

ANTIBODIES RECOGNIZING SORTILIN

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of US 63/043,481 filed June 24, 2020, which is incorporated by reference in its entirety for all purposes.

REFERENCE TO A SEQUENCE LISTING

[0002] The Sequence Listing written in file 561308SEQLIST.txt is 353,813 bytes, was created on June 22, 2021, and is hereby incorporated by reference.

BACKGROUND OF THE INVENTION

[0003] Frontotemporal dementia (FTD) is an early-onset dementia affecting 50,000-60,000 individuals in the United States. Reductions in brain levels of progranulin (PGRN) are associated with genetic forms of frontotemporal dementia (FTD). Heterozygous loss-of-function mutations in the human progranulin gene cause FTD by a mechanism of haploinsufficiency (Baker, M. et al. Nature 2006;442:916-9; Cruts, M. et al. Nature 2006, 442,: 920-924). Genetic forms of FTD with a missing functional copy of the Progranulin gene (FTD-GRN) and ~50% reduction on progranulin levels represent approximately 10% of all FTD population. Mutations that moderately reduce progranulin levels increase the risk for Alzheimer's disease and Parkinson's disease (Sheng, J. et al., Gene; 2014, 542(2):141-5; Mendsaikh, A., et al., Cells: 2019, 8(3), 230). Progranulin is a neurotrophic/ anti-inflammatory factor, and deficiencies may disrupt homeostasis between microglia and neurons and promote neurodegeneration. Progranulin may also play a role in regulating the formation and function of the lysosome.

[0004] Sortilin (SORT1) has been identified as a modifier of progranulin levels by mediating its endocytosis (Carrasquillo, M.M. et al. Am J Hum Genet 2010;87(6):890-7; Hu, F. et al. Neuron 2010;68(4):654-67). Targeted disruption of the sortilin-progranulin interaction may increase levels of extracellular progranulin, potentially reversing disease phenotype in FTD-GRN patients. Modulation of progranulin levels has been reported with an antibody targeting the progranulin receptor sortilin (SORT1) in healthy humans. For instance, Alector's

AL001 completed phase 1 and is currently in phase 2 (See, <https://investors.alector.com/static-files/7418b689-c5b7-43ac-a16a-3c64e1a14e80>). Increased progranulin in plasma and CSF relative to baseline has been reported in healthy volunteers administered an anti-sortilin antibody. Sortilin is also a receptor/transporter for other factors (e.g. neurotensin).

BRIEF SUMMARY OF THE CLAIMED INVENTION

[0005] In one aspect, the invention provides an isolated monoclonal antibody that competes for binding to human sortilin with antibody 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. Some antibodies bind to the same epitope on human sortilin as antibody 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2.

[0006] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 5E20, wherein 5E20 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:4 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:10. For example, the antibody can be a humanized antibody.

[0007] In some antibodies, the CDRs are of a definition selected from the group of Kabat, Chothia, Kabat/Chothia Composite, AbM and Contact. In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat/Chothia Composite heavy chain CDRs of 5E20 (SEQ ID NOS:5-7) and the humanized mature light chain variable region comprises the three Kabat/Chothia Composite light chain CDRs of 5E20 (SEQ ID NOS:11-13). In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 5E20 (SEQ ID NO:14, SEQ ID NO:6, and SEQ ID NO:7) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 5E20 (SEQ ID NOS:11-13). In some antibodies, the humanized mature heavy chain variable region comprises the three Chothia heavy chain CDRs of 5E20 (SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:7) and the humanized mature light chain variable region comprises the three Chothia light chain CDRs of 5E20 (SEQ ID NOS:11-13). In some antibodies, the humanized mature heavy chain variable region comprises the three AbM heavy chain CDRs of 5E20 (SEQ ID NO:5, SEQ ID NO:17, and SEQ ID NO:7) and the humanized mature light chain variable

region comprises the three AbM light chain CDRs of 5E20 (SEQ ID NOS:11-13). In some antibodies, the humanized mature heavy chain variable region comprises the three Contact heavy chain CDRs of 5E20 (SEQ ID NOS:18-20) and the humanized mature light chain variable region comprises the three Contact light chain CDRs of 5E20 (SEQ ID NOS:21-23).

[0008] Some antibodies comprise a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:163-169, and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:173-176.

[0009] In some antibodies, at least one of the following positions in the VH region is occupied by the amino acid as specified: H5 is occupied by L or V, H40 is occupied by A or T, H42 is occupied by G or D, H44 is occupied by G or R, H49 is occupied by A, H77 is occupied by T or S, H83 is occupied by R or K, H93 is occupied by S, H94 is occupied by R.

[0010] In some antibodies, positions H49, H93, and H94 in the VH region are occupied by A, S, and R, respectively. In some antibodies, positions H5, H49, H77, H93, and H94 in the VH region are occupied by V, A, S, S, and R, respectively. In some antibodies, positions H5, H44, H49, H77, H93, and H94 in the VH region are occupied by V, R, A, S, S, and R, respectively. In some antibodies, positions H5, H42, H44, H49, H77, H93, and H94 in the VH region are occupied by V, D, R, A, S, S, and R, respectively. In some antibodies, positions H5, H42, H44, H49, H77, H83, H93, and H94 in the VH region are occupied by V, D, R, A, S, K, S, and R, respectively. In some antibodies, positions H5, H40, H44, H49, H77, H93, and H94 in the VH region are occupied by V, T, R, A, S, S, and R, respectively. In some antibodies, positions H5, H40, H42, H44, H49, H77, H93, and H94 in the VH region are occupied by V, T, D, R, A, S, S, and R, respectively.

