	ADEYRPE	178	QVWDGSAAV	179
8H11	AGTSSDVGYGNYVS	180	AVSTRAS	181	ASYRSSNNYV	182
10A06	GLSSGSVTSSNYPA	183	SINSRHS	184	TLYMGTGSNNVV	185
10B02	AGTSSDVGYGNYVS	180	EVNKRAS	186	ASYRNSNNWV	187
10D02	AGTSSDVGYGNYVS	180	DVNKRAS	188	ASYRSPNNVV	189
11F02	GLSSGSVTSSNYPG	190	NTNSRYS	191	ALYMGSSSYNTV	192
11F12	AGTSSDVGYGNYVS	180	AVSTRAS	181	ASYSVRNNVV	193

Table 34: VH and VL sequences of scFv antibodies binding to galectin-10

scFv clone	VH	SEQ ID NO.	VL	SEQ ID NO.
7B07	QVQLVESGGGLVQPGGSLRLSCAASGFTFRSYDMSWVRQAPGKGPEWVSAIKNGGGITYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTAVYYCATTHGIGTLGFGSWGQGTQVTVSS	194	SYELTQSASVSVALTQTAKITCDGNNIGSKSAQWYQQKPGQAPALVIYADEYRPEGIPERFSGSNSGNTATLIISGAQAEDEADYYCQVWDGSAAVFGRGTHLTVL	195
8H11	ELQLVESGGGLVQPGGSLRLSCAASGFTFSTNYMNWVRQAPGKGLEWVSGITSGGGRTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARTDHAWLDAWGQGTLVTVSS	196	QAGLTQPPSVSGTLGKTVTISCAGTSSDVGYGNYVSWYQQLPGTAPKLLIYAVSTRASGIPDRFSGSKSGNTASLTISGLLSEDEADYYCASYRSSNNYVFGGGTKLTVL	197
10A06	QVQLVESGGGLVQPGGSLRLSCAASGFTFGNYDMSWVRQAPGKGPEWVSDINSGGGSTYYADSVKGRFTISRDNAKNSLLLQMNSLKPEDTAVYYCATGYTGYYYWGQGTQVTVSS	198	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPAWYQQTPGQAPRALIYSINSRHSGVPDRFSGSISGNKAALTITGAQAEDEADYYCTLYMGTGSNNVVFGGGTHLTVL	199
10B02	QVQLVESGGGLVQPGGSLRVSCAASGFTFSYHYMNWVRQAPGKGLEWVSGISAGGGRTYYADSVKGRFTISRDNAKNTLYLQMNSLNAEDTALYYCARVHGITNDYWGQGTQVTVSS	200	QSALTQPPSVSGSPGKTVTISCAGTSSDVGYGNYVSWYQQLPGMAPKLLIYEVNKRASGITDRFSGSKSGNTAFLTISGLQSEDEADYYCASYRNSNNWVFGGGTHLTVL	201
10D02	EVQLVESGGGLVQPGGSLRVSCAASGFTFSSYYMSWVRQAPGKGLEWVSGIVTGGGRTHYTDSVKGRFTITRDNAKNTLYLQMNSLRPEDTALYYCARVNGVVTNYDYWGQGTQVTVSS	202	QAVLTQPPSVSGSPGKTVTISCAGTSSDVGYGNYVSWYQQLPGMAPKLLIYDVNKRASGITDRFSGSKSGNTASLTISGLQSEDEADYFCASYRSPNNVVFGQGTHLTVL	203
11F02	ELQVVESGGGLVQPGGSLRLSCAASGFTFSTNYMNWVRQAPGKGLEWVSGITSHGARTYYADSVKGRFTISRDNSKNTLYLQMNSLKPEDTALYYCARTDHASLDAWGQGTLVTVSS	204	QTVVTQEPSLSVSPGGTVTLTCGLSSGSVTSSNYPGWYQQKPGQAPRTLIYNTNSRYSGVPNRFSGSISGNKAALTITGAQPEDEADYYCALYMGSSSYNTVFGGGTHLTVL	205
11F12	QVQLQESGGGLVQPGGSLRLSCAASGFTFSTNYMNWVRQAPGKGLEWVSGITSGGGRTYYADSVKGRFTISRDNAKNTLYLQMNSLKPEDTALYYCARTDHAWLDAWGQGTLVTVSS	206	HSAVTQPPSVSGTLGKTVTISCAGTSSDVGYGNYVSWYQHLPGTAPKLLIYAVSTRASGVPDRFSGSKSGNTASLTISGLQSEDEGDYYCASYSVRNNVVFGGGTRLTVL	207

Table 35: CDR sequences of VHH antibodies binding to galectin-10

VHH clone	CDR1	SEQ ID NO.	CDR2	SEQ ID NO.	CDR3	SEQ ID NO.
13G12	AYSMG	208	VISWSGGSHYDDSVKG	209	GTLYSFRYRDYDY	210
13H07	TYAMD	211	AISWHSAIYYADAVKG	212	ALRYRFNPSAMEY	213
14F10	GYAVG	214	IISWNGGTHYADSVKG	215	SPKYYFSPETYNY	216
13C06	SYSMG	217	TISWSGGFYYDDSVKG	218	GTRFSFSYREYHY	219
13C07	AYSMA	220	AITWSGGSHHDDSVKG	221	GTLYSFSYRDYDY	222
14E10	PYAMG	223	VISLSGAYTYNVNAVKG	224	SRTYYRTDESTYEY	225
13D12	SYHMM	226	ALAWRGGTYCANSVKG	227	SRRYVFDPSAMDY	228
13A05	SYSMS	229	IISWSGGTYYDDSVKG	230	GTQFSFSYREYDY	231
13C04	TYSMA	232	AITRSGGNTYADSVKG	233	GGTYSFVPRSYNY	234
13E09	SYHMM	226	AIAWRGGTYCANSVKG	235	SLRYVFDPSAMDY	236
14E02	TYSMA	232	AITWAGGYTYGADSEKG	237	GRLFTSQSSAYQY	238
13C10	PYTMG	239	VVSSGGGTYYANSVKG	240	GSIFRWSPMSYDY	241
13E07	SYHMM	226	AIAWRGGTYCANSVKG	235	SLRYVFDPSAMDY	236
13C03	ISRMG	242	IIFSDASTDYADSVKG	243	VLRAAGYGYFNQY	244
13B06	TYSMA	232	AITRSGGNTYADSVKG	233	GGTYSFVPRSYNY	234
1D6	TYAMG	245	AITRAGGNTYNADSVKG	246	GPRYSTISTMFPY	247
15A2	SYSMG	217	TISWSGGNYVDNSVKG	248	GTQFSFSYRQYDY	249

Table 36: VHH domain sequences of VHH antibodies binding to galectin-10

Clone	VHH sequence	SEQ ID NO.
13G12	QLQVVESGGGLVQAGGSLRLSCAASSSAYSMGWFRQAPGKEREFVAVISWSGGSHYDDSVKGRF TISRDGAKNTVYLQMNSLKPEDTAVYYCAVGTLYSFRYRDYDYWGQGTQVTVSS	250
13H07	QLQLVESGGGLVQAGGSLRLSCVASGRTFSTYAMDWFRQAPGKEREFVAAISWHSAIYYADAVKG RFTISRDNGKNTMYLQMNNLKPEDTAVYFCAAALRYRFNPSAMEYWGKGTLVTVSS	251
14F10	EVQLVESGGGLVQAGDSLRLSCAASGRTFSGYAVGWFRQAPGKEREFVTIISWNGGTHYADSVKG RFAISRDNAKNTVYLQMNNLKPEDTAVYYCAVSPKYYFSPETYNYWGQGTQVTVSS	252
13C06	QLQLVESGGGLVQAGGSLRLSCAASSSSYSMGWFRQAPGKEREFVATISWSGGFYYDDSVKGRFT VSRDNAKNTVYLQMNSLKPEDTAVYYCAAGTRFSFSYREYHYWGQGTQVTVSS	253
13C07	QLQVVESGGGLVQAGGSLRLSCAASSSAYSMAWFRQAPGKEREFVAAITWSGGSHHDDSVKGRF TISRDGAKNTVYLQMNSLKPEDTAVYYCAVGTLYSFSYRDYDYWGQGTQVTVSS	254
14E10	ELQLVESGGGLVQAGGSLRLSCAASEGTFRPYAMGWFRQAPRKEREFVAVISLSGAYTYNVNAVK GRFTISRDNAKNTVYLQMNSLTPEDTAIYYCGASRTYYRTDESTYEYWGQGTQVTVSS	255
13D12	QLQLVESGGGLVQAGDSLRLSCAASGRTFSSYHMMWFRQAPGKEREFVAALAWRGGTYCANSVK GRCTISRDNAQDTVYLQMNSLKPEDTAVYFCAASRRYVFDPSAMDYWAKGTLVTVSS	256
13A05	QVQLVESGGGLVQAGGSLRLSCAASSSSYSMSWFRQAPGKEREFVAIISWSGGTYYDDSVKGRFT VSRDNAKNTVYLQMNSLKPEDTAVYYCAAGTQFSFSYREYDYWGQGTQVTVSS	257
13C04	ELQVVESGGGLVQAGGSLRLSCAASGRTFSTYSMAWFRQAPGKEREFVAAITRSGGNTYADSVKG RFTISRDNAKNTVTLQMNSLKPEDTAAYHCAAGGTYSFVPRSYNYWGQGTQVTVSS	258
13E09	QVQVQESGGGLVQAGNSLRLSCAASGRTFSSYHMMWFRQAPGKEREFVAAIAWRGGTYCANSVK GRCTISRDNAKDTVYLQMNSLKPEDTAVYFCAASLRYVFDPSAMDYWGKGTLVTVSS	259
14E02	QVQLVESGGGLAQAGGSLRLSCVASGRAAGTYSMAWFRQAPGKEREFVAAITWAGGYTYGADSE KGRFTISRDNAKNTVYLQMNSLKPEDTAVYSCAGGRLFTSQSSAYQYWGQGTQVTVSS	260
13C10	QLQLVESGGGLVQAGGSLKLSCAASGRTFNPYTMGWFRQAPGKEREFVAVVSSGGGTYYANSVK GRFTISRDNAKATVYLQMNSLKPEDTAVYYCAAGSIFRWSPMSYDYWGQGTQVTVSS	261
13E07	ELQVVESGGGLVQAGDSLRLSCAVSGRTFSSYHMMWFRQAPGKEREFVAAIAWRGGTYCANSVK GRCTISRDNAKDTVYLQMNSLKPEDTAVYFCAASLRYVFDPSAMDYWGKGTLVTVSS	262
13C03	QVQLVESGGGLVQPGGSLRLSCAASGSIFSISRMGWYRQAPGKQRELVAIIFSDASTDYADSVKGRF TISRSNAKNTVYLQMNSLKPEDTAVYYCKSVLRAAGYGYFNQYWGQGTQVTVSS	263

13B06	QVQLVESGGGLVQAGGSLRLSCAASGRTFSTYSMAWFRQAPGKEREFVAAITRSGGNTYADSVKG RFTISRDNAKNTVTLQMNSLKPEDTAAYHCAAGGTYSFVPRSYNYWGQGTQVTVSS	264
1D6	QLQLVESGGGLVQPGGSLRLSCAASENSVSTYAMGWFRQAPGKEREFVAAITRAGGNTYNADSVK GRFTISRDNAENTIYLQMNSLKPEDTAVYSCAAGPRYSTISTMFPYWGQGTQVTVSS	265
15A2	QVQLVESGGGLVQAGGSLRLSCAASGSSYSMGWFRQAPGKEREFVATISWSGGNYVDNSVKGRF TVSRDNAKNTVYLQMNSLKPEDTAVYYCAAGTQFSFSYRQYDYWGQGTQVTVSS	266

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all embodiments described herein are considered to be broadly applicable and combinable with any and all other consistent embodiments, as appropriate.

Incorporation by Reference

Various publications are cited in the foregoing description and throughout the following examples, each of which is incorporated by reference herein in its entirety.

CLAIMS

1. An antagonist that binds to galectin-10, wherein the antagonist binds to an epitope of galectin-10 and thereby shields a crystal packing interface of galectin-10.

2. The antagonist of claim 1, which, when bound to soluble galectin-10, inhibits crystallization of galectin-10 and/or which, when bound to crystalline galectin-10, promotes dissolution of crystalline galectin-10.

3. An antagonist that binds to galectin-10, which, when bound to soluble galectin-10, inhibits crystallization of galectin-10.

4. An antagonist that binds to galectin-10, which, when bound to crystalline galectin-10, promotes dissolution of crystalline galectin-10.

5. The antagonist of any one of claims 1-4, wherein the antagonist binds to an epitope of galectin-10 comprising one or more amino acids from the crystal packing interfaces of galectin-10.

6. The antagonist of any one of claims 1-5, which binds to human galectin-10.

7. The antagonist of any one of claims 1-6, wherein the antagonist binds to an epitope comprising one or more amino acids selected from the group consisting of: Ser2, Leu3, Leu4, Tyr8, Thr9, Glu10, Ala11, Ala12, Ser13, Thr16, Thr42, Glu43, Met44, Lys45, Asp49, Ile50, Glu68, Tyr69, Gly70, Ala71, Lys73, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Leu96, Pro97, Asp98, Lys99, Gln101, Met103, Gly106, Gln107, Ser108, Ser109, Tyr110, Thr111, Asp113, His114, Arg115, Ile116, Lys117, Ala120, Gln125, Thr133, Lys134, Phe135, Asn136, Val137, Ser138, Tyr139, Leu140 and Lys141, wherein the residues are identified with reference to SEQ ID NO: 141.

8. The antagonist of any one of claims 1-7, wherein the antagonist binds to an epitope comprising Tyr69 or an epitope comprising an amino acid adjacent to Tyr69.

9. The antagonist of any one of claims 1-8, wherein the antagonist binds to an epitope comprising or consisting of the amino acids Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, His114, Arg115, Ile116, Lys117 and Ala120.

10. The antagonist of any one of claims 1-8, wherein the antagonist binds to an epitope comprising or consisting of the amino acids Thr42, Glu43, Lys45, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, His114, Arg115, Ile116, Lys117, Glu119, Ala120 and Lys122.

11. The antagonist of claim 10, wherein the epitope additionally comprises Gln74 and Asp98.

12. The antagonist of any one of claims 1-9, wherein the antagonist binds to an epitope consisting of amino acids from the crystal packing interfaces of galectin-10.

13. The antagonist of any one of claims 1-11, wherein the antagonist binds to an epitope comprising one or more amino acids from the dimerization interface of galectin-10.

14. The antagonist of claim 13, wherein the antagonist binds to an epitope comprising one or more amino acids selected from the group consisting of: Pro5, Pro7, Leu27, Ala28, Cys29, Leu31, Asn32, Glu33, Pro34, Tyr35, Gln37, His41, Glu46, Glu47, Gln55, Arg60, Arg61, Arg67, Trp72, Gln75, Trp127, Arg128 and Asp129.

15. The antagonist of any one of claims 1-6, wherein the antagonist binds to an epitope comprising Glu33, Gly59, Arg60 and Lys79.

16. The antagonist of claim 15, wherein the epitope comprises or consists of Leu31, Glu33, Gly59, Arg60, Ser78, Lys79, Asn80, Met81, Pro82 and Gln84.

17. The antagonist of claim 15, wherein the epitope additionally comprises Gln74, Gln75 and Glu77.

18. The antagonist of claim 17, wherein the epitope comprises or consists of Glu33, Gly59, Arg60, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Pro82 and Ser109.

19. The antagonist of claim 17, wherein the epitope comprises or consists of Glu33, Gly59, Arg60, Trp72, Gln74, Gln75, Val76, Glu77, Lys79, Asn80, Met81, Pro82, Gln84 and Ser109.

20. The antagonist of claim 17, wherein the epitope comprises or consists of Glu33, Gly59, Arg60, Gln74, Gln75, Val76, Glu77, Ser78, Lys79, Asn80, Met81, Pro82, Phe83, Gln84.

21. The antagonist of any one of claims 1-6, wherein the antagonist binds to an epitope comprising or consisting of Thr42, Asp49, Glu68, Tyr69, Gly70, Ala71, Lys73, Arg115, Ile116, Lys117, Glu119 and Ala120.

22. The antagonist of any one of claims 1-6, wherein the antagonist binds to an epitope comprising or consisting of Glu43, Asp49, Glu68, Tyr69, Lys73, Asp98, Asp113, His114, Arg115, Lys117, Glu119 and Ala120.

23. The antagonist of any one of claims 1-6, wherein the antagonist binds to an epitope comprising or consisting of Asp49, Glu68, Tyr69, Lys73, Gln74, Asp98, Asp113, His114, Arg115, Ile116 and Lys117.

24. The antagonist of any one of claims 1-6, wherein the antagonist binds to an epitope comprising or consisting of Ser2, Leu3, Leu4, Pro5, Pro7, Tyr8, Thr9, Glu10, Ala11, Lys23, Arg25, Met44, Gly86, Gln87, Glu88, Phe89, Glu90, Asn105, Gln125, Thr133, Lys134 and Phe135.

25. The antagonist of any one of claims 1-24, wherein the antagonist is selected from the group consisting of: a small molecule; an inhibitory polypeptide; an antibody or antigen binding fragment thereof; and an antibody mimetic selected from an affibody, an affilin, an affitin, an adnectin, an atrimer, an evasin, a DARPin, an anticalin, an avimer, a fynomer, a versabody and a duocalin.

26. The antagonist of any one of claims 1-24, wherein the antagonist is an antibody or antigen binding fragment thereof.

27. The antagonist of claim 26, wherein the antibody is an immunoglobulin, preferably an IgG.

28. The antagonist of claim 26, wherein the antagonist is a VHH antibody.

29. The antagonist of any one of claims 26-28, wherein the antibody is a humanised or germlined variant of a non-human antibody.

30. The antagonist of claim 29, wherein the non-human antibody is camelid-derived.

31. The antagonist of any one of claims 26-30, wherein the antibody comprises the CH1 domain, hinge region, CH2 domain and/or CH3 domain of a human IgG, preferably IgG1.

32. The antagonist of any one of claims 26-31, wherein the antigen binding fragment is selected from the group consisting of: an antibody light chain variable domain (VL); an antibody heavy chain variable domain (VH or VHH); a single chain antibody (scFv); a F(ab')2 fragment; a Fab fragment; an Fd fragment; an Fv fragment; a one-armed (monovalent) antibody; diabodies,

triabodies, tetrabodies, or any antigen binding molecule formed by combination, assembly or conjugation of such antigen binding fragments.

33. The antagonist of claim 32, wherein the antigen binding fragment is a Fab fragment.

34. The antagonist of any one of claims 26-33, wherein the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:
(i) HCDR3 comprising or consisting of SEQ ID NO: 3; HCDR2 comprising or consisting of SEQ ID NO: 2; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or consisting of SEQ ID NO: 58; LCDR2 comprising or consisting of SEQ ID NO: 57; LCDR1 comprising or consisting of SEQ ID NO: 56;
(ii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 5; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 61; LCDR2 comprising or consisting of SEQ ID NO: 60; LCDR1 comprising or consisting of SEQ ID NO: 59;
(iii) HCDR3 comprising or consisting of SEQ ID NO: 9; HCDR2 comprising or consisting of SEQ ID NO: 8; HCDR1 comprising or consisting of SEQ ID NO: 7; LCDR3 comprising or consisting of SEQ ID NO: 64; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 62;
(iv) HCDR3 comprising or consisting of SEQ ID NO: 12; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 10; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;
(v) HCDR3 comprising or consisting of SEQ ID NO: 15; HCDR2 comprising or consisting of SEQ ID NO: 14; HCDR1 comprising or consisting of SEQ ID NO: 13; LCDR3 comprising or consisting of SEQ ID NO: 70; LCDR2 comprising or consisting of SEQ ID NO: 69; LCDR1 comprising or consisting of SEQ ID NO: 68;
(vi) HCDR3 comprising or consisting of SEQ ID NO: 18; HCDR2 comprising or consisting of SEQ ID NO: 17; HCDR1 comprising or consisting of SEQ ID NO: 16; LCDR3 comprising or consisting of SEQ ID NO: 72; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 71;
(vii) HCDR3 comprising or consisting of SEQ ID NO: 20; HCDR2 comprising or consisting of SEQ ID NO: 19; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 75; LCDR2 comprising or consisting of SEQ ID NO: 74; LCDR1 comprising or consisting of SEQ ID NO: 73;
(viii) HCDR3 comprising or consisting of SEQ ID NO: 23; HCDR2 comprising or consisting of SEQ ID NO: 22; HCDR1 comprising or consisting of SEQ ID NO: 21; LCDR3 comprising or

consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;

(ix) HCDR3 comprising or consisting of SEQ ID NO: 25; HCDR2 comprising or consisting of SEQ ID NO: 24; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 77; LCDR1 comprising or consisting of SEQ ID NO: 76;

(x) HCDR3 comprising or consisting of SEQ ID NO: 28; HCDR2 comprising or consisting of SEQ ID NO: 27; HCDR1 comprising or consisting of SEQ ID NO: 26; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 79;

(xi) HCDR3 comprising or consisting of SEQ ID NO: 31; HCDR2 comprising or consisting of SEQ ID NO: 30; HCDR1 comprising or consisting of SEQ ID NO: 29; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 80;

(xii) HCDR3 comprising or consisting of SEQ ID NO: 33; HCDR2 comprising or consisting of SEQ ID NO: 32; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or consisting of SEQ ID NO: 84; LCDR2 comprising or consisting of SEQ ID NO: 83; LCDR1 comprising or consisting of SEQ ID NO: 82;

(xiii) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 35; HCDR1 comprising or consisting of SEQ ID NO: 34; LCDR3 comprising or consisting of SEQ ID NO: 87; LCDR2 comprising or consisting of SEQ ID NO: 86; LCDR1 comprising or consisting of SEQ ID NO: 85;

(xiv) HCDR3 comprising or consisting of SEQ ID NO: 38; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 37; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 88;

(xv) HCDR3 comprising or consisting of SEQ ID NO: 41; HCDR2 comprising or consisting of SEQ ID NO: 40; HCDR1 comprising or consisting of SEQ ID NO: 39; LCDR3 comprising or consisting of SEQ ID NO: 91; LCDR2 comprising or consisting of SEQ ID NO: 90; LCDR1 comprising or consisting of SEQ ID NO: 89;

(xvi) HCDR3 comprising or consisting of SEQ ID NO: 43; HCDR2 comprising or consisting of SEQ ID NO: 42; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 92;

(xvii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 44; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 97; LCDR2 comprising or consisting of SEQ ID NO: 96; LCDR1 comprising or consisting of SEQ ID NO: 95;

(xviii) HCDR3 comprising or consisting of SEQ ID NO: 47; HCDR2 comprising or consisting of SEQ ID NO: 46; HCDR1 comprising or consisting of SEQ ID NO: 45; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71;
(xix) HCDR3 comprising or consisting of SEQ ID NO: 50; HCDR2 comprising or consisting of SEQ ID NO: 49; HCDR1 comprising or consisting of SEQ ID NO: 48; LCDR3 comprising or consisting of SEQ ID NO: 96; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 95;
(xx) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 52; HCDR1 comprising or consisting of SEQ ID NO: 51; LCDR3 comprising or consisting of SEQ ID NO: 98; LCDR2 comprising or consisting of SEQ ID NO: 97; LCDR1 comprising or consisting of SEQ ID NO: 80; and
(xxi) HCDR3 comprising or consisting of SEQ ID NO: 55; HCDR2 comprising or consisting of SEQ ID NO: 54; HCDR1 comprising or consisting of SEQ ID NO: 53; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71.