[0011] In some antibodies, at least one of the following positions in the VL region is occupied by the amino acid as specified: L11 is L or V, L36 is L, L44 is F, L46 is G, L69 is A, L85 is T or D, L87 is F, L100 is G or Q, L106 is I or K. In some antibodies, positions L36, L44, L46, L69, and L87 in the VL region are occupied by L, F, G, A, and F, respectively. In some antibodies, positions L11, L36, L44, L46, L69, and L87 in the VL region are occupied by V, L, F, G, A, and F, respectively. In some antibodies, positions L11, L36, L44, L46, L69, L87, L100, and L106 in

the VL region are occupied by V, L, F, G, A, F, Q, and K, respectively. In some antibodies, positions L11, L36, L44, L46, L69, L85, L87, L100, and L106 in the VL region are occupied by V, L, F, G, A, D, F, Q, and K, respectively.

[0012] Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NO: 163-169 and a mature light chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NOS:173-176. Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:163-169 and a mature light chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:173-176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of any one of SEQ ID NOS:163-169, and the mature light chain variable region has an amino acid sequence of any one of SEQ ID NOS:173-176.

[0013] In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:163 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:164 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:165 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:166 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:167 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:168 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

[0014] In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:169 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:163 and the mature light chain variable region has an amino acid

sequence of SEQ ID NO:176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO: 164 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:165 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:166 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:167 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:168 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:169 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

[0017] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 8H24, wherein 8H24 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:28 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:34. For example, the antibody can be a humanized antibody.

[0018] In some antibodies, the CDRs are of a definition selected from the group of Kabat, Chothia, Kabat/Chothia Composite, AbM and Contact. In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat/Chothia Composite heavy chain CDRs of 8H24 (SEQ ID NOS:29-31) and the humanized mature light chain variable region comprises the three Kabat/Chothia Composite light chain CDRs of 8H24 (SEQ ID NOS:35-37). In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 8H24 (SEQ ID NO:38, SEQ ID NO:30, and SEQ ID NO:31) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 8H24 (SEQ ID NOS:35-37). In some antibodies, the humanized mature heavy chain variable region comprises the three Chothia heavy chain CDRs of 8H24 (SEQ ID NO:39, SEQ ID NO:40,

and SEQ ID NO:31) and the humanized mature light chain variable region comprises the three Chothia light chain CDRs of 8H24 (SEQ ID NOS:35-37). In some antibodies, the humanized mature heavy chain variable region comprises the three AbM heavy chain CDRs of 8H24 (SEQ ID NO:29, SEQ ID NO:41, and SEQ ID NO:31) and the humanized mature light chain variable region comprises the three AbM light chain CDRs of 8H24 (SEQ ID NOS:35-37). In some antibodies, the humanized mature heavy chain variable region comprises the three Contact heavy chain CDRs of 8H24 (SEQ ID NOS:42-44) and the humanized mature light chain variable region comprises the three Contact light chain CDRs of 8H24 (SEQ ID NOS:45-47).

[0019] Some antibodies comprise a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:180-181, and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:185-186.

[0020] In some antibodies, at least one of the following positions in the VH region is occupied by the amino acid as specified: H2 is occupied by A, H12 is occupied by K or V, H48 is occupied by I, H67 is occupied by A, H71 is occupied by V, H91 is occupied by F, H108 is occupied by T.

[0021] In some antibodies, positions H2, H48, H67, H71, H91, and H108 in the VH region are occupied by A, I, A, V, F, and T, respectively. In some antibodies, H2, H12, H48, H67, H71, H91, and H108 in the VH region are occupied by A, V, I, A, V, F, and T, respectively. In some antibodies, at least one of the following positions in the VL region is occupied by the amino acid as specified: L2 is V, L9 is L or S, L74 is K or T.

[0022] In some antibodies, position L2 in the VL region is occupied by V. In some antibodies, positions L2, L9, and L74 in the VL region are occupied by V, S, and T, respectively.

[0023] Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NO: 180-181 and a mature light chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NOS:185-186. Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:180-181 and a mature light chain variable

region having an amino acid sequence at least 98% identical to SEQ ID NOS:185-186. In some antibodies, the mature heavy chain variable region has an amino acid sequence of any one of SEQ ID NOS:180-181, and the mature light chain variable region has an amino acid sequence of any one of SEQ ID NOS:185-186.

[0024] In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:180 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:185. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:180 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:186. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:181 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:185. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:181 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:186.

[0025] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 11M14, wherein 11M14 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:52 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:58), except that position L54 can be L, G, or I. For example, the antibody can be a humanized antibody.

[0026] In some antibodies, the CDRs are of a definition selected from the group of Kabat, Chothia, Kabat/Chothia Composite, AbM and Contact. In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat/Chothia Composite heavy chain CDRs of 11M14 (SEQ ID NOS:53-55) and the humanized mature light chain variable region comprises the three Kabat/Chothia Composite light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I. In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 11M14 (SEQ ID NO:62, SEQ ID NO:54, and SEQ ID NO:55) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I. In some antibodies, the humanized mature heavy chain variable

region comprises the three Chothia heavy chain CDRs of 11M14 (SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:55) and the humanized mature light chain variable region comprises the three Chothia light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I. In some antibodies, the humanized mature heavy chain variable region comprises the three AbM heavy chain CDRs of 11M14 (SEQ ID NO:53, SEQ ID NO:65, and SEQ ID NO:55) and the humanized mature light chain variable region comprises the three AbM light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I. In some antibodies, the humanized mature heavy chain variable region comprises the three Contact heavy chain CDRs of 11M14 (SEQ ID NOS:66-68) and the humanized mature light chain variable region comprises the three Contact light chain CDRs of 11M14 (SEQ ID NOS:69-71), except that position L54 can be L, G, or I.

[0027] Some antibodies comprise a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:190-192, and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:196-199.

[0028] In some antibodies, at least one of the following positions in the VH region is occupied by the amino acid as specified: H49 is occupied by A, H80 is occupied by L or G, H82c is occupied by L or G. In some antibodies, position H49 in the VH region is occupied by A. In some antibodies, positions H49 and H82c in the VH region are occupied by A and G, respectively. In some antibodies, positions H49 and H80 in the VH region are occupied by A and G, respectively.

[0029] In some antibodies, at least one of the following positions in the VL region is occupied by the amino acid as specified: L43 is A or S, L48 is V, L54 is L, G, or I, L71 is Y, L76 is N or S. In some antibodies, positions L48 and L71 in the VL region are occupied by V and Y respectively. In some antibodies, positions L43, L48, L71, and L76 in the VL region are occupied by S, V, Y, and S, respectively. In some antibodies, positions L43, L48, L54, L71, and L76 in the VL region are occupied by S, V, G, Y, and S, respectively. In some antibodies, positions L43, L48, L54, L71, and L76 in the VL region are occupied by S, V, I, Y, and S, respectively.

[0030] Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NO: 190-192 and a mature light chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NOS:196-199. Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:190-192 and a mature light chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:196-199. In some antibodies, the mature heavy chain variable region has an amino acid sequence of any one of SEQ ID NOS:190-192, and the mature light chain variable region has an amino acid sequence of any one of SEQ ID NOS:196-199.

[0031] In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:196. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:197. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:198. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:199. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:196. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:197.

[0032] In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:198. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:199. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:196. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an

amino acid sequence of SEQ ID NO:197. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:198. In some antibodies, the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:199.

[0033] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 5M13, wherein 5M13 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:78 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:84. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:79-81) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:85-87).

[0034] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 2F18, wherein 2F18 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:90 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:96. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:91-93) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:97-99).

[0035] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 2P22, wherein 2P22 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:102 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:108. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:103-105) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:109-111).

[0036] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 6B15, wherein 6B15 is a mouse antibody characterized by a heavy chain variable region having

an amino acid sequence comprising SEQ ID NO:114 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:120. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:115-117) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:121-123).

[0037] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 2C14, wherein 2C14 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:126 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:132. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:127-129) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:133-135).

[0038] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 9N18, wherein 9N18 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:138 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:144. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:139-141) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:145-147).

[0039] In another aspect, the invention provides an antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 4N2, wherein 4N2 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:150 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:156. In some antibodies, the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:151-153) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:157-159).

[0040] For example, the antibody can be a humanized antibody.

[0041] The antibody can be an intact antibody or a binding fragment. Some of the antibodies have a human IgG1 isotype, while others may have a human IgG2 or IgG4 isotype. Some

antibodies have the mature light chain variable region fused to a light chain constant region and the mature heavy chain variable region fused to a heavy chain constant region. The heavy chain constant region of some antibodies is a mutant form of a natural human heavy chain constant region which has reduced binding to a Fcγ receptor relative to the natural human heavy chain constant region.

[0042] The heavy chain constant region of some antibodies is a mutant form of a natural human heavy chain constant region which has enhanced binding to a neonatal Fcγ receptor relative to the natural human heavy chain constant region.

[0043] Some antibodies comprise a heavy chain of SEQ ID NO:244 and a light chain of SEQ ID NO:245. Some antibodies comprise a heavy chain of SEQ ID NO:246 and a light chain of SEQ ID NO:247. Some antibodies comprise a heavy chain of SEQ ID NO:248 and a light chain of SEQ ID NO:249. Some antibodies comprise a heavy chain of SEQ ID NO:250 and a light chain of SEQ ID NO:245. Some antibodies comprise a heavy chain of SEQ ID NO:251 and a light chain of SEQ ID NO:247. Some antibodies comprise a heavy chain of SEQ ID NO:252 and a light chain of SEQ ID NO:249.

[0044] In another aspect, the invention provides a pharmaceutical composition comprising any of the antibodies disclosed herein and a pharmaceutically-acceptable carrier. In another aspect, the invention provides a nucleic acid encoding the heavy chain and/or light chain of any of the antibodies disclosed herein.

[0045] In yet another aspect, the invention provides methods of humanizing any non-human antibody described herein, for example mouse antibody 5E20, wherein 5E20 is characterized by a mature heavy chain variable region of SEQ ID NO:4 and a mature light chain variable region of SEQ ID NO:10; mouse antibody 8H24, wherein 8H24 is characterized by a mature heavy chain variable region of SEQ ID NO:28 and a mature light chain variable region of SEQ ID NO:34; mouse antibody 11M14, wherein 11M14 is characterized by a mature heavy chain variable region of SEQ ID NO:52 and a mature light chain variable region of SEQ ID NO:58; mouse antibody 5M13, wherein 5M13 is characterized by a mature heavy chain variable region of SEQ ID NO:78 and a mature light chain variable region of SEQ ID NO:84; mouse antibody 2F18, wherein 2F18 is characterized by a mature heavy chain variable region of SEQ ID NO:90

and a mature light chain variable region of SEQ ID NO:96; mouse antibody 2P22, wherein 2P22 is characterized by a mature heavy chain variable region of SEQ ID NO:102 and a mature light chain variable region of SEQ ID NO:108; mouse antibody 6B15, wherein 6B15 is characterized by a mature heavy chain variable region of SEQ ID NO:114 and a mature light chain variable region of SEQ ID NO:120; mouse antibody 2C14, wherein 2C14 is characterized by a mature heavy chain variable region of SEQ ID NO:126 and a mature light chain variable region of SEQ ID NO:132; mouse antibody 9N18, wherein 9N18 is characterized by a mature heavy chain variable region of SEQ ID NO:138 and a mature light chain variable region of SEQ ID NO:144; or mouse antibody 4N2, wherein 4N2 is characterized by a mature heavy chain variable region of SEQ ID NO:150 and a mature light chain variable region of SEQ ID NO:156. Such methods can involve selecting one or more acceptor antibody sequences; identifying amino acid residues of the mouse antibody to be retained; synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and expressing the nucleic acids in a host cell to produce a humanized antibody.