35. The antagonist of any one of claims 26-34, wherein the antibody or antigen binding fragment thereof comprises a combination of a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the following:
(i) a VH comprising the amino acid sequence of SEQ ID NO: 99 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 100 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(ii) a VH comprising the amino acid sequence of SEQ ID NO: 101 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 102 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iii) a VH comprising the amino acid sequence of SEQ ID NO: 103 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 104 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iv) a VH comprising the amino acid sequence of SEQ ID NO: 105 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 106 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(v) a VH comprising the amino acid sequence of SEQ ID NO: 107 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

sequence of SEQ ID NO: 108 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(vi) a VH comprising the amino acid sequence of SEQ ID NO: 109 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 110 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(vii) a VH comprising the amino acid sequence of SEQ ID NO: 111 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 112 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(viii) a VH comprising the amino acid sequence of SEQ ID NO: 113 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(ix) a VH comprising the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 116 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(x) a VH comprising the amino acid sequence of SEQ ID NO: 117 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 118 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xi) a VH comprising the amino acid sequence of SEQ ID NO: 119 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 120 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xii) a VH comprising the amino acid sequence of SEQ ID NO: 121 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 122 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xiii) a VH comprising the amino acid sequence of SEQ ID NO: 123 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 124 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xiv) a VH comprising the amino acid sequence of SEQ ID NO: 125 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 126 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xv) a VH comprising the amino acid sequence of SEQ ID NO: 127 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 128 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xvi) a VH comprising the amino acid sequence of SEQ ID NO: 129 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 130 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xvii) a VH comprising the amino acid sequence of SEQ ID NO: 131 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 132 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xviii) a VH comprising the amino acid sequence of SEQ ID NO: 133 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 134 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xix) a VH comprising the amino acid sequence of SEQ ID NO: 135 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 136 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(xx) a VH comprising the amino acid sequence of SEQ ID NO: 137 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and
(xxi) a VH comprising the amino acid sequence of SEQ ID NO: 139 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 140 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

36. The antagonist of any one of claims 26-33, wherein the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:
(i) HCDR3 comprising or consisting of SEQ ID NO: 162; HCDR2 comprising or consisting of SEQ ID NO: 161; HCDR1 comprising or consisting of SEQ ID NO: 160; LCDR3 comprising or consisting of SEQ ID NO: 179; LCDR2 comprising or consisting of SEQ ID NO: 178; LCDR1 comprising or consisting of SEQ ID NO: 177;
(ii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or

consisting of SEQ ID NO: 182; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1 comprising or consisting of SEQ ID NO: 180;
(iii) HCDR3 comprising or consisting of SEQ ID NO: 168; HCDR2 comprising or consisting of SEQ ID NO: 167; HCDR1 comprising or consisting of SEQ ID NO: 166; LCDR3 comprising or consisting of SEQ ID NO: 185; LCDR2 comprising or consisting of SEQ ID NO: 184; LCDR1 comprising or consisting of SEQ ID NO: 183;
(iv) HCDR3 comprising or consisting of SEQ ID NO: 171; HCDR2 comprising or consisting of SEQ ID NO: 170; HCDR1 comprising or consisting of SEQ ID NO: 169; LCDR3 comprising or consisting of SEQ ID NO: 187; LCDR2 comprising or consisting of SEQ ID NO: 186; LCDR1 comprising or consisting of SEQ ID NO: 180;
(v) HCDR3 comprising or consisting of SEQ ID NO: 174; HCDR2 comprising or consisting of SEQ ID NO: 173; HCDR1 comprising or consisting of SEQ ID NO: 172; LCDR3 comprising or consisting of SEQ ID NO: 189; LCDR2 comprising or consisting of SEQ ID NO: 188; LCDR1 comprising or consisting of SEQ ID NO: 180;
(vi) HCDR3 comprising or consisting of SEQ ID NO: 176; HCDR2 comprising or consisting of SEQ ID NO: 175; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 192; LCDR2 comprising or consisting of SEQ ID NO: 191; LCDR1 comprising or consisting of SEQ ID NO: 190; and
(vii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 193; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1 comprising or consisting of SEQ ID NO: 180.

37. The antagonist of claim 36, wherein the antibody or antigen binding fragment thereof comprises a combination of a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the following:
(i) a VH comprising the amino acid sequence of SEQ ID NO: 194 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 195 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(ii) a VH comprising the amino acid sequence of SEQ ID NO: 196 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 197 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iii) a VH comprising the amino acid sequence of SEQ ID NO: 198 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 199 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(iv) a VH comprising the amino acid sequence of SEQ ID NO: 200 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 201 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(v) a VH comprising the amino acid sequence of SEQ ID NO: 202 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 203 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(vi) a VH comprising the amino acid sequence of SEQ ID NO: 204 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 205 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and
(vii) a VH comprising the amino acid sequence of SEQ ID NO: 206 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 207 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

38. The antagonist of any one of claims 26-33, wherein the antibody comprises a VHH domain comprising the CDR sequences selected from the group consisting of:
(i) CDR3 comprising or consisting of SEQ ID NO: 210; CDR2 comprising or consisting of SEQ ID NO: 209; CDR1 comprising or consisting of SEQ ID NO: 208;
(ii) CDR3 comprising or consisting of SEQ ID NO: 213; CDR2 comprising or consisting of SEQ ID NO: 212; CDR1 comprising or consisting of SEQ ID NO: 211;
(iii) CDR3 comprising or consisting of SEQ ID NO: 216; CDR2 comprising or consisting of SEQ ID NO: 215; CDR1 comprising or consisting of SEQ ID NO: 214;
(iv) CDR3 comprising or consisting of SEQ ID NO: 219; CDR2 comprising or consisting of SEQ ID NO: 218; CDR1 comprising or consisting of SEQ ID NO: 217;
(v) CDR3 comprising or consisting of SEQ ID NO: 222; CDR2 comprising or consisting of SEQ ID NO: 221; CDR1 comprising or consisting of SEQ ID NO: 220;
(vi) CDR3 comprising or consisting of SEQ ID NO: 225; CDR2 comprising or consisting of SEQ ID NO: 224; CDR1 comprising or consisting of SEQ ID NO: 223;
(vii) CDR3 comprising or consisting of SEQ ID NO: 228; CDR2 comprising or consisting of SEQ ID NO: 227; CDR1 comprising or consisting of SEQ ID NO: 226;
(viii) CDR3 comprising or consisting of SEQ ID NO: 231; CDR2 comprising or consisting of SEQ ID NO: 230; CDR1 comprising or consisting of SEQ ID NO: 229;
(ix) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;

(x) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xi) CDR3 comprising or consisting of SEQ ID NO: 238; CDR2 comprising or consisting of SEQ ID NO: 237; CDR1 comprising or consisting of SEQ ID NO: 232;
(xii) CDR3 comprising or consisting of SEQ ID NO: 241; CDR2 comprising or consisting of SEQ ID NO: 240; CDR1 comprising or consisting of SEQ ID NO: 239;
(xiii) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xiv) CDR3 comprising or consisting of SEQ ID NO: 244; CDR2 comprising or consisting of SEQ ID NO: 243; CDR1 comprising or consisting of SEQ ID NO: 242;
(xv) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
(xvi) CDR3 comprising or consisting of SEQ ID NO: 247; CDR2 comprising or consisting of SEQ ID NO: 246; CDR1 comprising or consisting of SEQ ID NO: 245; and
(xvii) CDR3 comprising or consisting of SEQ ID NO: 249; CDR2 comprising or consisting of SEQ ID NO: 248; CDR1 comprising or consisting of SEQ ID NO: 217.

39. The antagonist of claim 38, wherein the VHH domain comprises or consists of the amino acid sequence represented by any one of SEQ ID NOs: 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265 or 266, or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

40. An antibody or antigen binding fragment thereof which binds to galectin-10, wherein the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:
(i) HCDR3 comprising or consisting of SEQ ID NO: 3; HCDR2 comprising or consisting of SEQ ID NO: 2; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or consisting of SEQ ID NO: 58; LCDR2 comprising or consisting of SEQ ID NO: 57; LCDR1 comprising or consisting of SEQ ID NO: 56;
(ii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 5; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 61; LCDR2 comprising or consisting of SEQ ID NO: 60; LCDR1 comprising or consisting of SEQ ID NO: 59;
(iii) HCDR3 comprising or consisting of SEQ ID NO: 9; HCDR2 comprising or consisting of SEQ ID NO: 8; HCDR1 comprising or consisting of SEQ ID NO: 7; LCDR3 comprising or consisting of SEQ ID NO: 64; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 62;

(iv) HCDR3 comprising or consisting of SEQ ID NO: 12; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 10; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;
(v) HCDR3 comprising or consisting of SEQ ID NO: 15; HCDR2 comprising or consisting of SEQ ID NO: 14; HCDR1 comprising or consisting of SEQ ID NO: 13; LCDR3 comprising or consisting of SEQ ID NO: 70; LCDR2 comprising or consisting of SEQ ID NO: 69; LCDR1 comprising or consisting of SEQ ID NO: 68;
(vi) HCDR3 comprising or consisting of SEQ ID NO: 18; HCDR2 comprising or consisting of SEQ ID NO: 17; HCDR1 comprising or consisting of SEQ ID NO: 16; LCDR3 comprising or consisting of SEQ ID NO: 72; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 71;
(vii) HCDR3 comprising or consisting of SEQ ID NO: 20; HCDR2 comprising or consisting of SEQ ID NO: 19; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 75; LCDR2 comprising or consisting of SEQ ID NO: 74; LCDR1 comprising or consisting of SEQ ID NO: 73;
(viii) HCDR3 comprising or consisting of SEQ ID NO: 23; HCDR2 comprising or consisting of SEQ ID NO: 22; HCDR1 comprising or consisting of SEQ ID NO: 21; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 65;
(ix) HCDR3 comprising or consisting of SEQ ID NO: 25; HCDR2 comprising or consisting of SEQ ID NO: 24; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 77; LCDR1 comprising or consisting of SEQ ID NO: 76;
(x) HCDR3 comprising or consisting of SEQ ID NO: 28; HCDR2 comprising or consisting of SEQ ID NO: 27; HCDR1 comprising or consisting of SEQ ID NO: 26; LCDR3 comprising or consisting of SEQ ID NO: 67; LCDR2 comprising or consisting of SEQ ID NO: 66; LCDR1 comprising or consisting of SEQ ID NO: 79;
(xi) HCDR3 comprising or consisting of SEQ ID NO: 31; HCDR2 comprising or consisting of SEQ ID NO: 30; HCDR1 comprising or consisting of SEQ ID NO: 29; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 80;
(xii) HCDR3 comprising or consisting of SEQ ID NO: 33; HCDR2 comprising or consisting of SEQ ID NO: 32; HCDR1 comprising or consisting of SEQ ID NO: 1; LCDR3 comprising or consisting of SEQ ID NO: 84; LCDR2 comprising or consisting of SEQ ID NO: 83; LCDR1 comprising or consisting of SEQ ID NO: 82;
(xiii) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 35; HCDR1 comprising or consisting of SEQ ID NO: 34; LCDR3 comprising or

consisting of SEQ ID NO: 87; LCDR2 comprising or consisting of SEQ ID NO: 86; LCDR1 comprising or consisting of SEQ ID NO: 85;
(xiv) HCDR3 comprising or consisting of SEQ ID NO: 38; HCDR2 comprising or consisting of SEQ ID NO: 11; HCDR1 comprising or consisting of SEQ ID NO: 37; LCDR3 comprising or consisting of SEQ ID NO: 78; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 88;
(xv) HCDR3 comprising or consisting of SEQ ID NO: 41; HCDR2 comprising or consisting of SEQ ID NO: 40; HCDR1 comprising or consisting of SEQ ID NO: 39; LCDR3 comprising or consisting of SEQ ID NO: 91; LCDR2 comprising or consisting of SEQ ID NO: 90; LCDR1 comprising or consisting of SEQ ID NO: 89;
(xvi) HCDR3 comprising or consisting of SEQ ID NO: 43; HCDR2 comprising or consisting of SEQ ID NO: 42; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 92;
(xvii) HCDR3 comprising or consisting of SEQ ID NO: 6; HCDR2 comprising or consisting of SEQ ID NO: 44; HCDR1 comprising or consisting of SEQ ID NO: 4; LCDR3 comprising or consisting of SEQ ID NO: 97; LCDR2 comprising or consisting of SEQ ID NO: 96; LCDR1 comprising or consisting of SEQ ID NO: 95;
(xviii) HCDR3 comprising or consisting of SEQ ID NO: 47; HCDR2 comprising or consisting of SEQ ID NO: 46; HCDR1 comprising or consisting of SEQ ID NO: 45; LCDR3 comprising or consisting of SEQ ID NO: 94; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71;
(xix) HCDR3 comprising or consisting of SEQ ID NO: 50; HCDR2 comprising or consisting of SEQ ID NO: 49; HCDR1 comprising or consisting of SEQ ID NO: 48; LCDR3 comprising or consisting of SEQ ID NO: 96; LCDR2 comprising or consisting of SEQ ID NO: 63; LCDR1 comprising or consisting of SEQ ID NO: 95;
(xx) HCDR3 comprising or consisting of SEQ ID NO: 36; HCDR2 comprising or consisting of SEQ ID NO: 52; HCDR1 comprising or consisting of SEQ ID NO: 51; LCDR3 comprising or consisting of SEQ ID NO: 98; LCDR2 comprising or consisting of SEQ ID NO: 97; LCDR1 comprising or consisting of SEQ ID NO: 80; and
(xxi) HCDR3 comprising or consisting of SEQ ID NO: 55; HCDR2 comprising or consisting of SEQ ID NO: 54; HCDR1 comprising or consisting of SEQ ID NO: 53; LCDR3 comprising or consisting of SEQ ID NO: 81; LCDR2 comprising or consisting of SEQ ID NO: 93; LCDR1 comprising or consisting of SEQ ID NO: 71.

41. An antibody or antigen binding fragment thereof which binds to galectin-10, wherein the antibody or antigen binding fragment thereof comprises a combination of a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the following:

(i) a VH comprising the amino acid sequence of SEQ ID NO: 99 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 100 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(ii) a VH comprising the amino acid sequence of SEQ ID NO: 101 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 102 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iii) a VH comprising the amino acid sequence of SEQ ID NO: 103 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 104 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iv) a VH comprising the amino acid sequence of SEQ ID NO: 105 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 106 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(v) a VH comprising the amino acid sequence of SEQ ID NO: 107 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 108 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(vi) a VH comprising the amino acid sequence of SEQ ID NO: 109 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 110 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(vii) a VH comprising the amino acid sequence of SEQ ID NO: 111 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 112 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(viii) a VH comprising the amino acid sequence of SEQ ID NO: 113 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 114 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(ix) a VH comprising the amino acid sequence of SEQ ID NO: 115 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 116 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(x) a VH comprising the amino acid sequence of SEQ ID NO: 117 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid

sequence of SEQ ID NO: 118 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xi) a VH comprising the amino acid sequence of SEQ ID NO: 119 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 120 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xii) a VH comprising the amino acid sequence of SEQ ID NO: 121 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 122 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xiii) a VH comprising the amino acid sequence of SEQ ID NO: 123 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 124 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xiv) a VH comprising the amino acid sequence of SEQ ID NO: 125 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 126 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xv) a VH comprising the amino acid sequence of SEQ ID NO: 127 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 128 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xvi) a VH comprising the amino acid sequence of SEQ ID NO: 129 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 130 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xvii) a VH comprising the amino acid sequence of SEQ ID NO: 131 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 132 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xviii) a VH comprising the amino acid sequence of SEQ ID NO: 133 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 134 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xix) a VH comprising the amino acid sequence of SEQ ID NO: 135 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 136 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;

(xx) a VH comprising the amino acid sequence of SEQ ID NO: 137 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 138 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and
(xxi) a VH comprising the amino acid sequence of SEQ ID NO: 139 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 140 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

42. An antibody or antigen binding fragment thereof which binds to galectin-10, wherein the antibody or antigen binding fragment comprises a variable heavy chain domain (VH) and a variable light chain domain (VL) wherein the VH and VL domains comprise the CDR sequences selected from the group consisting of:
(i) HCDR3 comprising or consisting of SEQ ID NO: 162; HCDR2 comprising or consisting of SEQ ID NO: 161; HCDR1 comprising or consisting of SEQ ID NO: 160; LCDR3 comprising or consisting of SEQ ID NO: 179; LCDR2 comprising or consisting of SEQ ID NO: 178; LCDR1 comprising or consisting of SEQ ID NO: 177;
(ii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 182; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1 comprising or consisting of SEQ ID NO: 180;
(iii) HCDR3 comprising or consisting of SEQ ID NO: 168; HCDR2 comprising or consisting of SEQ ID NO: 167; HCDR1 comprising or consisting of SEQ ID NO: 166; LCDR3 comprising or consisting of SEQ ID NO: 185; LCDR2 comprising or consisting of SEQ ID NO: 184; LCDR1 comprising or consisting of SEQ ID NO: 183;
(iv) HCDR3 comprising or consisting of SEQ ID NO: 171; HCDR2 comprising or consisting of SEQ ID NO: 170; HCDR1 comprising or consisting of SEQ ID NO: 169; LCDR3 comprising or consisting of SEQ ID NO: 187; LCDR2 comprising or consisting of SEQ ID NO: 186; LCDR1 comprising or consisting of SEQ ID NO: 180;
(v) HCDR3 comprising or consisting of SEQ ID NO: 174; HCDR2 comprising or consisting of SEQ ID NO: 173; HCDR1 comprising or consisting of SEQ ID NO: 172; LCDR3 comprising or consisting of SEQ ID NO: 189; LCDR2 comprising or consisting of SEQ ID NO: 188; LCDR1 comprising or consisting of SEQ ID NO: 180;
(vi) HCDR3 comprising or consisting of SEQ ID NO: 176; HCDR2 comprising or consisting of SEQ ID NO: 175; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 192; LCDR2 comprising or consisting of SEQ ID NO: 191; LCDR1 comprising or consisting of SEQ ID NO: 190; and

(vii) HCDR3 comprising or consisting of SEQ ID NO: 165; HCDR2 comprising or consisting of SEQ ID NO: 164; HCDR1 comprising or consisting of SEQ ID NO: 163; LCDR3 comprising or consisting of SEQ ID NO: 193; LCDR2 comprising or consisting of SEQ ID NO: 181; LCDR1 comprising or consisting of SEQ ID NO: 180.