[0046] Methods of producing antibodies, for example humanized, chimeric or veneered form of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2, are also provided. In such methods, cells transformed with nucleic acids encoding the heavy and light chains of the antibody are cultured so that the cells secrete the antibody. The antibody can then be purified from the cell culture media.

[0047] Cell lines producing any of the antibodies disclosed herein, for example a humanized, chimeric, or veneered form of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2, can be produced by introducing a vector encoding heavy and light chains of the antibody and a selectable marker into cells, propagating the cells under conditions to select for cells having increased copy number of the vector, isolating single cells from the selected cells; and banking cells cloned from a single cell selected based on yield of antibody.

[0048] Some cells can be propagated under selective conditions and screened for cell lines naturally expressing and secreting at least 100 mg/L/10⁶ cells/24 hours.

[0049] The invention also provides methods of increasing progranulin levels in a subject having or at risk of developing a disease or disorder associated with changes in progranulin levels, comprising administering to the subject an effective dose of an antibody disclosed herein, thereby increasing progranulin levels in the subject.

[0050] The invention also provides methods of treating or effecting prophylaxis of a disease or disorder associated with changes in progranulin levels in a subject, comprising administering an effective dose of an antibody disclosed herein, and thereby treating or effecting prophylaxis of the disease or disorder. Some methods further comprise detecting progranulin levels in a subject. Some methods further comprise monitoring progranulin levels in a subject.

[0051] In some methods, the disease or disorder associated with changes in progranulin levels is frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging. In some methods, the disease or disorder associated with changes in progranulin levels is frontotemporal dementia.

[0052] The invention also provides methods of detecting sortilin in a subject having or at risk of a disease associated with a disease or disorder associated with changes in progranulin levels, comprising administering to a subject an antibody disclosed herein, and detecting the antibody bound to sortilin in the subject.

[0053] In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to a peptide consisting of residues FTESFLT (SEQ ID NO:202). In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to a peptide consisting of residues ESFL (SEQ ID NO:203). In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to human sortilin at an epitope within a motif of the formula E(S/Q/D)FL (SEQ ID NO:206). In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to a peptide consisting of residues DGCILGYKEQFL (SEQ ID NO:204). In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to a peptide consisting of residues PSICLCSLEDL (SEQ ID NO:205).

[0051] In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to a peptide consisting of residues RTEFGMAIGP (SEQ ID NO:213). In another aspect, the invention provides an isolated monoclonal antibody that specifically binds to a peptide consisting of residues WGFTEFLTS (SEQ ID NO:214).

[0051] In another aspect, the invention provides an isolated monoclonal antibody that specifically binds an epitope defined by amino acid residues D74, R76, F97, K110, Y535, L560, and E557 of SEQ ID NO:215. In another aspect, the invention provides an isolated monoclonal antibody that specifically binds an epitope defined by amino acid residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of SEQ ID NO:215. In another aspect, the invention provides an isolated monoclonal antibody that specifically binds an epitope defined by amino acid residues E557, S558, F559, L560, P510, and Y535 of SEQ ID NO:215.

[0054] The invention also provides methods of treating or effecting prophylaxis of a disease or disorder associated with changes in progranulin levels in a subject comprising administering an immunogen comprising a sortilin peptide of up to 20 contiguous amino acids of SEQ ID NO:1 to which antibody 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 specifically binds, wherein the peptide induces formation of antibodies specifically binding to sortilin in the subject.

BRIEF DESCRIPTION OF THE DRAWINGS

[0055] Figure 1 depicts an alignment of heavy chain variable regions of the mouse 5E20 antibody (5E20VH; SEQ ID NO:4) and humanized versions of the 5E20 antibody (hu5E20VHv1, hu5E20VHv2, hu5E20VHv3, hu5E20VHv4, hu5E20VHv5, hu5E20VHv6, and hu5E20VHv7) with human germline heavy chain variable region sequence IGHV3-21*01 (SEQ ID NO:162), with human acceptor heavy chain variable region sequence AEX29086-VH_huFrwk (AEX29086 VH; SEQ ID NO:161). hu5E20VHv1 (5E20VHv1) is SEQ ID NO:163, hu5E20VHv2 (5E20VHv2) is SEQ ID NO:164, hu5E20VHv3 (5E20VHv3) is SEQ ID NO:165, hu5E20VHv4 (5E20VHv4) is SEQ ID NO:166, hu5E20VHv5 (5E20VHv5) is SEQ ID NO:167, hu5E20VHv6 (5E20VHv6) is SEQ ID NO:168, and hu5E20VHv7 (5E20VHv7) is SEQ

ID NO:169. The CDRs of mouse 5E20 VH, as defined by Kabat/Chothia Composite, are in boldface.

[0056] Figure 2 depicts an alignment of light chain variable regions of the mouse 5E20 antibody (5E20VL, SEQ ID NO:10) and humanized versions of the 5E20 antibody (hu5E20VLv1, hu5E20VLv2, hu5E20VLv3, and hu5E20VLv4) with human germline light chain variable region sequence # IGKV1-12*01 (SEQ ID NO:172) and with human acceptor BAH04687-VL_huFrwk (BAH04687 VL; SEQ ID NO:171). hu5E20VLv1 (5D20VLv1) is SEQ ID NO:173, hu5E20VLv2 (5D20VLv2) is SEQ ID NO:174, hu5E20VLv3 (5E20VLv3) is SEQ ID NO:175, and hu5E20VLv4 (5E20VLv4) is SEQ ID NO:176. The CDRs of mouse 5E20 VL, as defined by Kabat/Chothia Composite, are in boldface.