43. An antibody or antigen binding fragment thereof which binds to galectin-10, wherein the antibody or antigen binding fragment thereof comprises a combination of a heavy chain variable domain (VH) and a light chain variable domain (VL) selected from the following:
(i) a VH comprising the amino acid sequence of SEQ ID NO: 194 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 195 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(ii) a VH comprising the amino acid sequence of SEQ ID NO: 196 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 197 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iii) a VH comprising the amino acid sequence of SEQ ID NO: 198 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 199 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(iv) a VH comprising the amino acid sequence of SEQ ID NO: 200 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 201 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(v) a VH comprising the amino acid sequence of SEQ ID NO: 202 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 203 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto;
(vi) a VH comprising the amino acid sequence of SEQ ID NO: 204 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 205 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto; and
(vii) a VH comprising the amino acid sequence of SEQ ID NO: 206 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto and a VL comprising the amino acid sequence of SEQ ID NO: 207 or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

44. An antibody which binds to galectin-10, wherein the antibody comprises a VHH domain comprising the CDR sequences selected from the group consisting of:
(i) CDR3 comprising or consisting of SEQ ID NO: 210; CDR2 comprising or consisting of SEQ ID NO: 209; CDR1 comprising or consisting of SEQ ID NO: 208;
(ii) CDR3 comprising or consisting of SEQ ID NO: 213; CDR2 comprising or consisting of SEQ ID NO: 212; CDR1 comprising or consisting of SEQ ID NO: 211;
(iii) CDR3 comprising or consisting of SEQ ID NO: 216; CDR2 comprising or consisting of SEQ ID NO: 215; CDR1 comprising or consisting of SEQ ID NO: 214;
(iv) CDR3 comprising or consisting of SEQ ID NO: 219; CDR2 comprising or consisting of SEQ ID NO: 218; CDR1 comprising or consisting of SEQ ID NO: 217;
(v) CDR3 comprising or consisting of SEQ ID NO: 222; CDR2 comprising or consisting of SEQ ID NO: 221; CDR1 comprising or consisting of SEQ ID NO: 220;
(vi) CDR3 comprising or consisting of SEQ ID NO: 225; CDR2 comprising or consisting of SEQ ID NO: 224; CDR1 comprising or consisting of SEQ ID NO: 223;
(vii) CDR3 comprising or consisting of SEQ ID NO: 228; CDR2 comprising or consisting of SEQ ID NO: 227; CDR1 comprising or consisting of SEQ ID NO: 226;
(viii) CDR3 comprising or consisting of SEQ ID NO: 231; CDR2 comprising or consisting of SEQ ID NO: 230; CDR1 comprising or consisting of SEQ ID NO: 229;
(ix) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
(x) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xi) CDR3 comprising or consisting of SEQ ID NO: 238; CDR2 comprising or consisting of SEQ ID NO: 237; CDR1 comprising or consisting of SEQ ID NO: 232;
(xii) CDR3 comprising or consisting of SEQ ID NO: 241; CDR2 comprising or consisting of SEQ ID NO: 240; CDR1 comprising or consisting of SEQ ID NO: 239;
(xiii) CDR3 comprising or consisting of SEQ ID NO: 236; CDR2 comprising or consisting of SEQ ID NO: 235; CDR1 comprising or consisting of SEQ ID NO: 226;
(xiv) CDR3 comprising or consisting of SEQ ID NO: 244; CDR2 comprising or consisting of SEQ ID NO: 243; CDR1 comprising or consisting of SEQ ID NO: 242;
(xv) CDR3 comprising or consisting of SEQ ID NO: 234; CDR2 comprising or consisting of SEQ ID NO: 233; CDR1 comprising or consisting of SEQ ID NO: 232;
(xvi) CDR3 comprising or consisting of SEQ ID NO: 247; CDR2 comprising or consisting of SEQ ID NO: 246; CDR1 comprising or consisting of SEQ ID NO: 245; and
(xvii) CDR3 comprising or consisting of SEQ ID NO: 249; CDR2 comprising or consisting of SEQ ID NO: 248; CDR1 comprising or consisting of SEQ ID NO: 217.

45. An antibody which binds to galectin-10, wherein the antibody comprises a VHH domain and wherein the VHH domain comprises or consists of the amino acid sequence represented by any one of SEQ ID NOs: 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265 or 266, or an amino acid sequence at least 90%, 95%, 97%, 98% or 99% identical thereto.

46. An antibody or antigen binding fragment thereof, which binds to the same epitope as the antibody or antigen binding fragment of any one of claims 34-45.

47. An isolated polynucleotide which encodes the antibody or antigen binding fragment of any of claims 34-45 or a VH, VL or VHH domain thereof.

48. An expression vector comprising the polynucleotide of claim 47 operably linked to regulatory sequences which permit expression of the antibody, antigen binding fragment, variable heavy chain domain, variable light chain domain or VHH domain in a host cell or cell-free expression system.

49. A host cell or cell-free expression system containing the expression vector of claim 48.

50. A pharmaceutical composition comprising an antagonist according to any one of claims 1-39 or an antibody or antigen binding fragment according to any one of claims 40-46 and at least one pharmaceutically acceptable carrier or excipient.

51. An antagonist according to any one of claims 1-39, an antibody or antigen binding fragment according to any one of claims 40-46, or a pharmaceutical composition according to claim 50 for use as a medicament.

52. A method of treating a subject in need thereof, wherein the method comprises administering to the subject a therapeutically effective amount of an antagonist according to any one of claims 1-29, an antibody or antigen binding fragment according to any one of claims 40-46, or a pharmaceutical composition according to claim 50.

53. The method of claim 52, wherein the antagonist, antibody or antigen binding fragment, or pharmaceutical composition is administered to prevent or treat a disease or condition associated with the presence or formation of galectin-10 crystals.

54. The method of claim 52 or claim 53, wherein the antagonist, antibody or antigen binding fragment, or pharmaceutical composition is administered to prevent or treat a disease or condition selected from the group consisting of: asthma; chronic rhinosinusitis; celiac disease;

helminth infection; gastrointestinal eosinophilic inflammation; cystic fibrosis (CF); allergic bronchopulmonary aspergillosis (ABPA); Churg-Straus vasculitis; chronic eosinophilic pneumonia; and acute myeloid leukemia.

55. The method of claim 54, wherein the disease or condition is asthma.

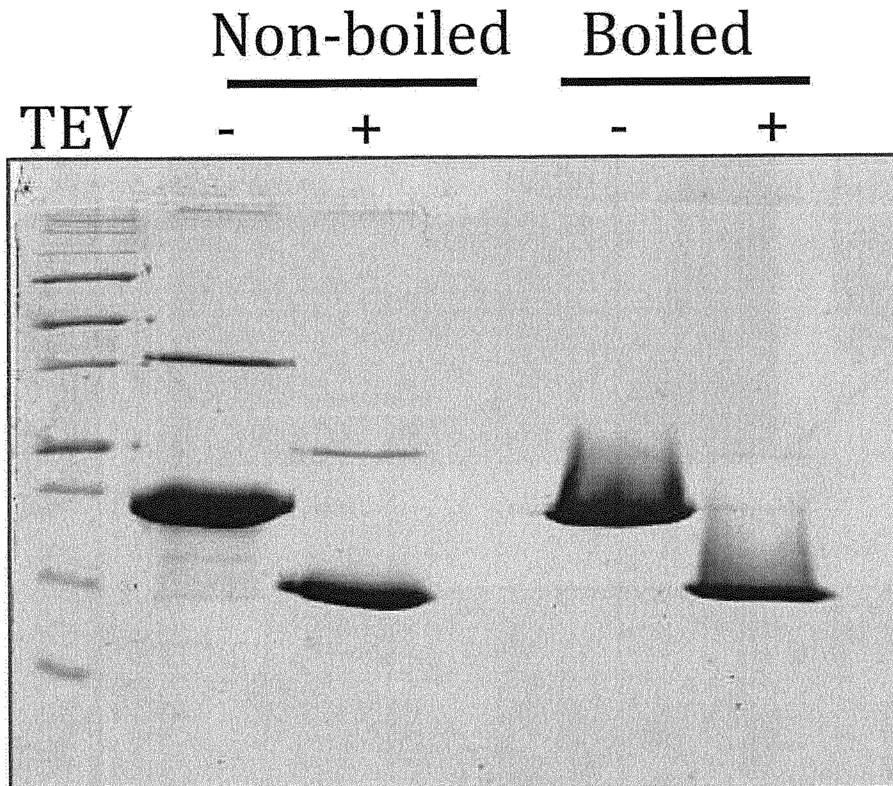
56. Use of an antagonist according to any one of claims 1-39 or an antibody or antigen binding fragment according to any one of claims 40-46 for the detection of galectin-10 in a sample obtained from a patient.

57. The use according to claim 56, wherein the patient sample is a mucus sample or a sputum sample.

58. A kit comprising an antagonist according to any one of claims 1-39 or an antibody or antigen binding fragment according to any one of claims 40-46.

Fig. 1

A



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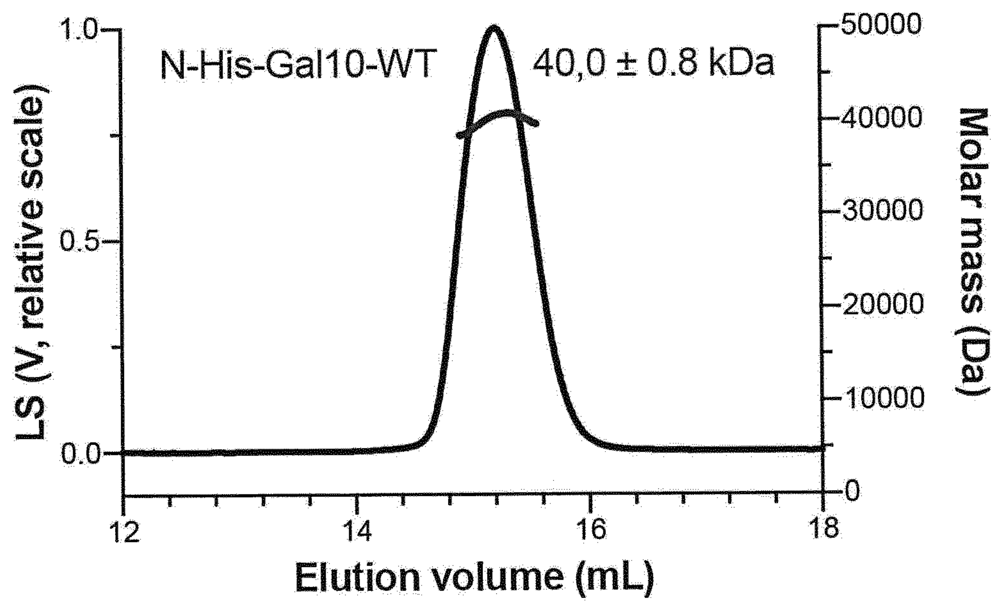
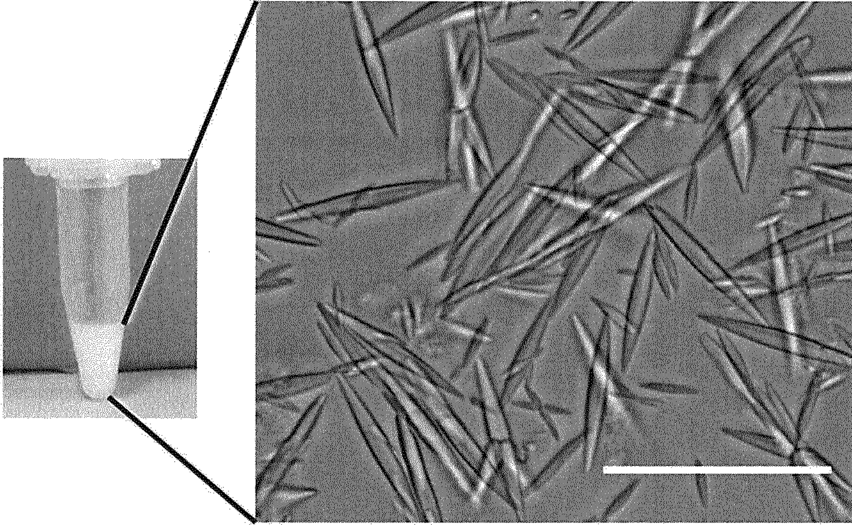


Fig. 1 (continued)

C



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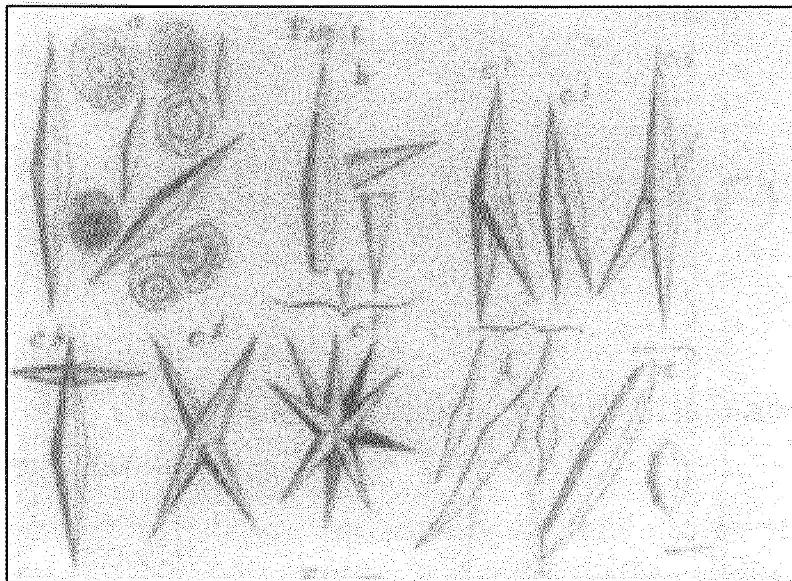

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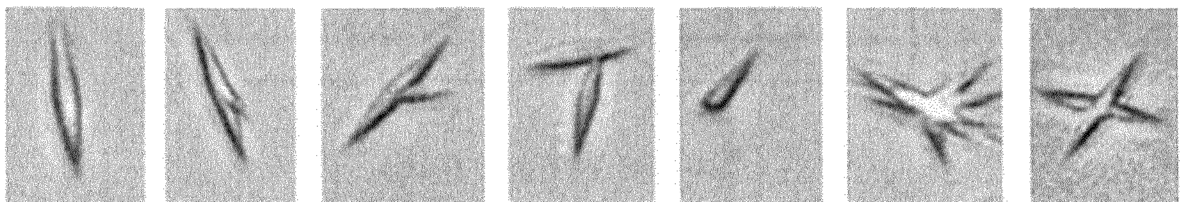
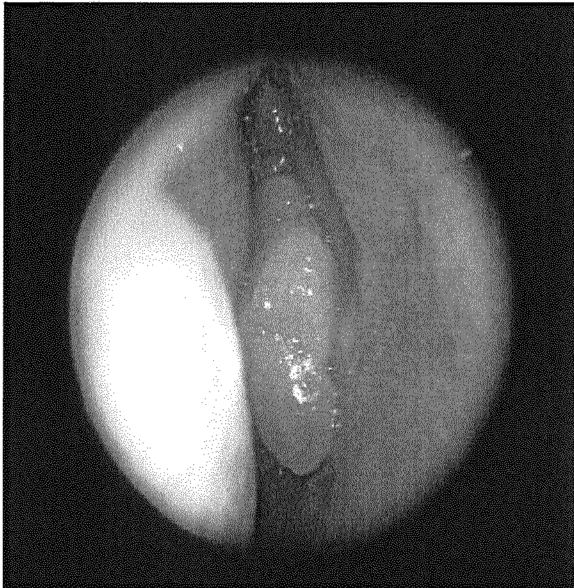


Fig. 2

A



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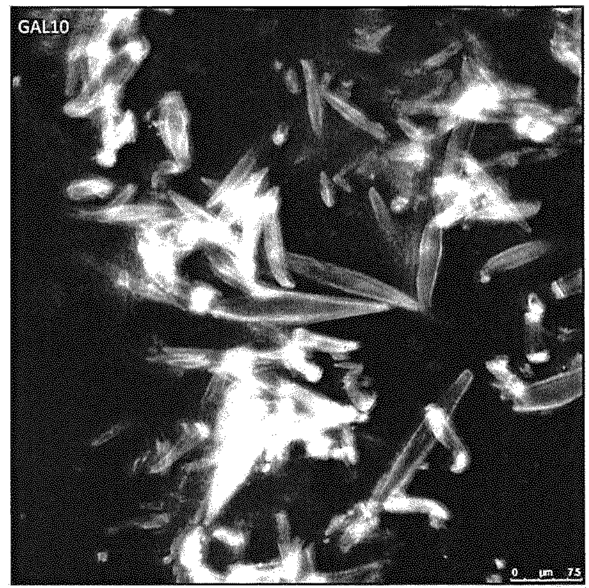

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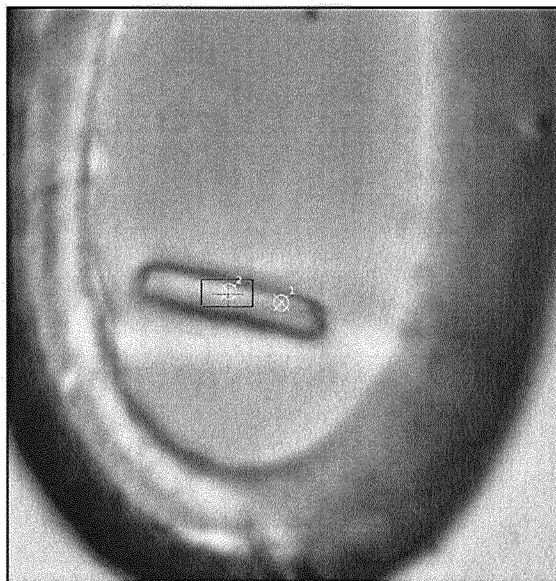

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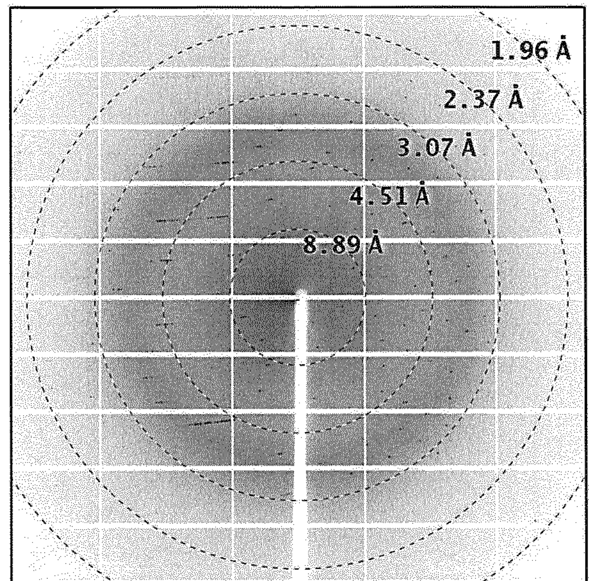


Fig. 2 (continued)

E

F

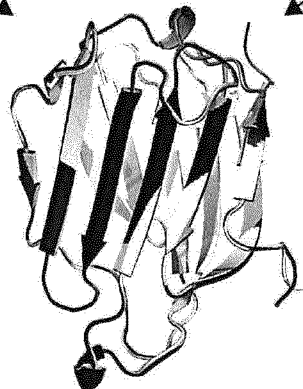
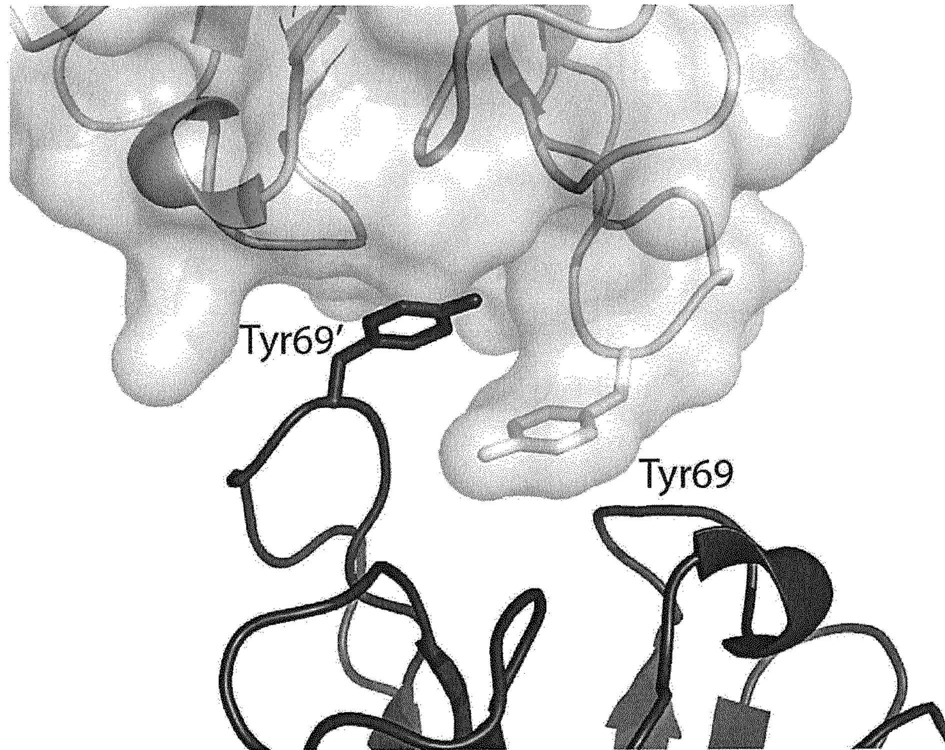