[0057] Figure 3 depicts an alignment of heavy chain variable regions of the mouse 8H24 antibody (8H24_VH, SEQ ID NO:28) and humanized versions of the 8H24 antibody (hu8H24VHv1 and hu8H24VHv2) with human germline heavy chain variable region sequence IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179) and with human acceptor heavy chain variable region sequence AAC51714-VH_huFrwk (AAC51714VH Frwk, SEQ ID NO:178). hu8H24VHv1 (h8H24VLv1) is SEQ ID NO:180 and hu8H24VHv2 (h8H24VHv2) is SEQ ID NO:181. The CDRs of mouse 8H24 VH, as defined by Kabat/Chothia Composite, are in boldface.

[0058] Figure 4 depicts an alignment of light chain variable regions of the mouse 8H24 (8H24 VL) SEQ ID NO:34) and humanized versions of the 8H24 antibody (hu8H24VLv1 and hu8H24VLv2) with human germline light chain variable region sequence IGKV2-40*01 (IGKV2-40*01_IGKJ4*01, SEQ ID NO:184) and with human acceptor ABC66914-VL_huFrwk (ABC66914VL Frwk SEQ ID NO:183). hu8H24VLv1 (hH824VLv1) is SEQ ID NO:185 and hu8H24VLv2 (hH824VLv2) is SEQ ID NO:186. The CDRs of mouse 8H24 VL, as defined by Kabat/Chothia Composite, are in boldface.

[0059] Figure 5 depicts an alignment of heavy chain variable regions of the mouse 11M14 antibody (11M14_VH; SEQ ID NO:52) and humanized versions of the 11M14 antibody (hu11M14VHv1b, hu11M14VHv2b, and hu11M14VHv3b) with human germline heavy chain variable region sequence IGHV3-48*03 (SEQ ID NO:189) and with human acceptor heavy chain variable region sequence ACS96198-VH_huFrwk (ASC96198 VH hFrwk, SEQ ID NO:188)

hu11M14VHv1b (11M14VHv1b) is SEQ ID NO:190, hu11M14VHv2b (11M14VHv2b) is SEQ ID NO:191, and hu11M14VHv3b (11M14VHv3b) is SEQ ID NO:192. The CDRs of mouse 11M14 VH, as defined by Kabat/Chothia Composite, are in boldface.

[0060] Figure 6 depicts an alignment of light chain variable regions of the mouse 11M14 antibody (11M14 VL, SEQ ID NO:58) and humanized versions of the 11M14 antibody (hu11M14VLv1b, hu11M14VLv2b, hu11M14VLv3b, and hu11M14VLv4b,) with human germline light chain variable region sequence IGKV1-39*01 (SEQ ID NO:195) and with human acceptor CBZ39892-VL_huFrwk (CBZ39892VL hFrwk, SEQ ID NO:194). hu11M14VLv1b (11M14VLv1b) is SEQ ID NO:196, hu11M14VLv2b (11M14VLv2b) is SEQ ID NO:197, hu11M14VLv3b (11M14VLv3b) is SEQ ID NO:198, and hu11M14VLv4b (11M14VLv4b) is SEQ ID NO:199. The CDRs of mouse 11M14 VL, as defined by Kabat/Chothia Composite, are in boldface.

[0061] Figure 7 depicts inhibition of binding of human progranulin (in percentage PGRN binding) to human sortilin by ELISA by non-clonal mouse antibody supernatants.

[0062] Figure 8 depicts binding of selected purified monoclonal antibodies to sortilin (left graphs) and inhibition of progranulin binding to sortilin (in percentage PGRN blocking, right graphs) by ELISA. The right-hand table shows EC₅₀ (nM) of Ab binding to human sortilin-ECD and percentage blocking at 7.4 nM mAb for selected purified antibodies.

[0063] Figure 9 depicts results of a competition assay by Biacore to determine whether selected monoclonal antibodies block the binding of neurotensin to sortilin. The table summarizes the results of the assays for selected monoclonal antibodies. Examples of data are shown in the 3 graphs: no competition for 8H24, weak competition with 1M16, and competition with 4J22.

[0064] Figure 10 depicts results of binding of selected purified monoclonal antibodies to human sortilin overexpressed by HEK 293 cells vs parental HEK cell line, and to U251MG cells (human glioblastoma cells line) which endogenously express sortilin. The left-hand plots depict binding of monoclonal antibodies 6B15, 7B18, 10B6, and 10O16 in the HEK 293 cells assay (left column of plots) and in the U251MG cells assay (right column of plots). The right-hand table summarizes the results of the assays for selected monoclonal antibodies.

[0065] Figure 11 depicts inhibition of hu PGRN binding to human Sortilin-expressing HEK293 cells (in percentage PGRN blocking) by selected purified monoclonal antibodies.

[0066] Figure 12 depicts a dose response of inhibition of hu PGRN binding to cell surface human Sortilin-expressing HEK293 cells. The left-hand plots depict binding curves for antibodies with different efficacy. The right-hand table summarizes the results of the assay for selected monoclonal antibodies. (% maximum binding at 11 nM mAb)

[0067] Figure 13 depicts extracellular PGRN and cell surface sortilin levels after treatment of U251MG cells with selected purified monoclonal antibodies. The left-hand plot depicts the assay results as a correlation graph for selected monoclonal antibodies. The right-hand table summarizes the results of the assay for selected monoclonal antibodies.

[0068] Figure 14 depicts anti sortilin antibody hu8H24 H1L2 IgG1 LALA, hu5E20 H7L4 IgG1 LALA, or hu11M14 H1bL3b IgG1 LALA levels in plasma after 60 mg/kg dose and after 30 mg/kg dose in cynomolgus monkeys (pharmacokinetic studies). Anti-sortilin antibody levels in plasma after a 30mg/kg or 60mg/kg dose of Antibodies in cynomolgus monkeys. N=3 animals per Ab group. Animals received a 30 mg/kg dose followed 48 days later by a 60 mg/kg dose of anti-sortilin antibody.