Fig. 3

A



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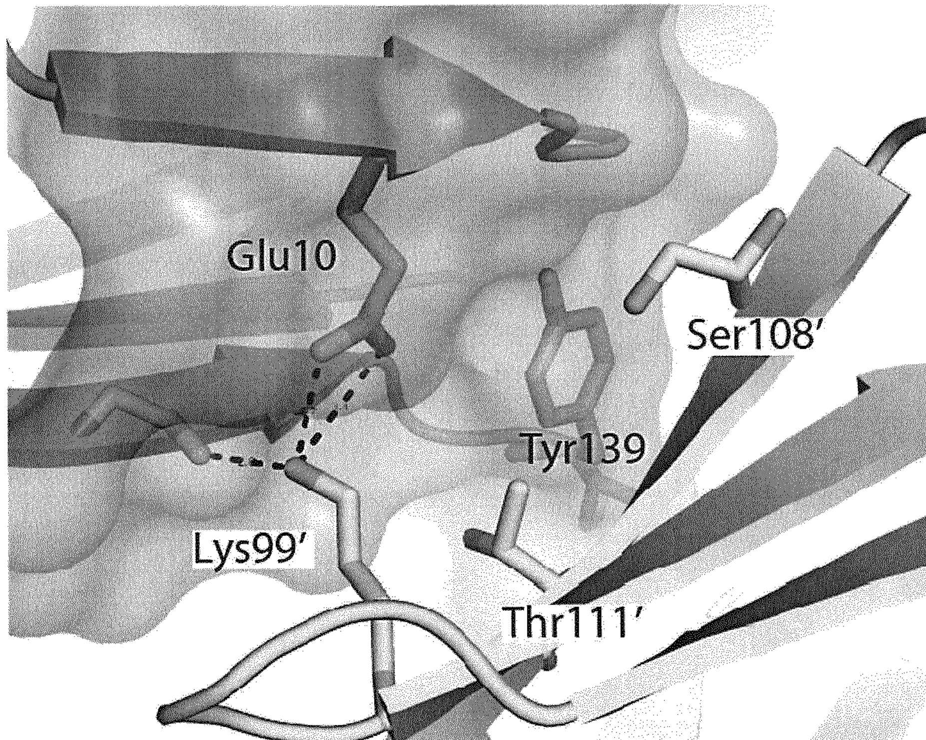

Fig. 3 (continued)

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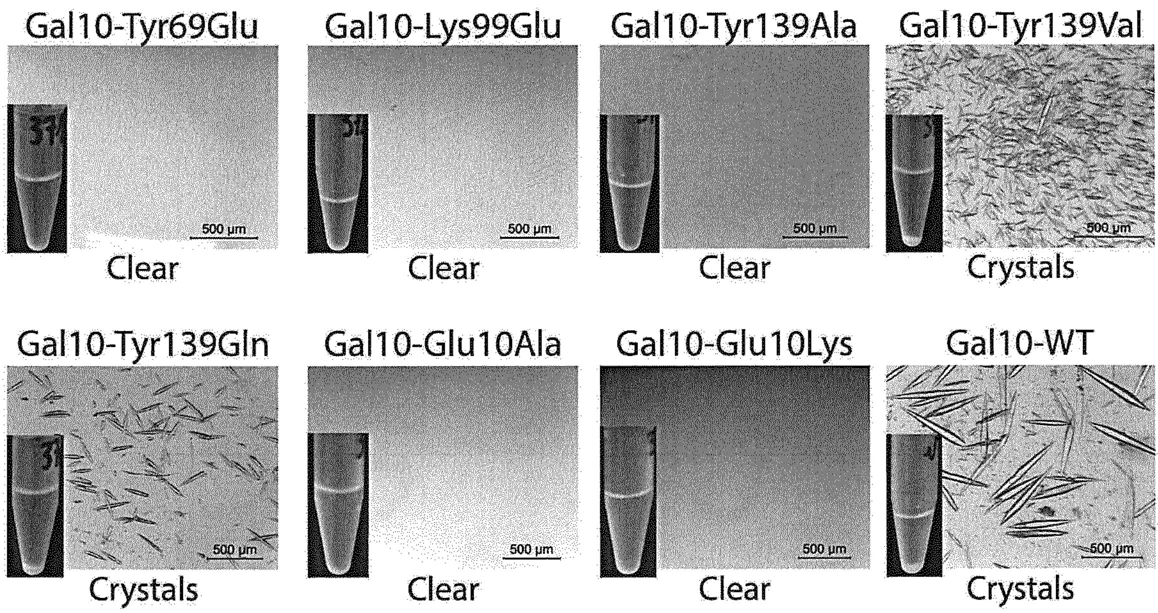

Fig. 3 (continued)

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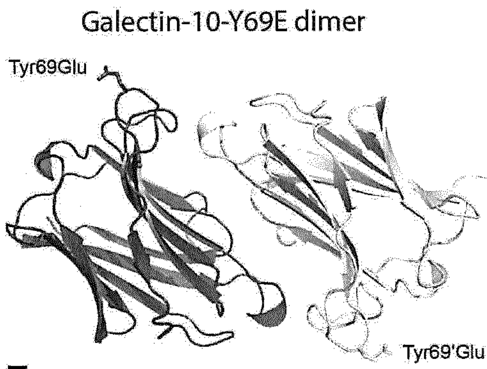

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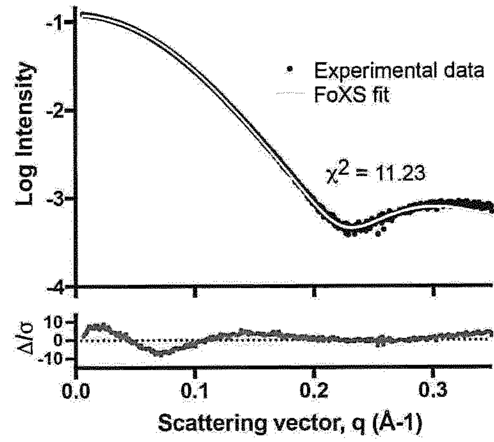

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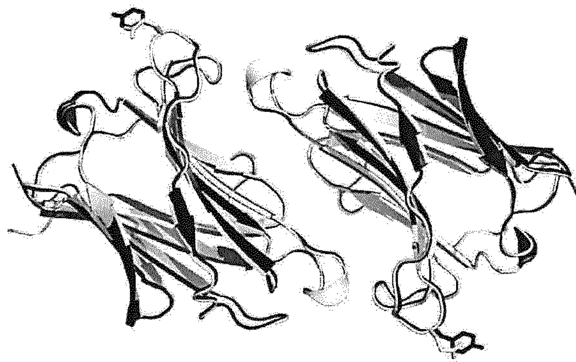
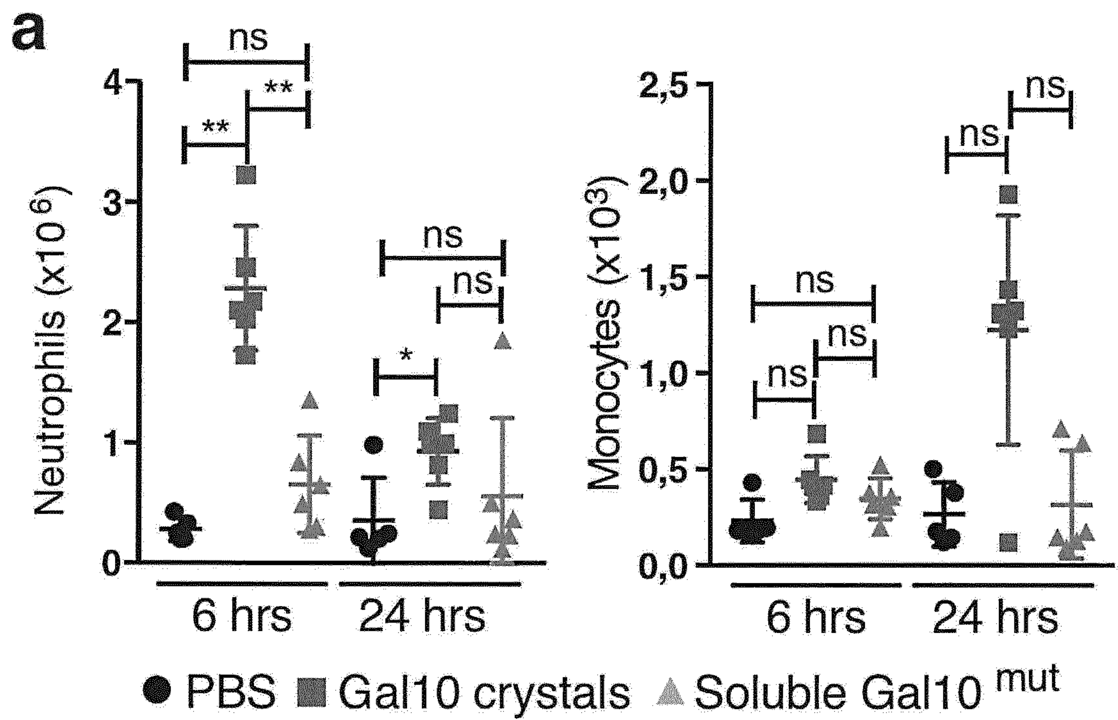


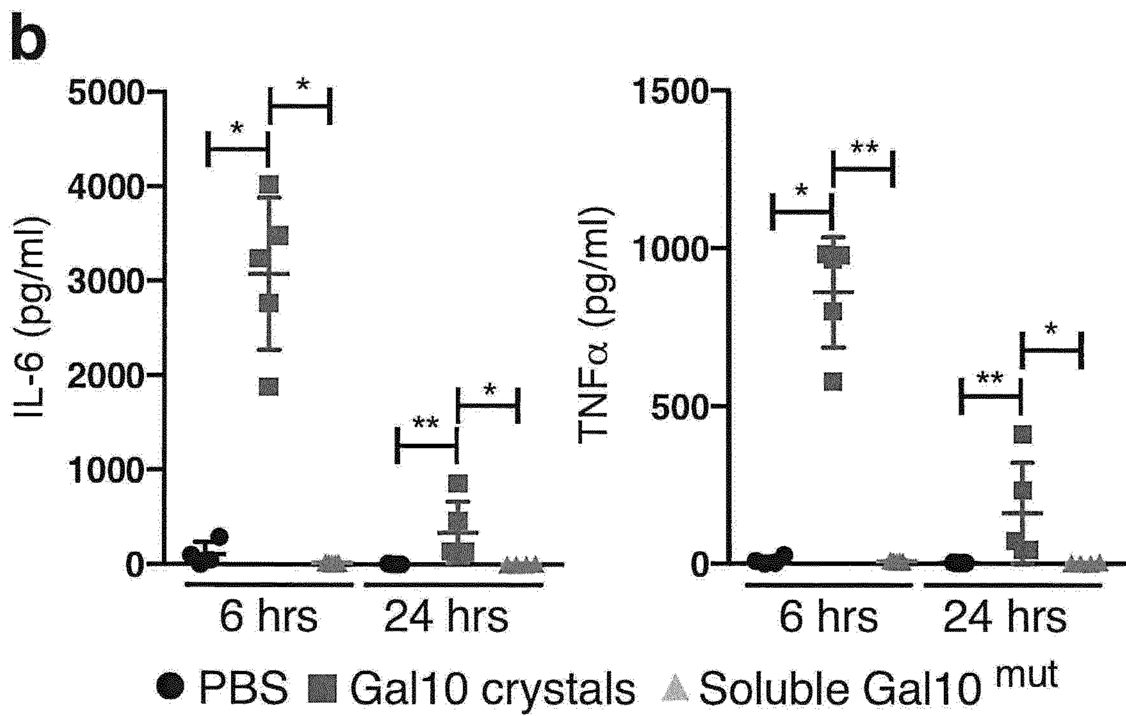
Crystallographic galectin-10 dimer (pdb 1LCL)

Galectin-10-Y69E dimer

RMSD < 0.3 Å

Fig. 4





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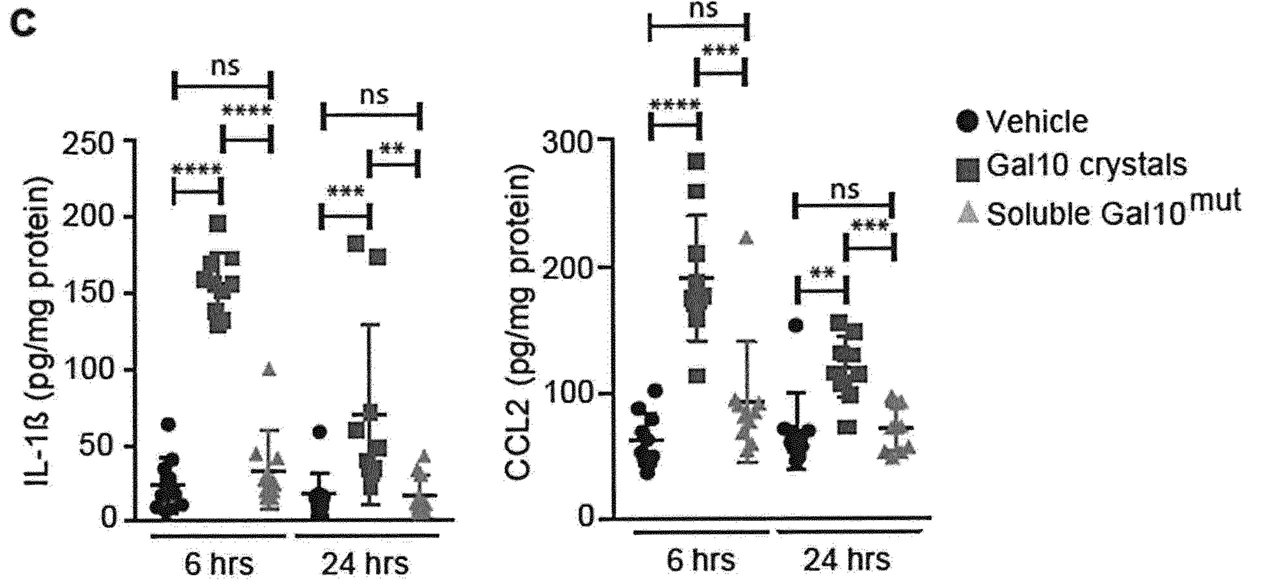


Fig. 5

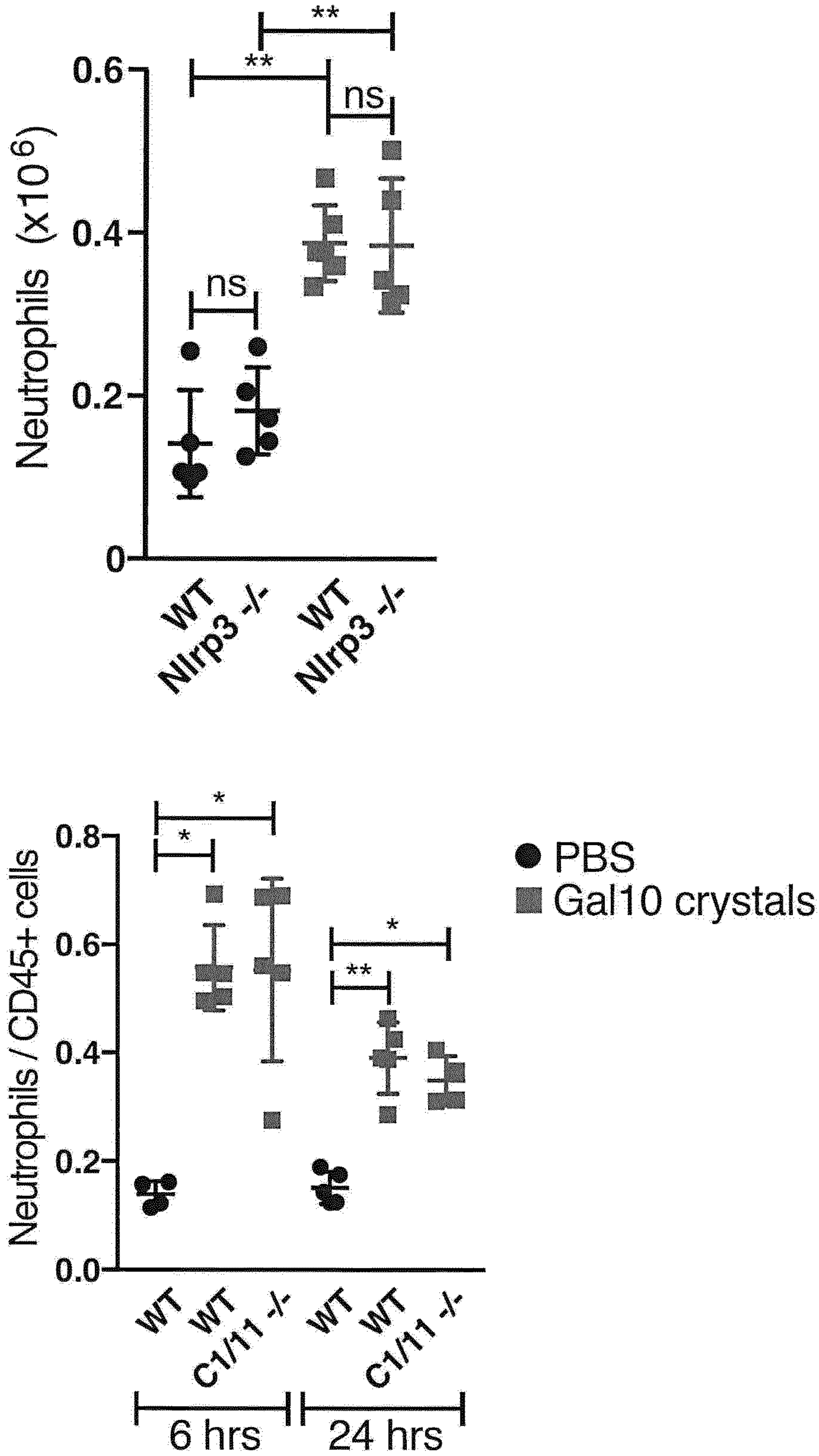


Fig. 6

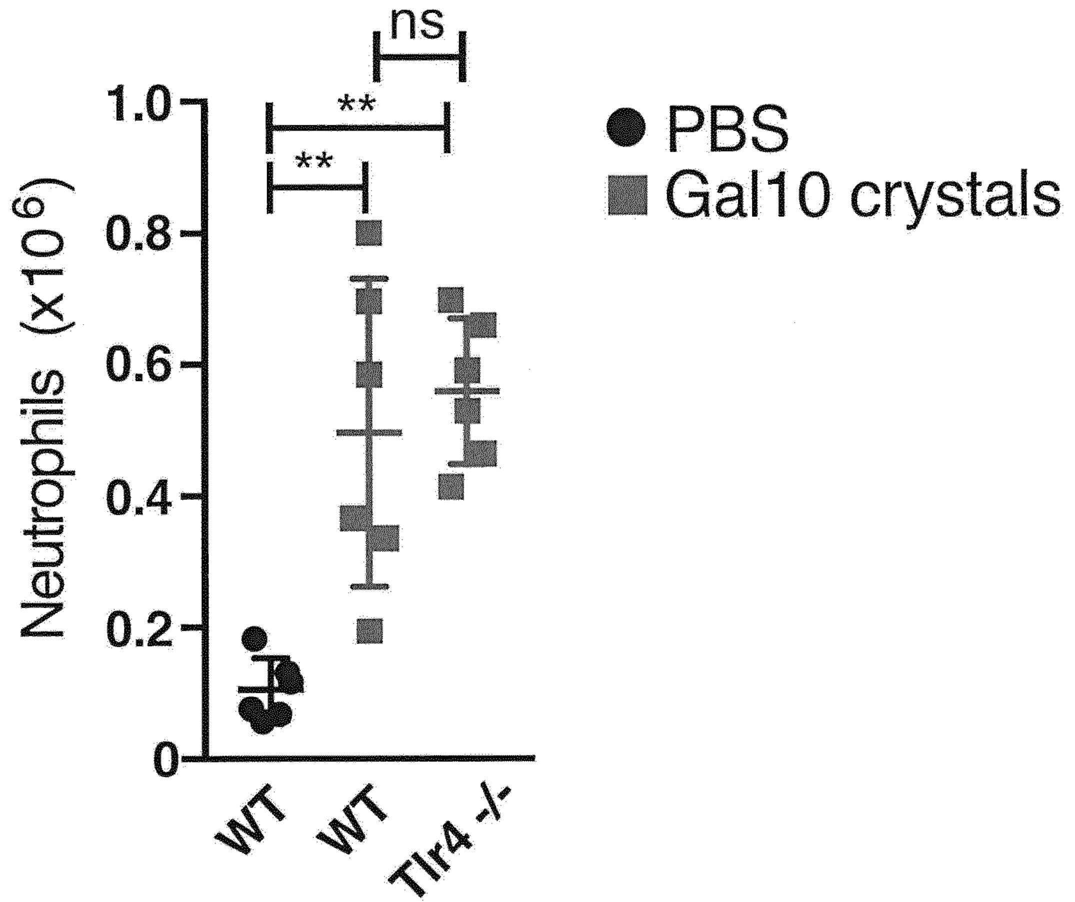
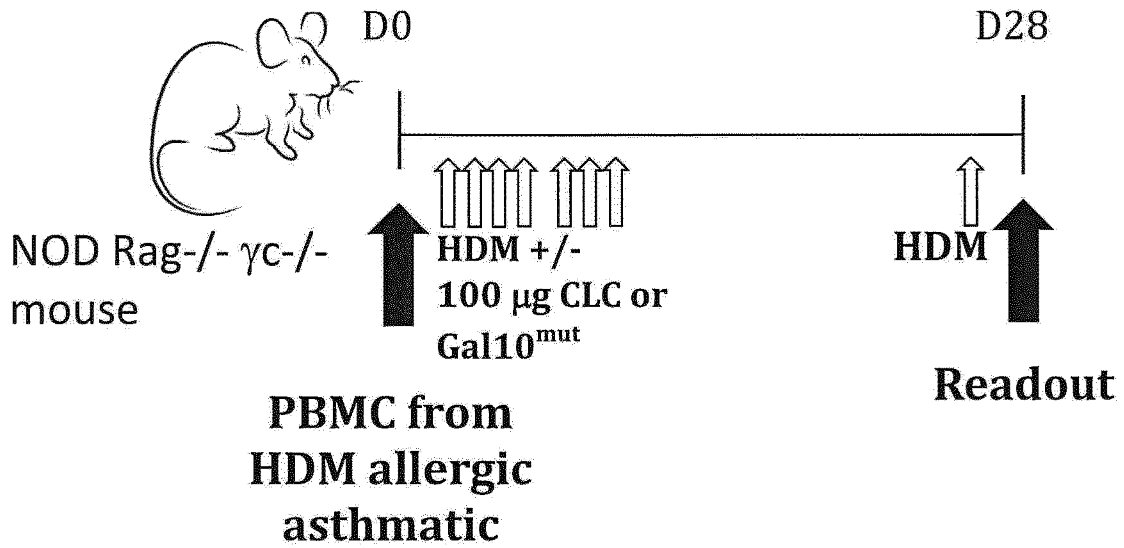


Fig. 7

A



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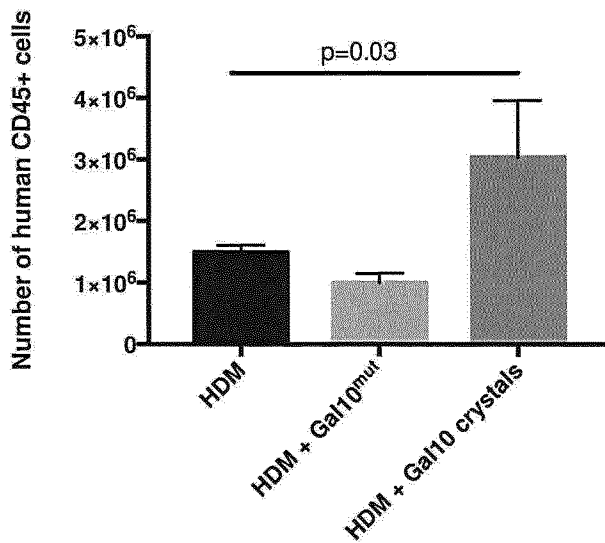

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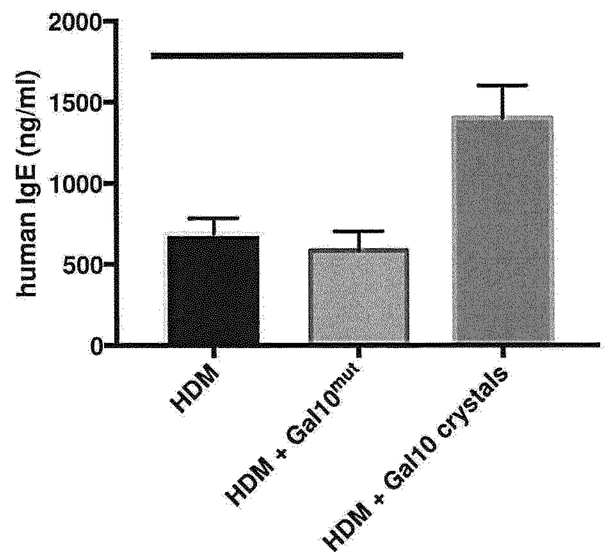


Fig. 8

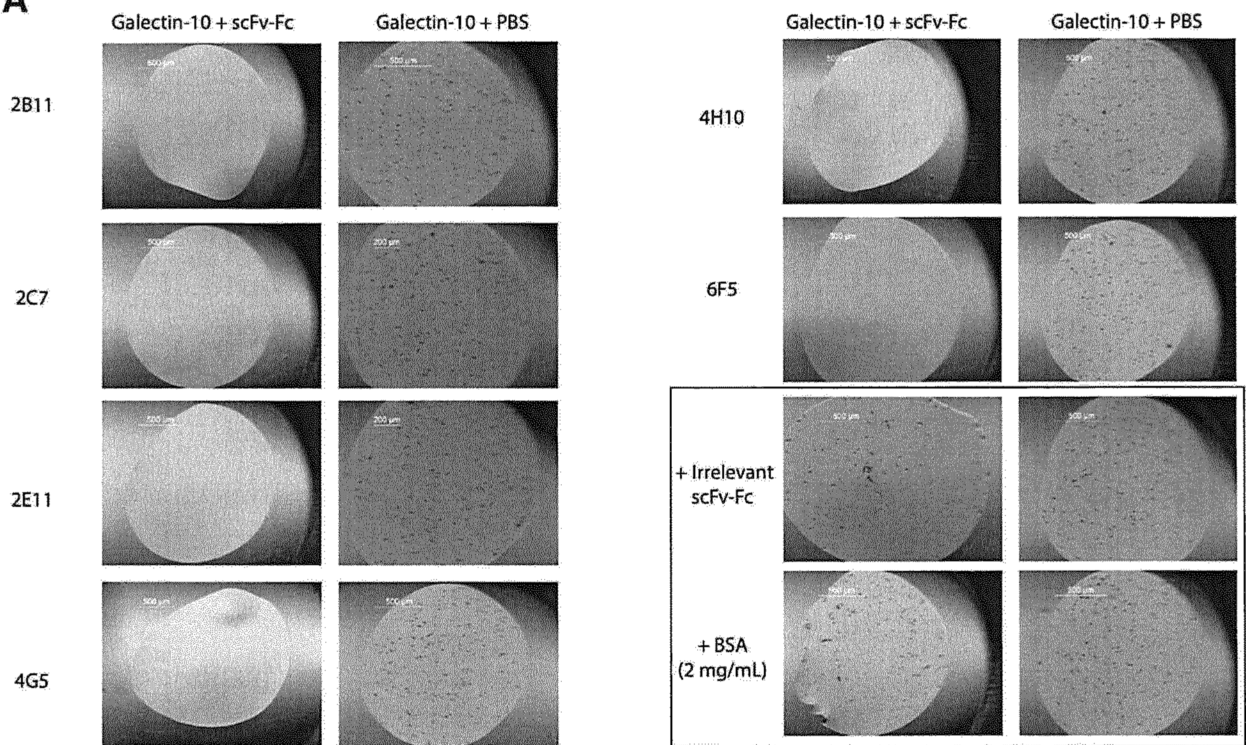
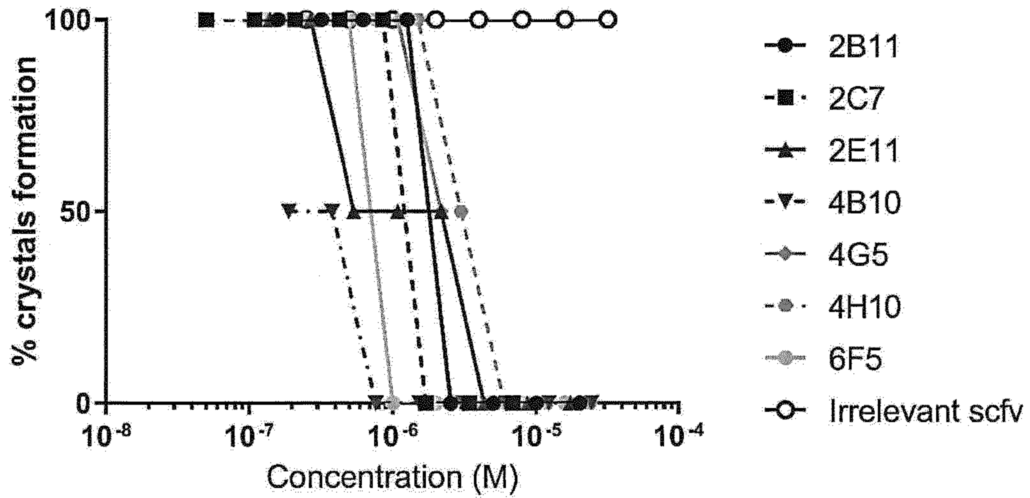


Fig. 8 (continued)

B



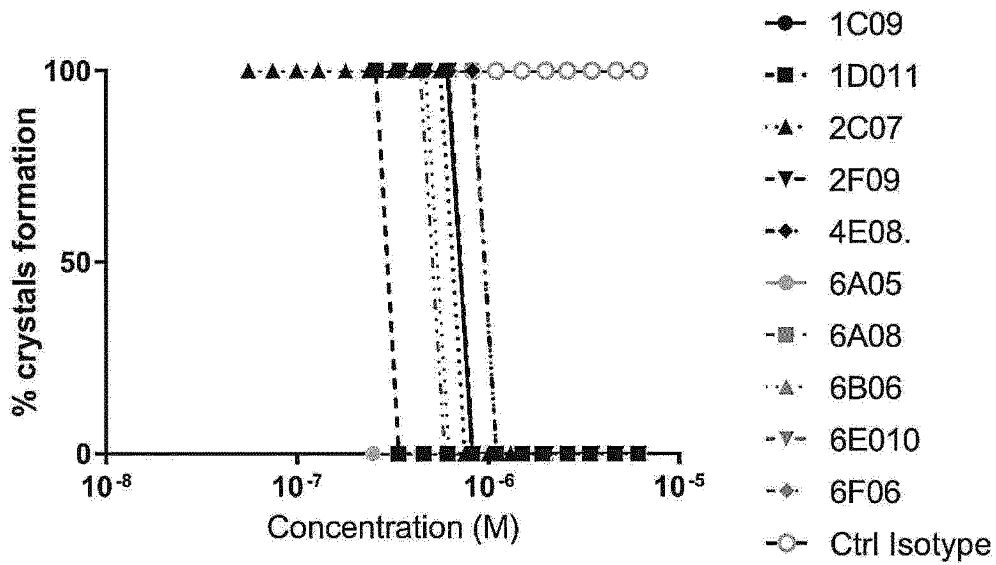


Fig. 9

A

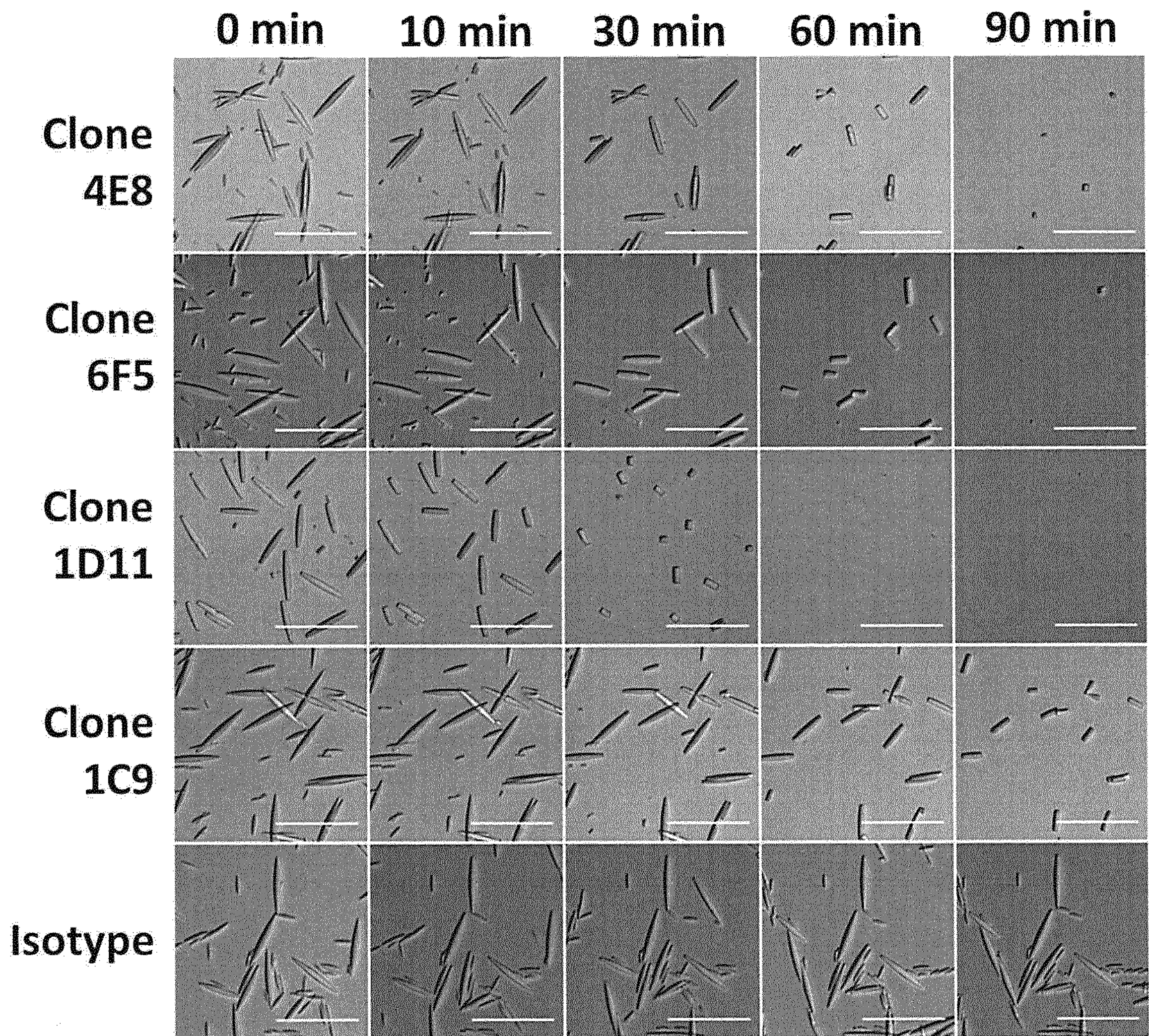


Fig. 9 (continued)

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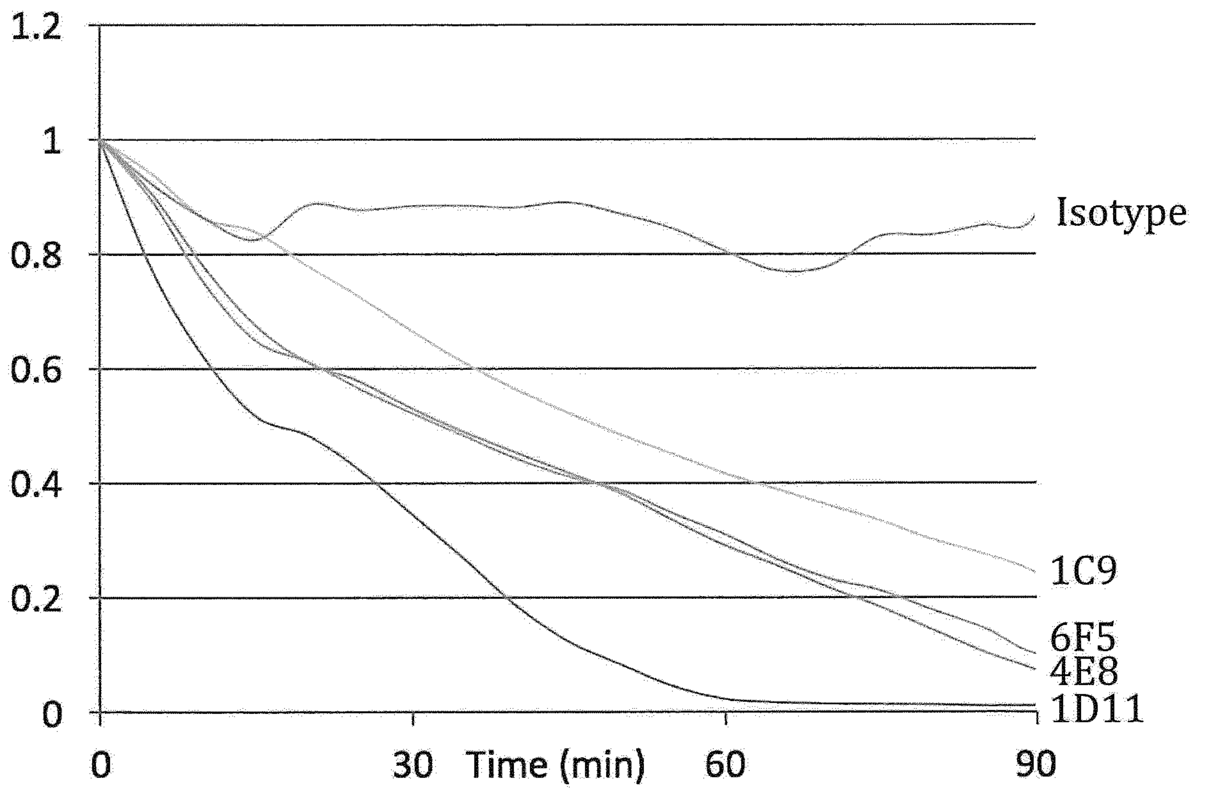