[0069] Figure 15 depicts plasma PGRN levels after 60 mg/kg dose and after 30 mg/kg dose of hu8H24 H1L2 IgG1 LALA, hu5E20 H7L4 IgG1 LALA, or hu11M14 H1bL3b IgG1 LALA in non-human primates (pharmacodynamic studies). Fold PGRN levels in plasma after a single 30mg/kg or 60mg/kg dose of Antibodies in cynomolgus monkeys. N=3 animals per Ab group. Triangles denote animals which showed detectable levels of anti-drug antibodies.

[0070] Figure 16 depicts CSF PGRN levels after 60 mg/kg dose and after 30 mg/kg dose of hu8H24 H1L2 IgG1 LALA, hu5E20 H7L4 IgG1 LALA, or hu11M14 H1bL3b IgG1 LALA in cynomolgus monkeys (pharmacodynamic studies). Fold PGRN levels in CSF after a single 30mg/kg or 60mg/kg dose of Antibodies in cynomolgus monkeys. N=3 animals per Ab group. Triangles denote animals which showed the presence of anti-drug antibodies. No CSF samples were collected at Day 7 in animals treated with 30 mg/kg anti-sortilin antibodies.

[0071] Figure 17 depicts anti sortilin antibody levels in plasma after 4 weekly repeat 60mg/kg

doses of hu11M14 H1bL3b_IgG1_LALA or hu11M14 H1bL3b_IgG1_LALA_YTE in cynomolgus monkeys (pharmacokinetic studies). Mean $\pm$ SD Anti-sortilin antibody plasma levels with 4 weekly repeat doses of 60mg/kg of Ab. N=4.

[0072] Figure 18 depicts anti sortilin antibody levels in CSF after 4 weekly repeat 60mg/kg doses of hu11M14 H1bL3b_IgG1_LALA or hu11M14 H1bL3b_IgG1_LALA_YTE in cynomolgus monkeys (pharmacokinetic studies). Mean $\pm$ SD Anti-Sortilin Ab CSF levels with 4 weekly repeat doses of 60mg/kg of Ab. N=4 for hu11M14 H1bL3b_IgG1_LALA and N=3 for hu11M14 H1bL3b_IgG1_LALA_YTE.

[0073] Figure 19 depicts plasma progranulin levels in cynomolgus monkeys after 4 weekly repeat 60mg/kg doses of hu11M14 H1bL3b_IgG1_LALA or hu11M14 H1bL3b_IgG1_LALA_YTE (pharmacodynamic studies). Mean $\pm$ SD Fold PGRN levels in plasma in cynomolgus monkeys. N=4 animals per Ab group.

[0074] Figure 20 depicts CSF progranulin levels in cynomolgus monkeys after 4 weekly repeat 60mg/kg doses of hu11M14 H1bL3b_IgG1_LALA or hu11M14 H1bL3b_IgG1_LALA_YTE (pharmacodynamic studies). Mean $\pm$ SD Fold PGRN levels in CSF in cynomolgus monkeys. N=4 animals in hu11M14 H1bL3b_IgG1_LALA group and N=3 in hu11M14 H1bL3b_IgG1_LALA_YTE group.

[0075] Figure 21 depicts sortilin levels in cynomolgus monkey PBMCs after 4 weekly repeat 60mg/kg doses of hu11M14 H1bL3b_IgG1_LALA or hu11M14 H1bL3b_IgG1_LALA_YTE. Mean $\pm$ SD Sortilin levels in cynomolgus monkey PBMCs as a percent of Baseline with 4 weekly doses of 60 mg/kg anti-sortilin antibodies. N=4 animals per group. Sortilin levels normalized to total protein levels.

BRIEF DESCRIPTION OF THE SEQUENCES

[0076] SEQ ID NO:1 sets forth the amino acid sequence of human sortilin extracellular domain.

[0077] SEQ ID NO:2 sets forth the nucleotide sequence of a murine 5E20VH (mIgG1):

- [0078] SEQ ID NO:3 sets forth the amino acid sequence of signal peptide for murine 5E20VH.
- [0079] SEQ ID NO: 4 sets forth the amino acid sequence of murine 5E20VH.
- [0080] SEQ ID NO:5 sets forth the amino acid sequence of murine 5E20_Kabat Chothia Composite CDR-H1.
- [0081] SEQ ID NO:6 sets forth the amino acid sequence of murine 5E20_Kabat Chothia Composite CDR-H2.
- [0082] SEQ ID NO:7 sets forth the amino acid sequence of murine 5E20_Kabat Chothia Composite CDR-H3.
- [0083] SEQ ID NO:8 sets forth the nucleotide sequence of a murine 5E20VL (kappa):
- [0084] SEQ ID NO:9 sets forth the amino acid sequence of signal peptide for murine 5E20VL.
- [0085] SEQ ID NO:10 sets forth the amino acid sequence of murine 5E20VL Vk.
- [0086] SEQ ID NO:11 sets forth the amino acid sequence of murine 5E20_Kabat Chothia Composite CDR-L1.
- [0087] SEQ ID NO:12 sets forth the amino acid sequence of murine 5E20_Kabat Chothia Composite CDR-L2.
- [0088] SEQ ID NO:13 sets forth the amino acid sequence of murine 5E20_Kabat Chothia Composite CDR-L3.
- [0089] SEQ ID NO:14 sets forth the amino acid sequence of Kabat CDR-H1 of mouse 5E20 antibody.
- [0090] SEQ ID NO:15 sets forth the amino acid sequence of Chothia CDR-H1 of mouse 5E20 antibody.
- [0091] SEQ ID NO:16 sets forth the amino acid sequence of Chothia CDR-H2 of mouse 5E20 antibody.
- [0092] SEQ ID NO:17 sets forth the amino acid sequence of AbM CDR-H2 of mouse 5E20 antibody.