Fig. 10

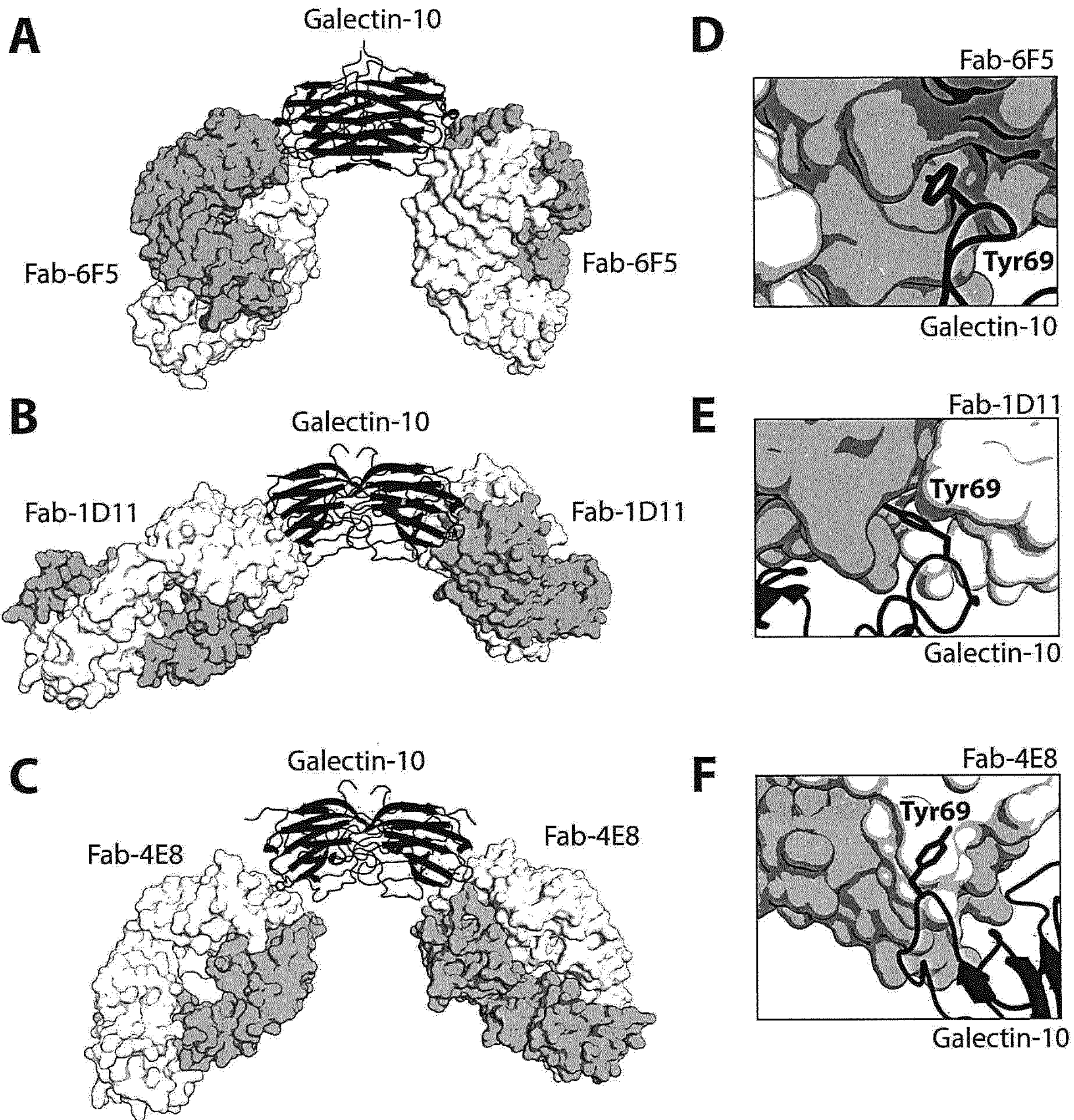

Fig. 11

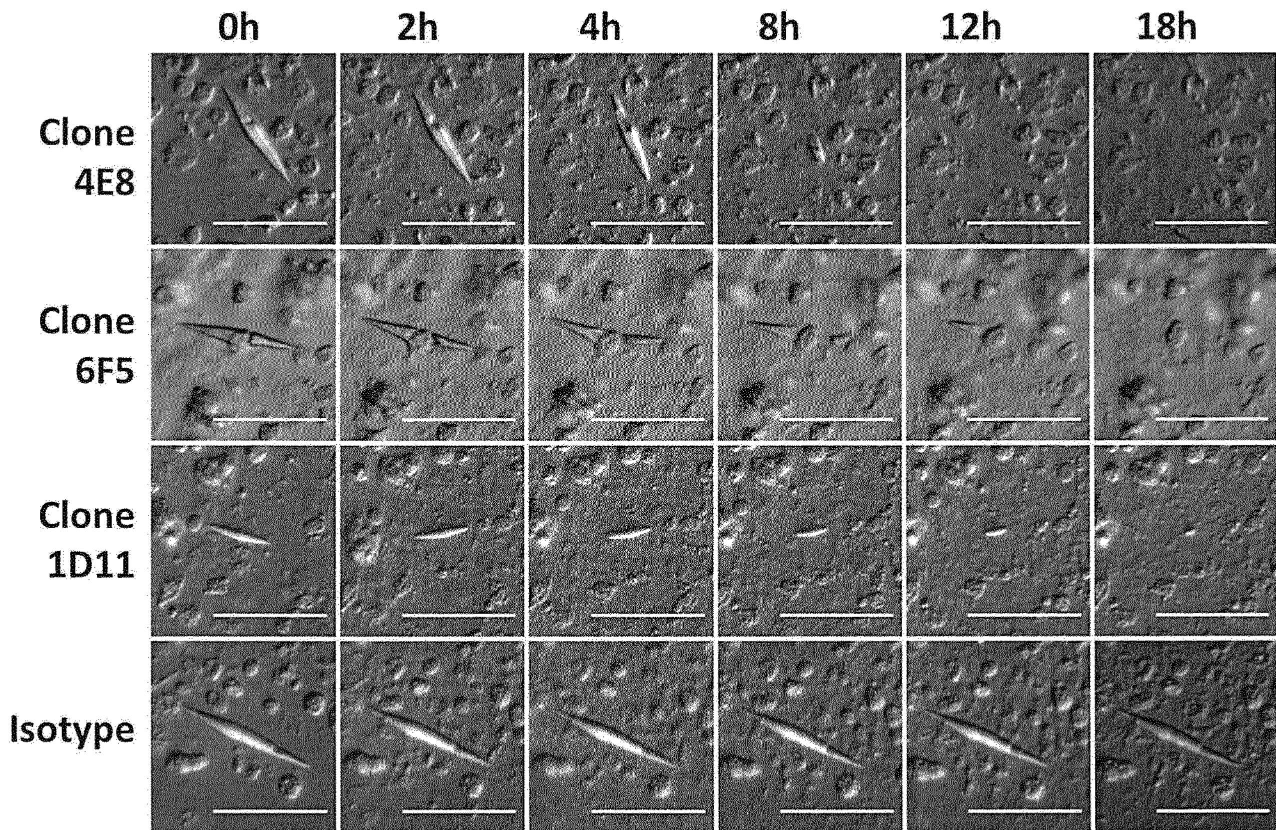

Fig. 12

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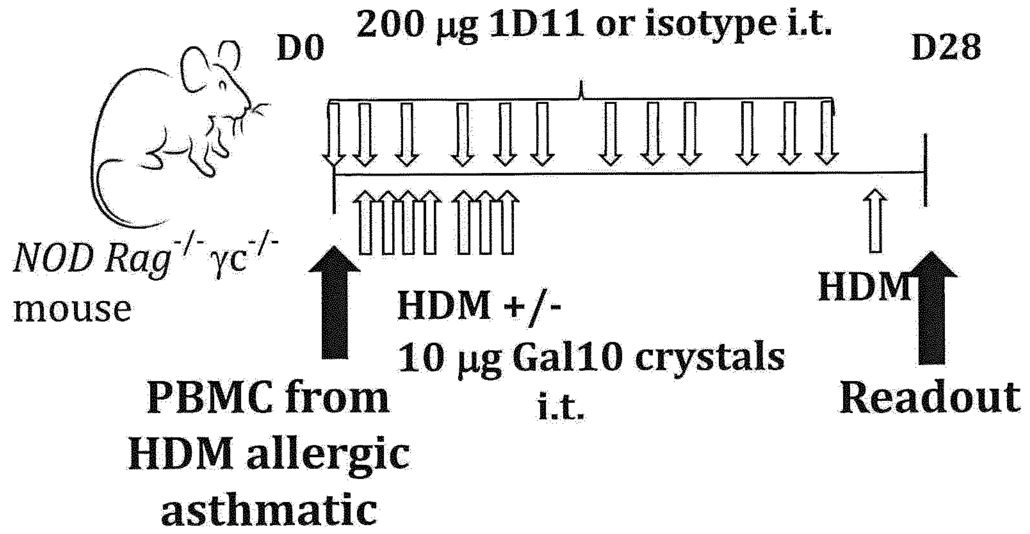

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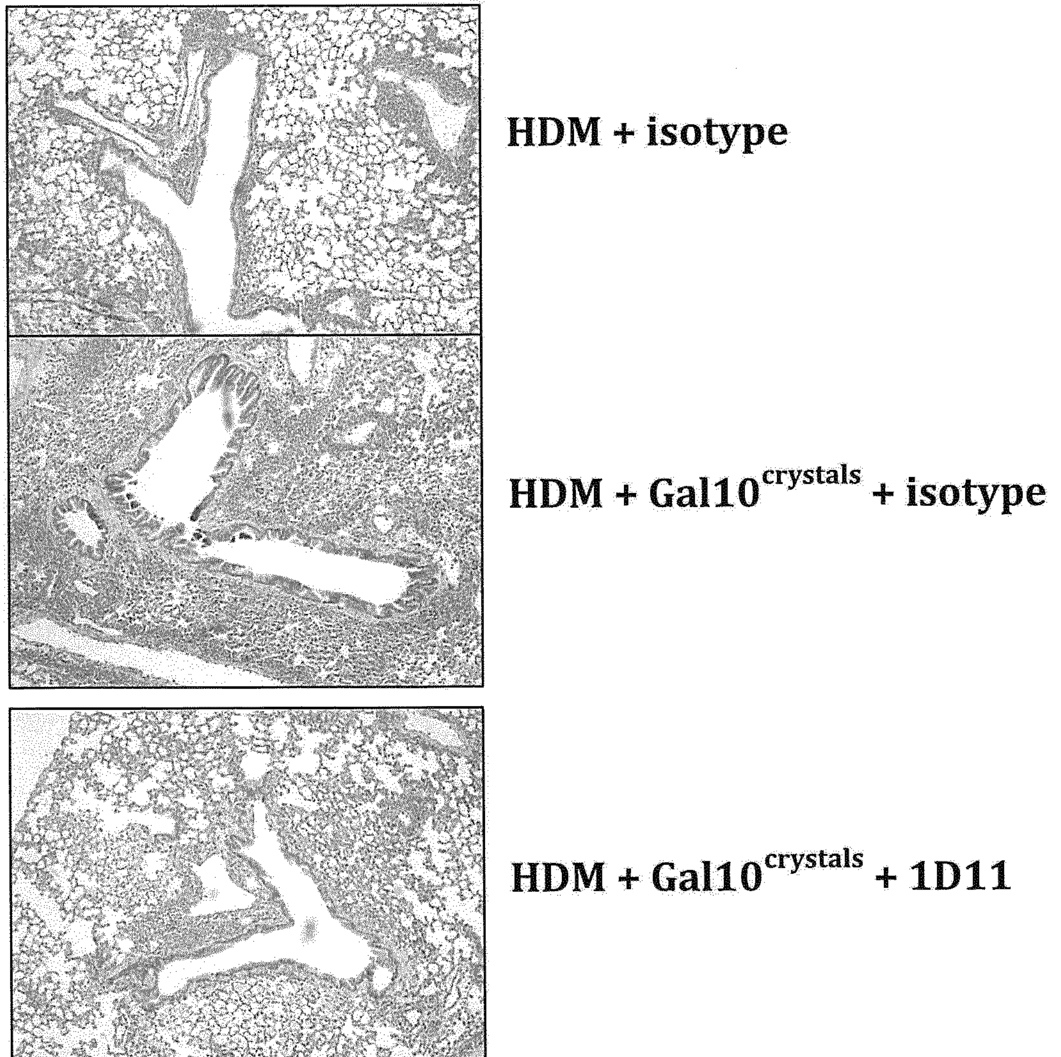

Fig. 12 (continued)

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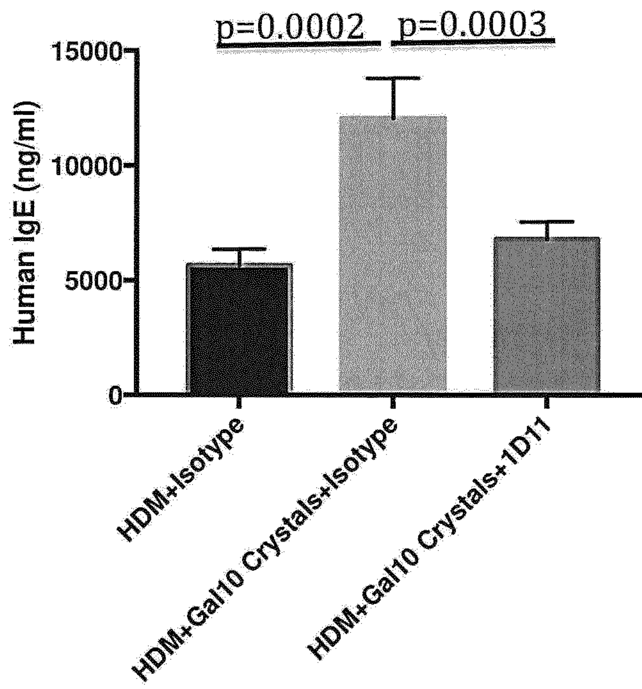

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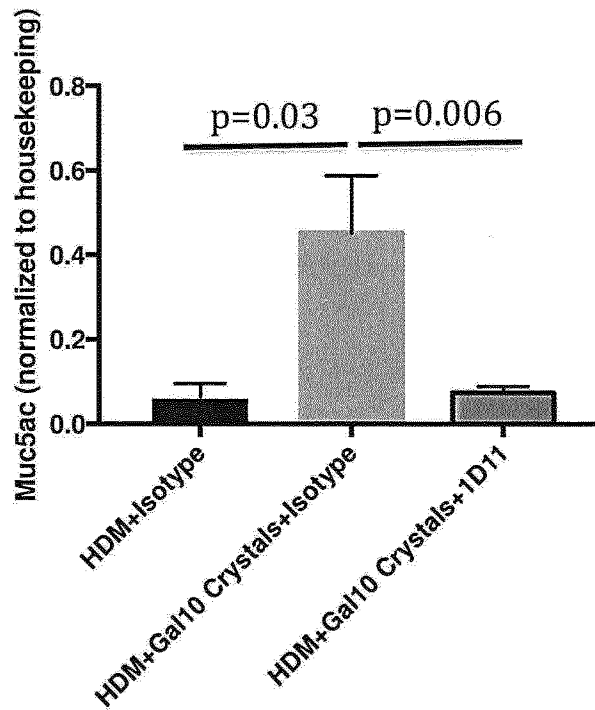
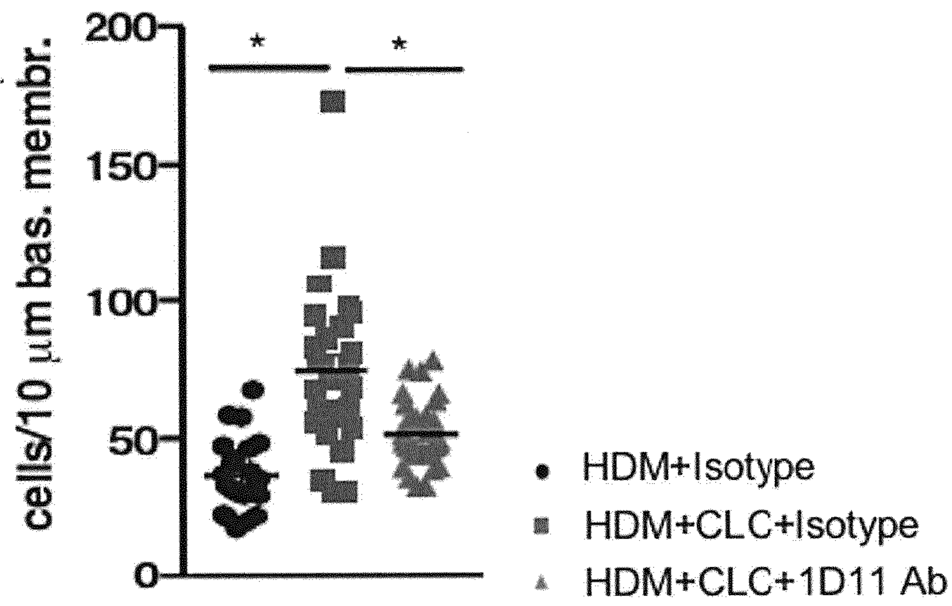


Fig. 12 (continued)

E



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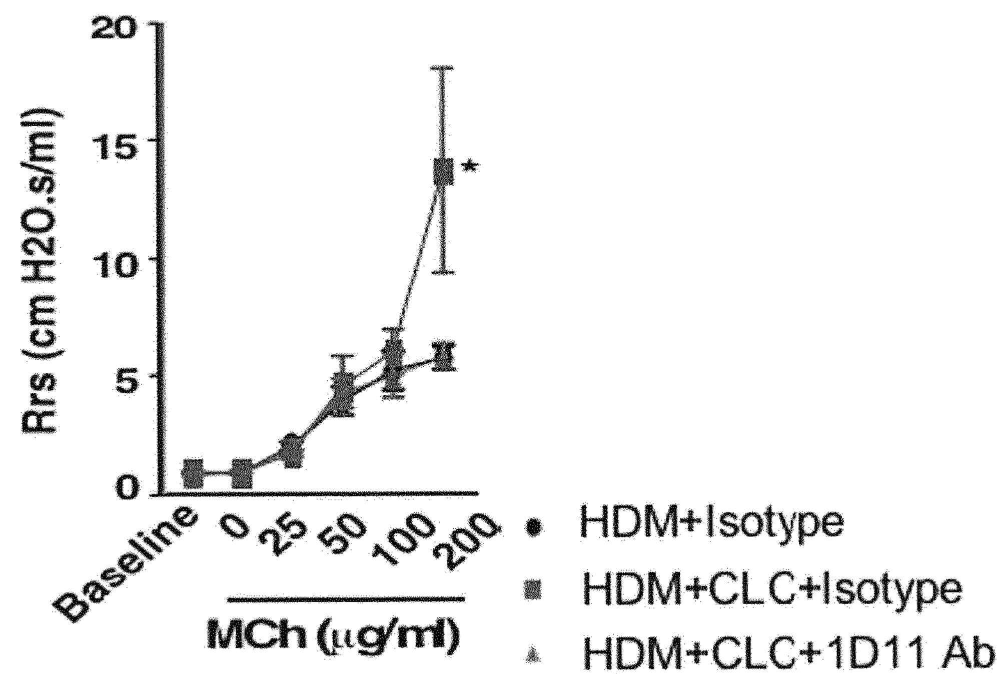


Fig. 13

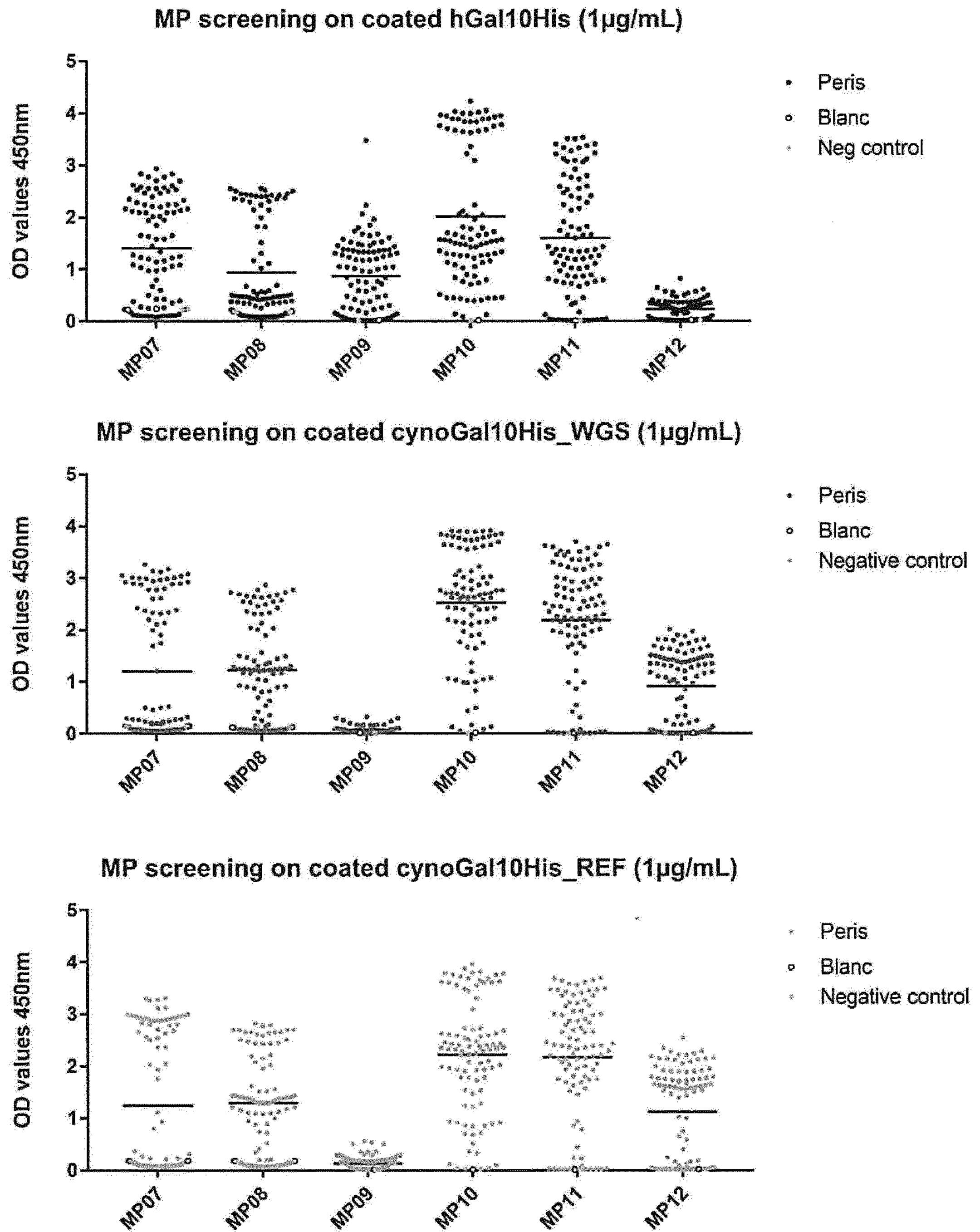


Fig. 14

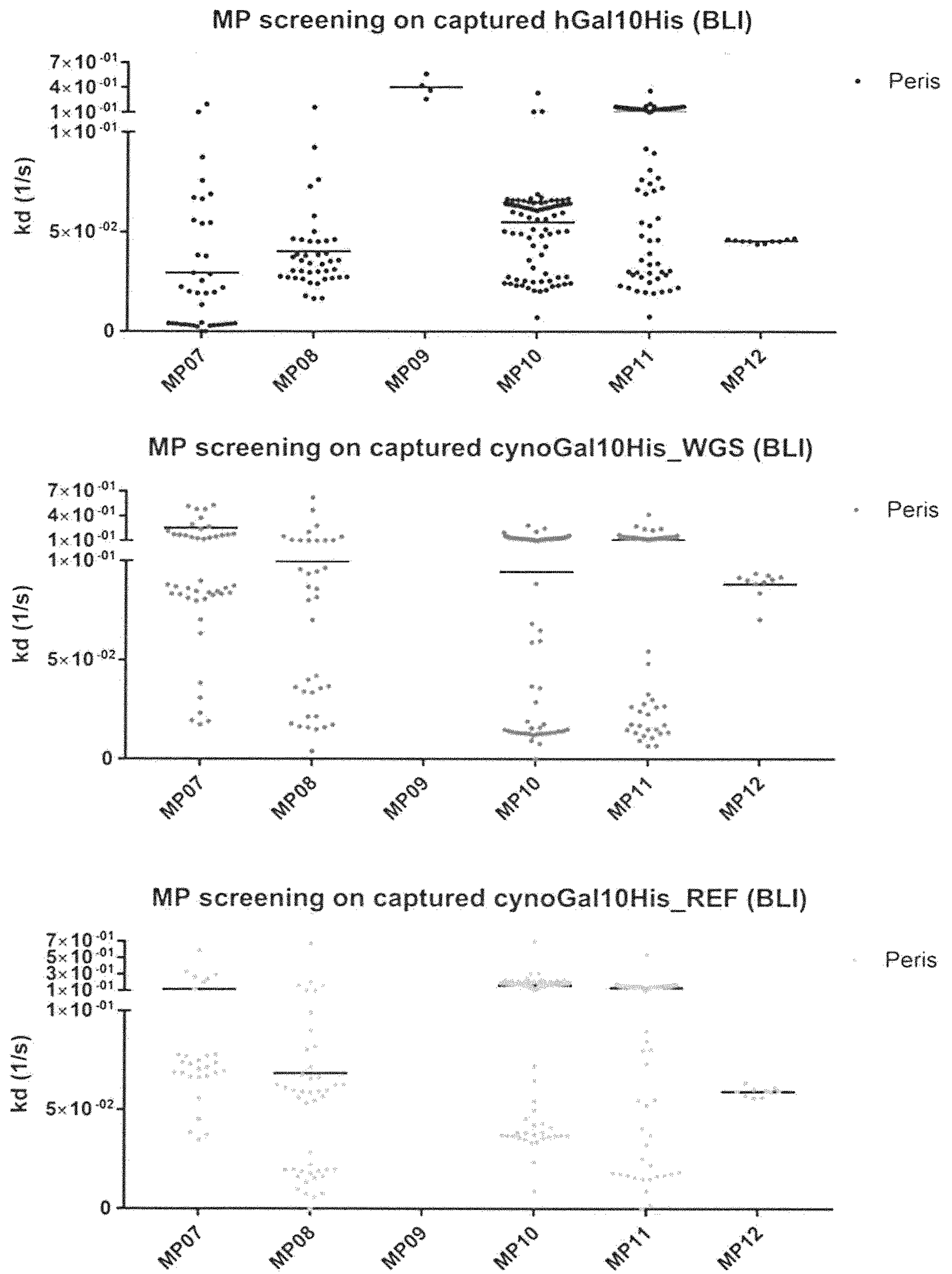


Fig. 15

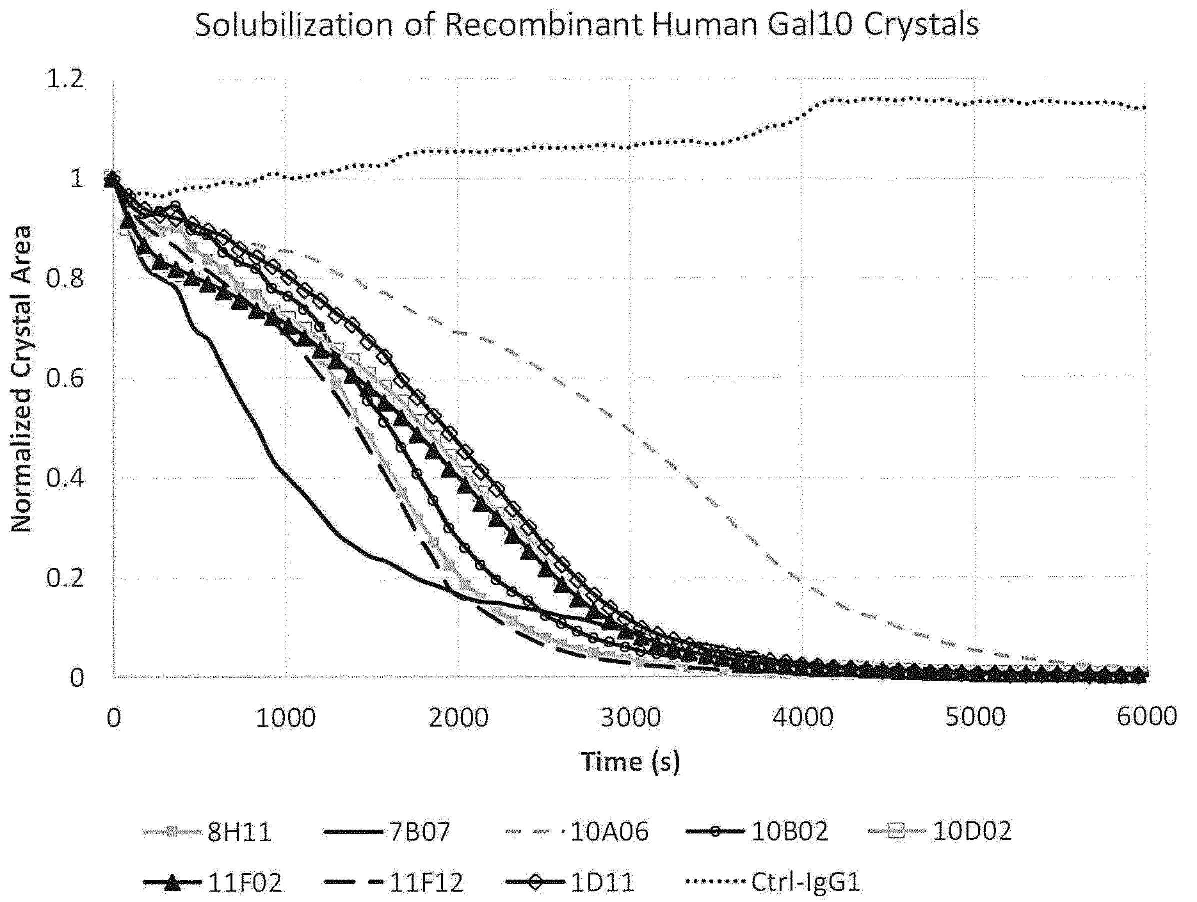
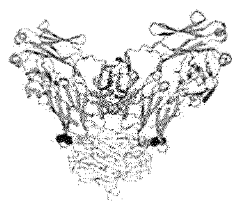


Fig. 16



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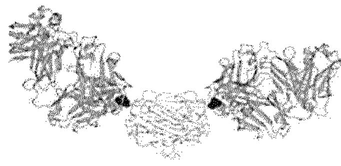

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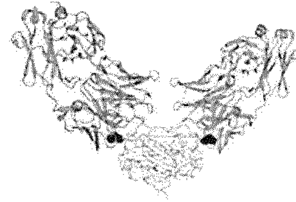

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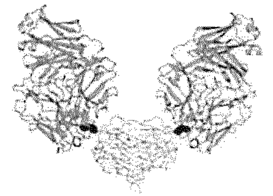

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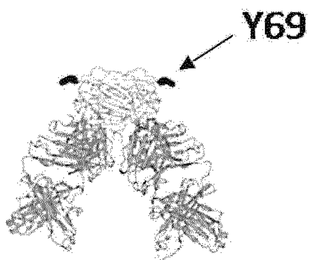

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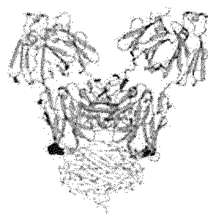

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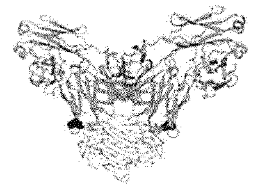

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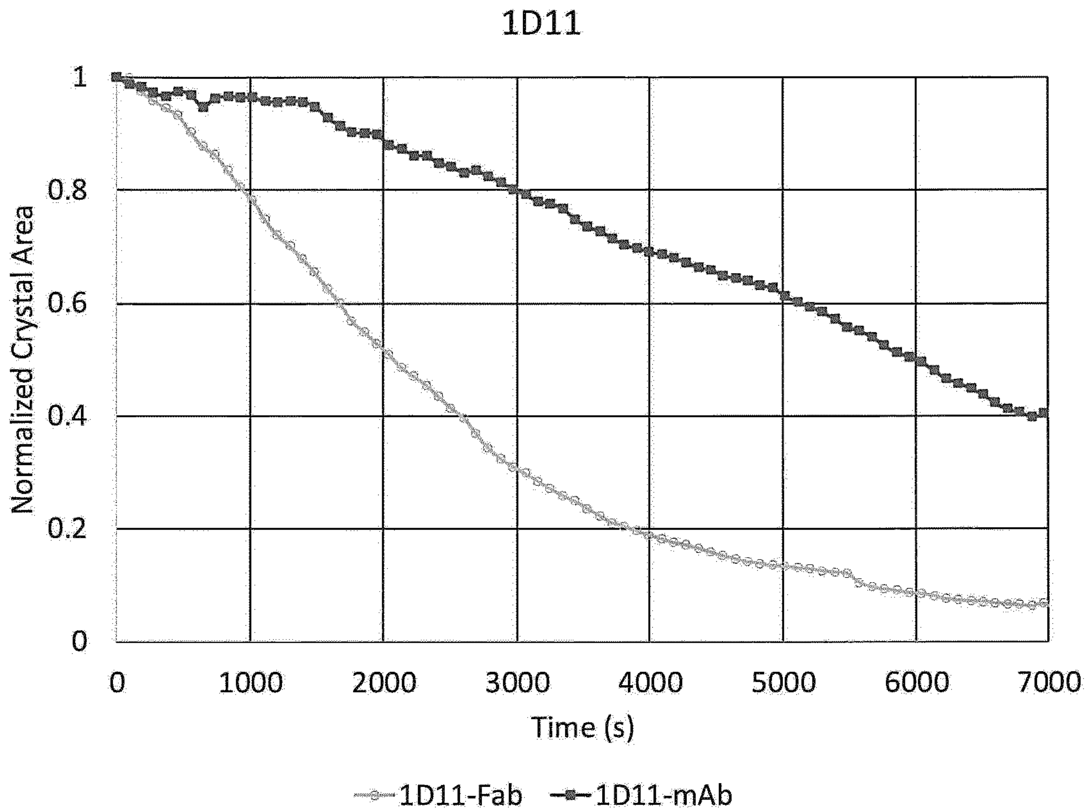
Fig. 17



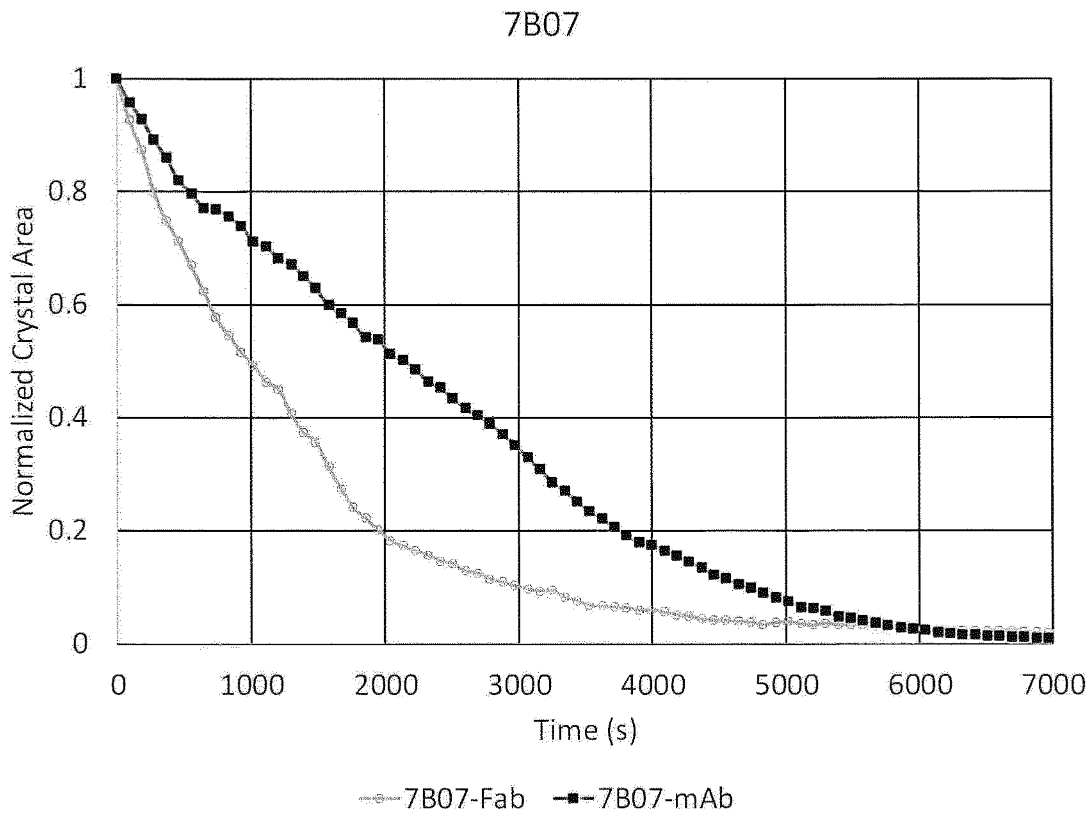
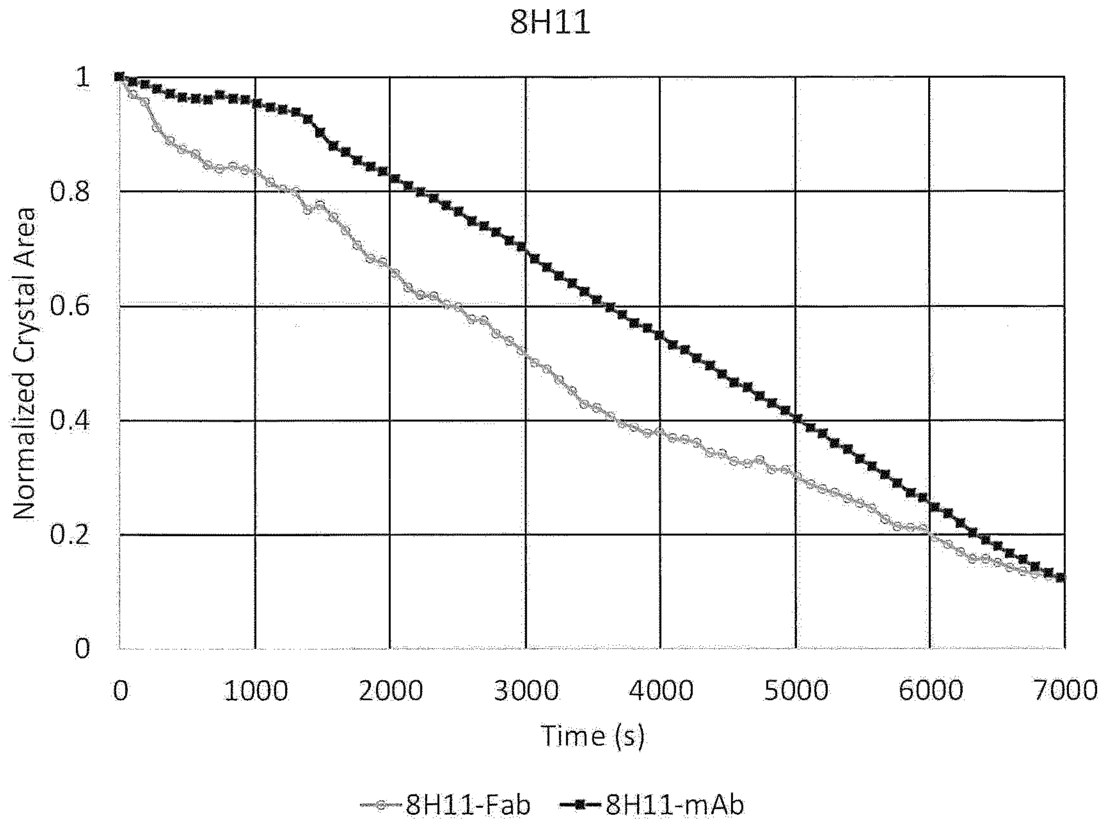


Fig. 17 continued



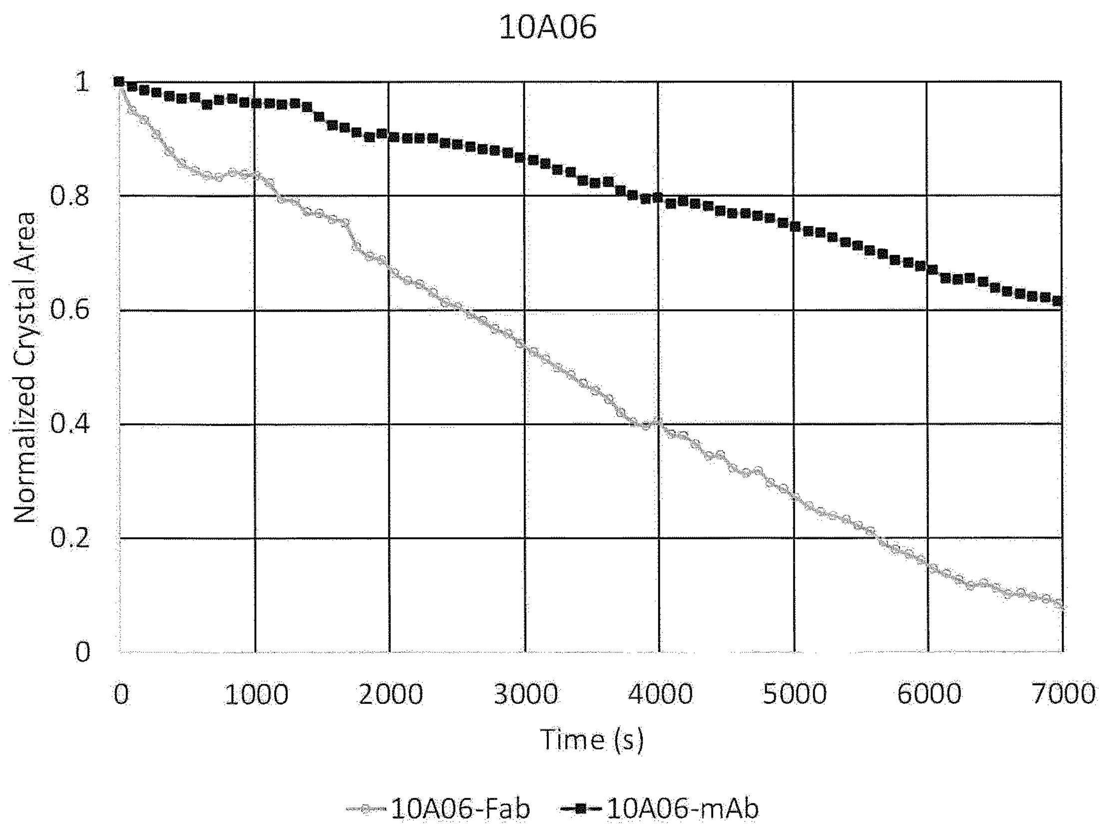
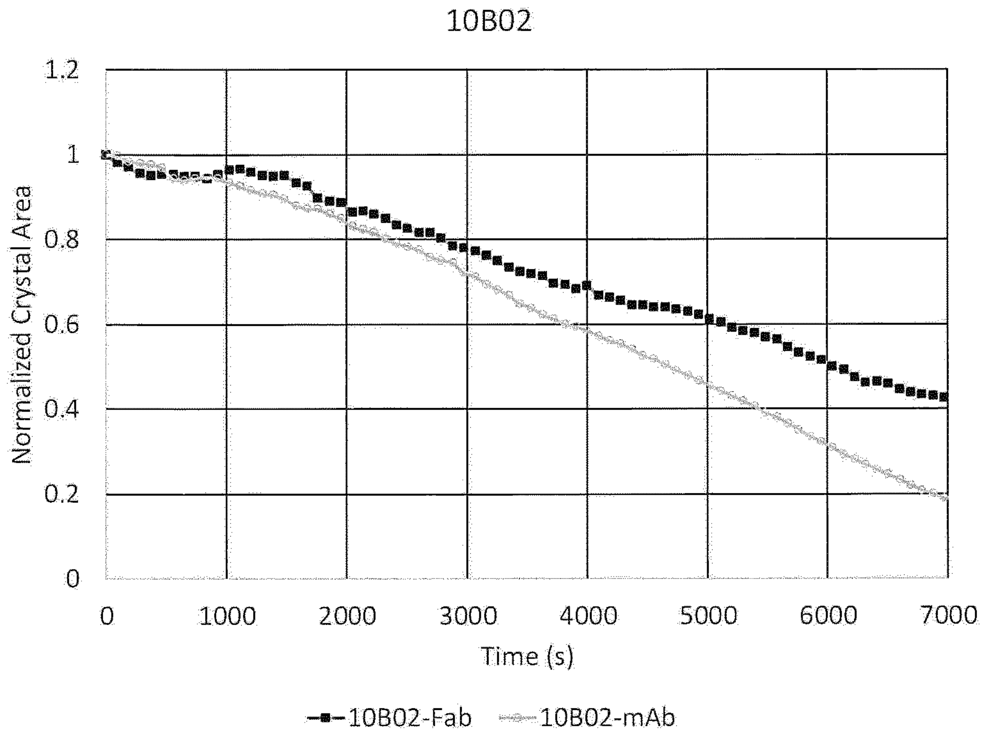