- [0093] SEQ ID NO:18 sets forth the amino acid sequence of Contact CDR-H1 of mouse 5E20 antibody.
- [0094] SEQ ID NO:19 sets forth the amino acid sequence of Contact CDR-H2 of mouse 5E20 antibody.
- [0095] SEQ ID NO:20 sets forth the amino acid sequence of Contact CDR-H3 of mouse 5E20 antibody.
- [0096] SEQ ID NO:21 sets forth the amino acid sequence of Contact CDR-L1 of mouse 5E20 antibody.
- [0097] SEQ ID NO:22 sets forth the amino acid sequence of Contact CDR-L2 of mouse 5E20 antibody.
- [0098] SEQ ID NO:23> Contact CDR-L3 of mouse 5E20 antibody.
- [0099] SEQ ID NO:24 sets forth the amino acid sequence of a chimeric 5E20 heavy chain.
- [0100] SEQ ID NO:25 sets forth the amino acid sequence of a chimeric 5E20 light chain.
- [0101] SEQ ID NO: 26 sets forth the nucleotide sequence of a murine 8H24VH (IgG2c).
- [0102] SEQ ID NO:27 sets forth the amino acid sequence of murine 8H24VH signal peptide.
- [0103] SEQ ID NO:28 sets forth the amino acid sequence of murine 8H24Vh.
- [0104] SEQ ID NO:29 sets forth the amino acid sequence of murine 8H24_ Kabat Chothia Composite CDR-H1.
- [0105] SEQ ID NO:30 sets forth the amino acid sequence of murine 8H24_ Kabat Chothia Composite CDR-H2.
- [0106] SEQ ID NO:31 sets forth the amino acid sequence of murine 8H24_ Kabat Chothia Composite CDR-H3.
- [0107] SEQ ID NO:32 sets forth the nucleotide sequence of a murine 8H24VL (kappa).
- [0108] SEQ ID NO:33 sets forth the amino acid sequence of murine 8H24VL signal peptide.
- [0109] SEQ ID NO:34 sets forth the amino acid sequence of murine 8H24Vk.

[0110] SEQ ID NO:35 sets forth the amino acid sequence of murine 8H24_Kabat Chothia Composite CDR-L1.

[0111] SEQ ID NO:36 sets forth the amino acid sequence of murine 8H24_Kabat Chothia Composite CDR-L2.

[0112] SEQ ID NO:37 sets forth the amino acid sequence of murine 8H24_Kabat Chothia Composite CDR-L3.

[0113] SEQ ID NO:38 sets forth the amino acid sequence of Kabat CDR-H1 of mouse 8H24 antibody.

[0114] SEQ ID NO:39 sets forth the amino acid sequence of Chothia CDR-H1 of mouse 8H24 antibody.

[0115] SEQ ID NO:40 sets forth the amino acid sequence of Chothia CDR-H2 of mouse 8H24 antibody.

[0116] SEQ ID NO:41 sets forth the amino acid sequence of AbM CDR-H2 of mouse 8H24 antibody.

[0117] SEQ ID NO:42 sets forth the amino acid sequence of Contact CDR-H1 of mouse 8H24 antibody.

[0118] SEQ ID NO:43 sets forth the amino acid sequence of Contact CDR-H2 of mouse 8H24 antibody.

[0119] SEQ ID NO:44 sets forth the amino acid sequence of Contact CDR-H3 of mouse 8H24 antibody.

[0120] SEQ ID NO:45 sets forth the amino acid sequence of Contact CDR-L1 of mouse 8H24 antibody.

[0121] SEQ ID NO:46 sets forth the amino acid sequence of Contact CDR-L2 of mouse 8H24 antibody.

[0122] SEQ ID NO:47 sets forth the amino acid sequence of Contact CDR-L3 of mouse 8H24 antibody.

[0123] SEQ ID NO:48 sets forth the amino acid sequence of a chimeric 8H24 heavy chain.

- [0124] SEQ ID NO:49 sets forth the amino acid sequence of a chimeric 8H24 light chain.
- [0125] SEQ ID NO:50 sets forth the nucleotide sequence of a murine 11M14VH (IgG1).
- [0126] SEQ ID NO:51 sets forth the amino acid sequence of murine 11M14VH signal peptide.
- [0127] SEQ ID NO:52 sets forth the amino acid sequence of murine 11M14Vh.
- [0128] SEQ ID NO:53 sets forth the amino acid sequence of murine 11M14_ Kabat Chothia Composite CDR-H1.
- [0129] SEQ ID NO:54 sets forth the amino acid sequence of murine 11M14_ Kabat Chothia Composite CDR-H2.
- [0130] SEQ ID NO:55 sets forth the amino acid sequence of murine 11M14_ Kabat Chothia Composite CDR-H3.
- [0131] SEQ ID NO:56 sets forth the nucleotide sequence of a murine 11M14Vk (kappa).
- [0132] SEQ ID NO:57 sets forth the amino acid sequence of murine 11M14Vk signal peptide.
- [0133] SEQ ID NO:58 sets forth the amino acid sequence of murine 11M14Vk.
- [0134] SEQ ID NO:59 sets forth the amino acid sequence of murine 11M14_ Kabat Chothia Composite CDR-L1.
- [0135] SEQ ID NO:60 sets forth the amino acid sequence of murine 11M14_ Kabat Chothia Composite CDR-L2.
- [0136] SEQ ID NO:61 sets forth the amino acid sequence of murine 11M14_ Kabat Chothia Composite CDR-L3.
- [0137] SEQ ID NO:62 sets forth the amino acid sequence of Kabat CDR-H1 of mouse 11M14 antibody.
- [0138] SEQ ID NO:63 sets forth the amino acid sequence of Chothia CDR-H1 of mouse 11M14 antibody.
- [0139] SEQ ID NO:64 sets forth the amino acid sequence of Chothia CDR-H2 of mouse 11M14 antibody.
- [0140] SEQ ID NO:65 sets forth the amino acid sequence of AbM CDR-H2 of mouse 11M14 antibody.