Fig. 17 continued



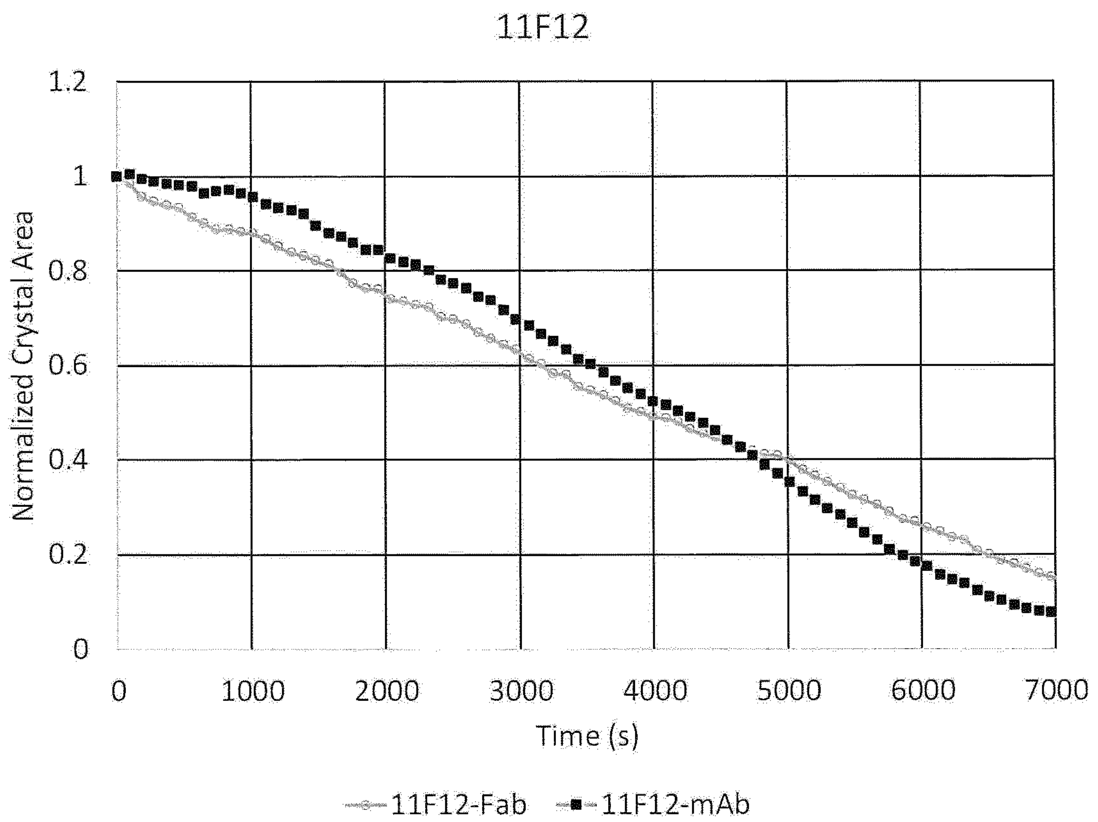
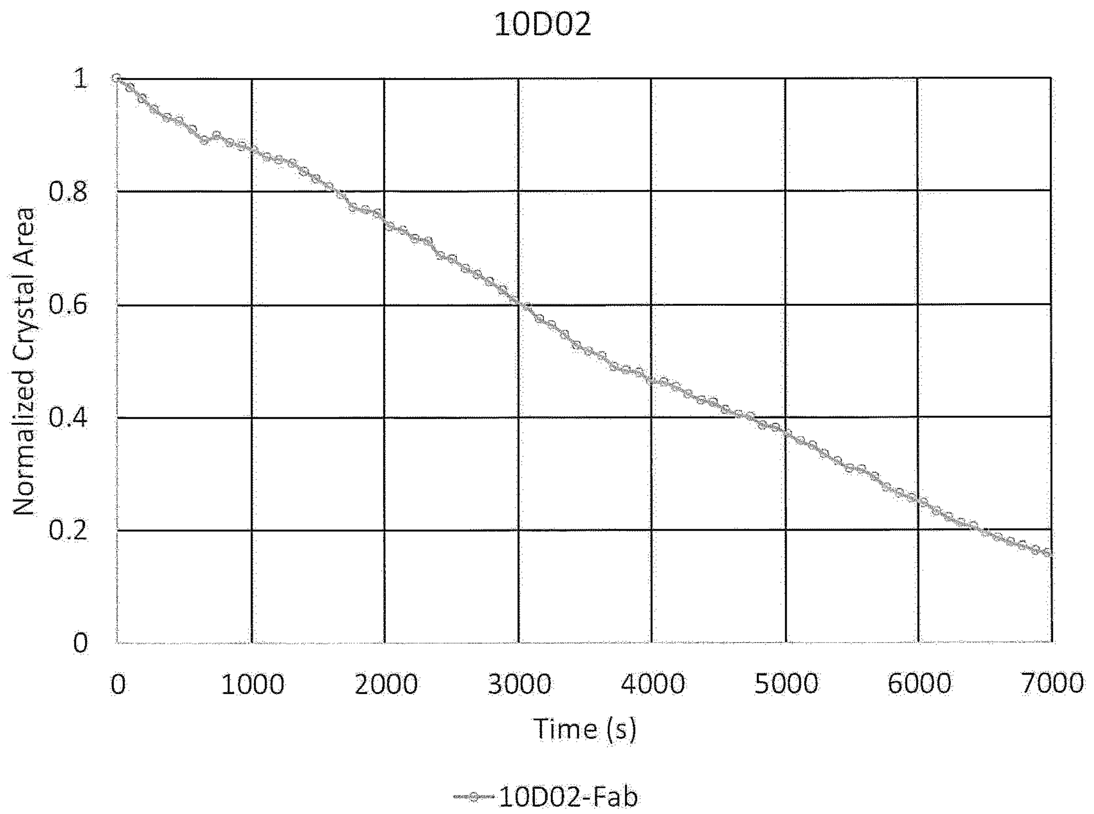


Fig. 17 continued



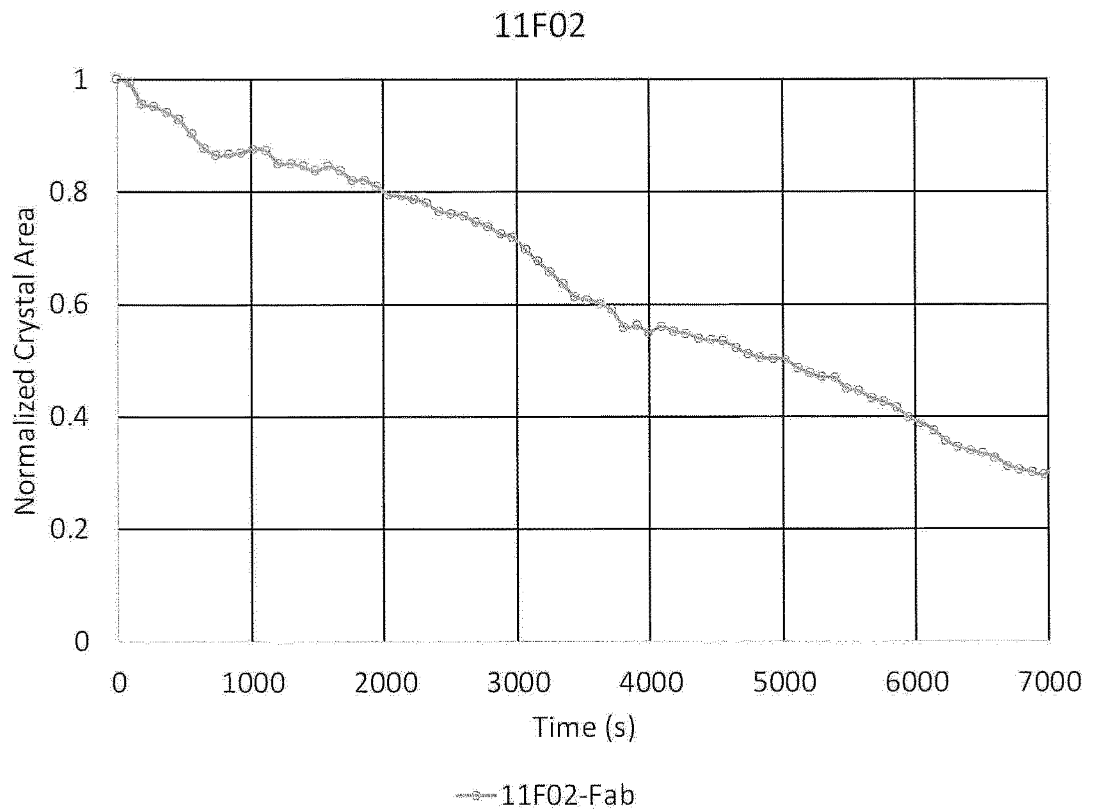


Fig.18

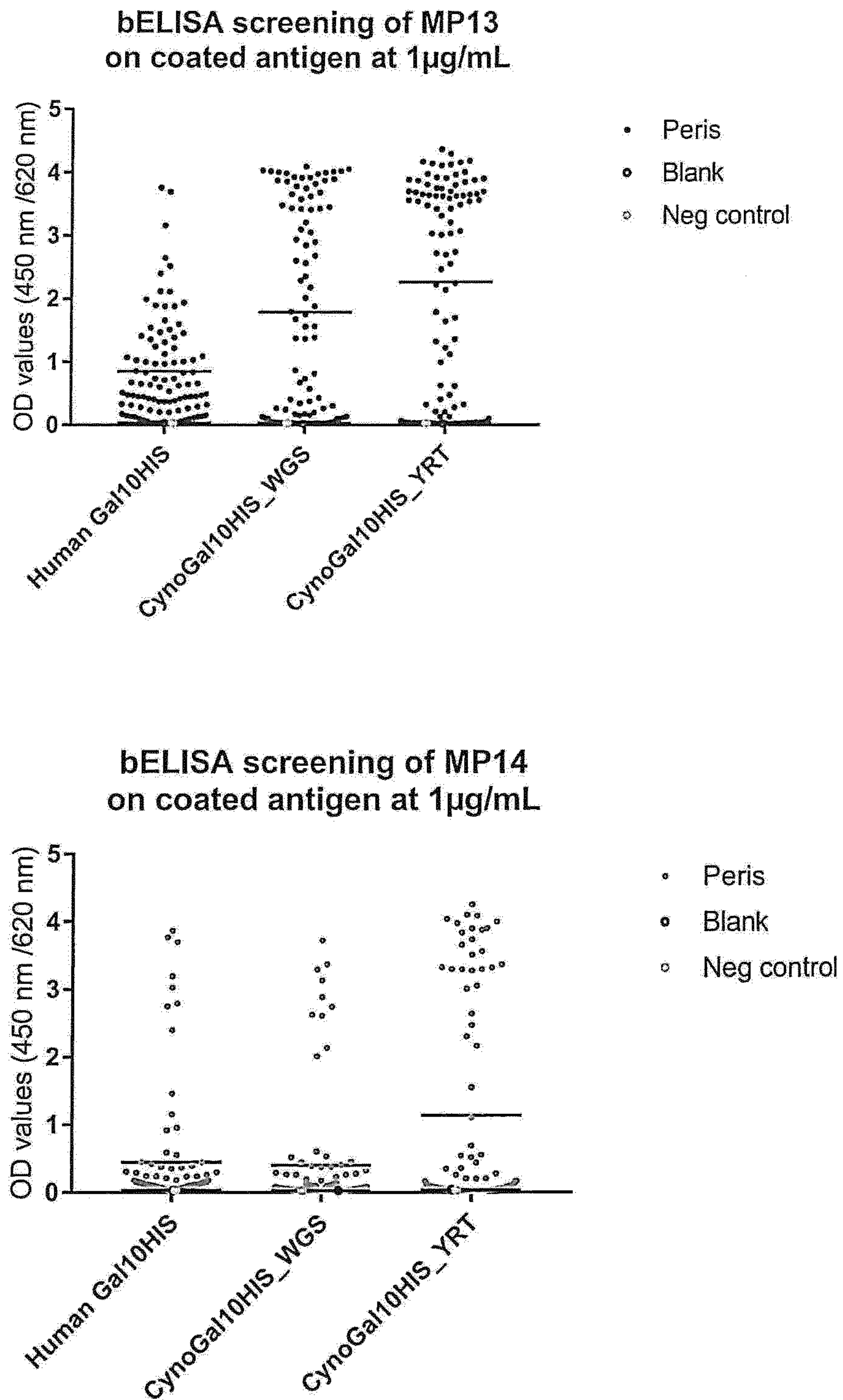
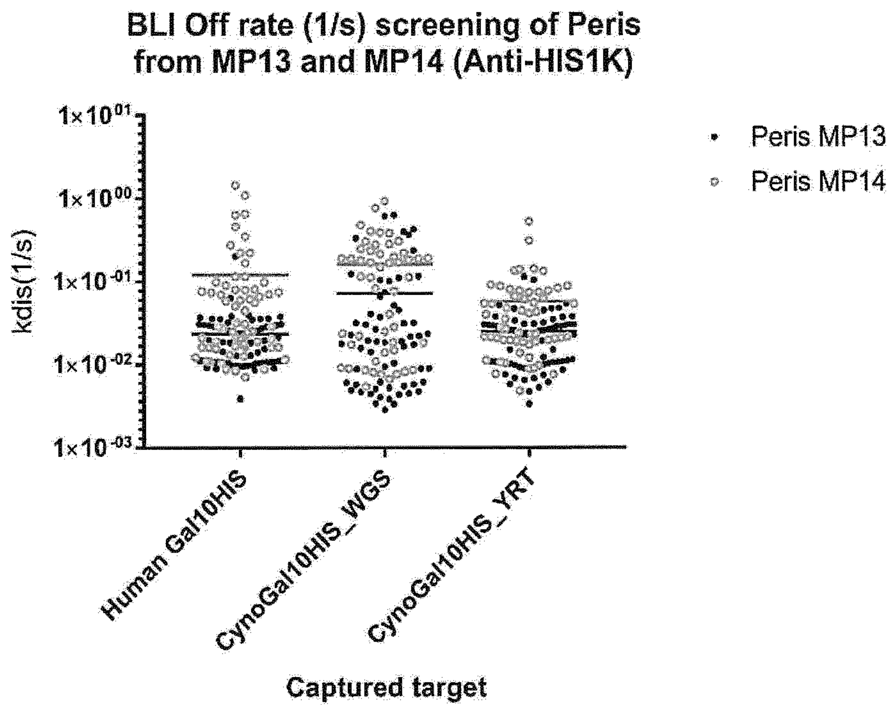


Fig. 19



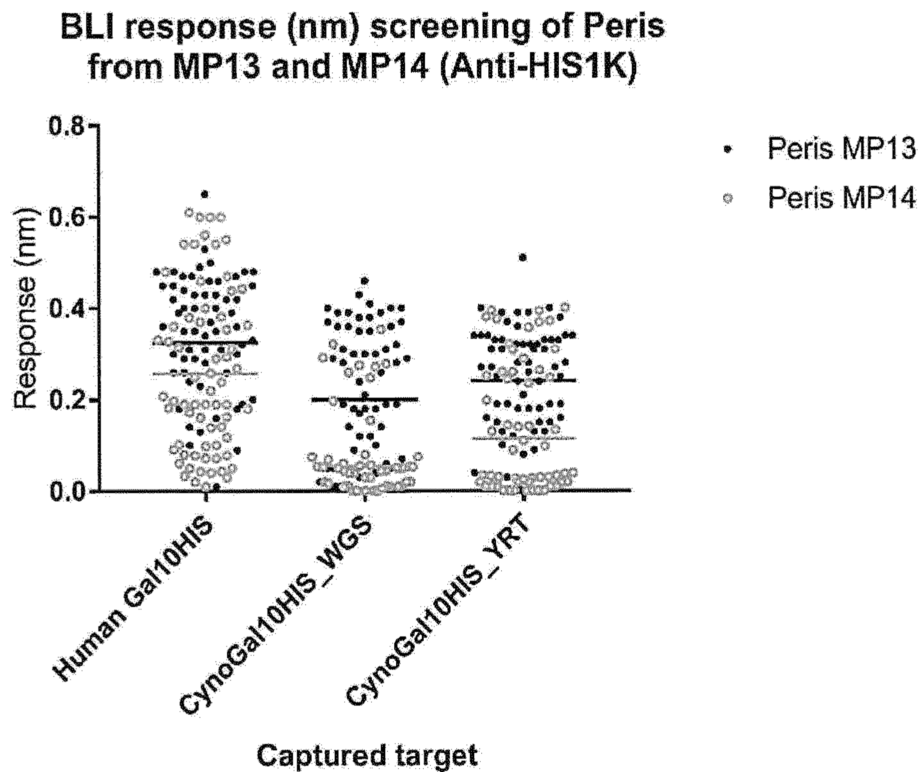
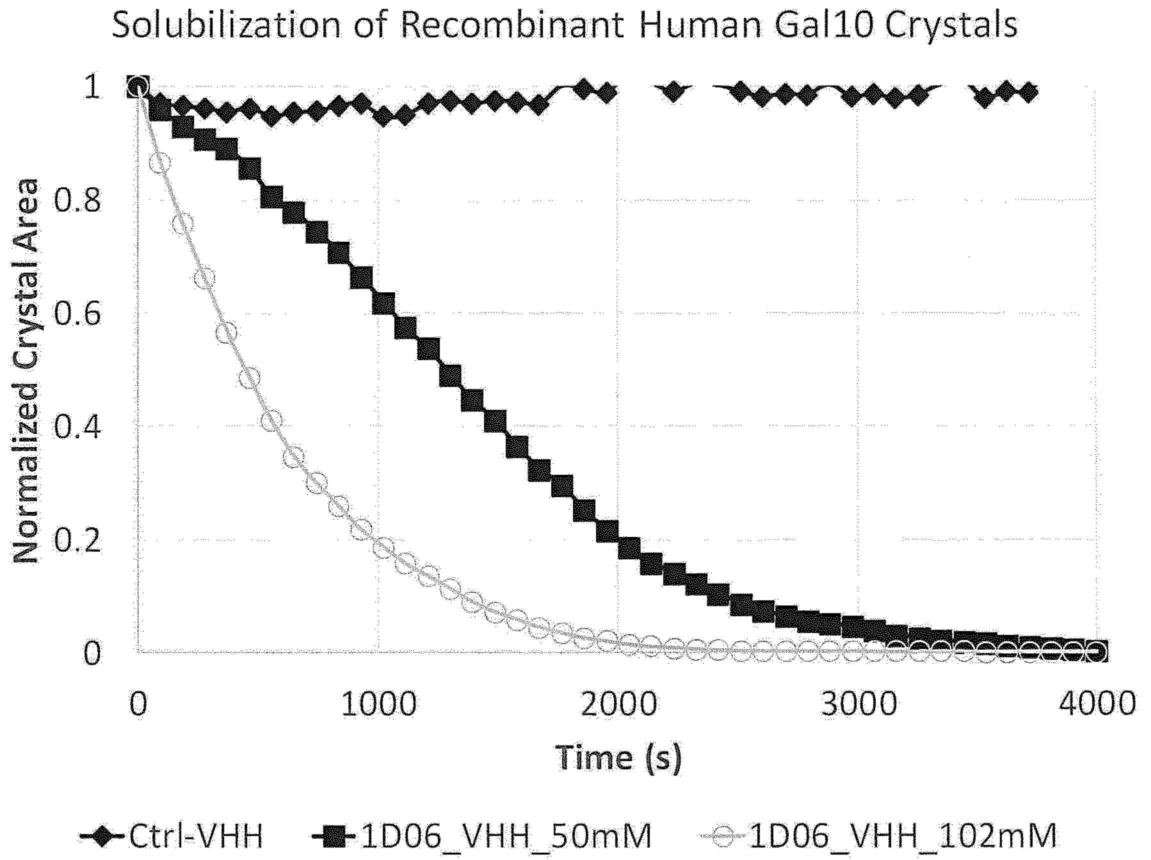


Fig. 20



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/059570

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P11/00
ADD. A61K39/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

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

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

EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STEVEN J. ACKERMAN ET AL: "Charcot-Leyden Crystal Protein (Galectin-10) Is Not a Dual Function Galectin with Lysophospholipase Activity but Binds a Lysophospholipase Inhibitor in a Novel Structural Fashion", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 277, no. 17, 7 February 2002 (2002-02-07), pages 14859-14868, XP55594773, US ISSN: 0021-9258, DOI: 10.1074/jbc.M200221200 abstract -----	1-58
X	CN 106 645 752 A (XIANGYA HOSPITAL CENTRAL SOUTH UNIV) 10 May 2017 (2017-05-10) the whole document -----	1-58

☐ Further documents are listed in the continuation of Box C.

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17 June 2019	01/07/2019

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/059570

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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