- [0141] SEQ ID NO:66 sets forth the amino acid sequence of Contact CDR-H1 of mouse 11M14 antibody.
- [0142] SEQ ID NO:67 sets forth the amino acid sequence of Contact CDR-H2 of mouse 11M14 antibody.
- [0143] SEQ ID NO:68 sets forth the amino acid sequence of Contact CDR-H3 of mouse 11M14 antibody.
- [0144] SEQ ID NO:69 sets forth the amino acid sequence of Contact CDR-L1 of mouse 11M14 antibody.
- [0145] SEQ ID NO:70 sets forth the amino acid sequence of Contact CDR-L2 of mouse 11M14 antibody.
- [0146] SEQ ID NO:71 sets forth the amino acid sequence of Contact CDR-L3 of mouse 11M14 antibody.
- [0147] SEQ ID NO:72 sets forth the amino acid sequence of Alternate Kabat-Chothia CDR-L2 (present in Hu11M14VLv3b, SEQ ID NO:198).
- [0148] SEQ ID NO:73 sets forth the amino acid sequence of Alternate Kabat-Chothia CDR-L2 (present in Hu11M14VLv4b, SEQ ID NO:199).
- [0149] SEQ ID NO:74 sets forth the amino acid sequence of Alternate Contact CDR-L2 (present in Hu11M14VLv3b, SEQ ID NO:198).
- [0150] SEQ ID NO:75 sets forth the amino acid sequence of Alternate Contact CDR-L2 (present in Hu11M14VLv4b, SEQ ID NO:199).
- [0151] SEQ ID NO:76 sets forth the nucleotide sequence of a murine 5M13VH (IgG1).
- [0152] SEQ ID NO:77 sets forth the amino acid sequence of murine 5M13VH signal peptide.
- [0153] SEQ ID NO:78 >murine 5M13VH amino acid sequence.
- [0154] SEQ ID NO:79 sets forth the amino acid sequence of murine 5M13_Kabat Chothia Composite CDR-H1.
- [0155] SEQ ID NO:80 sets forth the amino acid sequence of murine 5M13_Kabat Chothia Composite CDR-H2.

[0156] SEQ ID NO:81 sets forth the amino acid sequence of murine 5M13_Kabat Chothia Composite CDR-H3.

[0157] SEQ ID NO: 82 sets forth the nucleotide sequence of a murine 5M13VL (kappa).

[0158] SEQ ID NO:83 sets forth the amino acid sequence of murine 5M13VL signal peptide.

[0159] SEQ ID NO:84 sets forth the amino acid sequence of murine 5M13VL Vk.

[0160] SEQ ID NO:85 sets forth the amino acid sequence of murine 5M13_Kabat Chothia Composite CDR-L1.

[0161] SEQ ID NO:86 sets forth the amino acid sequence of murine 5M13_Kabat Chothia Composite CDR-L2.

[0162] SEQ ID NO:87 sets forth the amino acid sequence of murine 5M13_Kabat Chothia Composite CDR-L3.

[0163] SEQ ID NO:88 sets forth the nucleotide sequence of a murine 2F18VH (mIgG1).

[0164] SEQ ID NO:89 sets forth the amino acid sequence of murine 2F18VH signal peptide.

[0165] SEQ ID NO:90 sets forth the amino acid sequence of murine 2F18VH.

[0166] SEQ ID NO:91 sets forth the amino acid sequence of murine 2F18_Kabat Chothia Composite CDR-H1.

[0167] SEQ ID NO:92 sets forth the amino acid sequence of murine 2F18_Kabat Chothia Composite CDR-H2.

[0168] SEQ ID NO:93 sets forth the amino acid sequence of murine 2F18_Kabat Chothia Composite CDR-H3.

[0169] SEQ ID NO:94 sets forth the nucleotide sequence of a murine 2F18VL (kappa).

[0170] SEQ ID NO: 95 sets forth the amino acid sequence of murine 2F18VL signal peptide.

[0171] SEQ ID NO:96 sets forth the amino acid sequence of murine Vk_2F18VL.

[0172] SEQ ID NO:97 sets forth the amino acid sequence of murine 2F18_Kabat Chothia Composite CDR-L1.

- [0173] SEQ ID NO:98 sets forth the amino acid sequence of murine 2F18_ Kabat Chothia Composite CDR-L2.
- [0174] SEQ ID NO:99 sets forth the amino acid sequence of murine 2F18_ Kabat Chothia Composite CDR-L3.
- [0175] SEQ ID NO:100 sets forth the nucleotide sequence of a murine 2P22VH (IgG2b).
- [0176] SEQ ID NO:101 sets forth the amino acid sequence of murine 2P22VH signal peptide.
- [0177] SEQ ID NO:102 sets forth the amino acid sequence of murine 2P22VH.
- [0178] SEQ ID NO:103 sets forth the amino acid sequence of murine 2P22_ Kabat Chothia Composite CDR-H1.
- [0179] SEQ ID NO:104 sets forth the amino acid sequence of murine 2P22_ Kabat Chothia Composite CDR-H2.
- [0180] SEQ ID NO:105 sets forth the amino acid sequence of murine 2P22_ Kabat Chothia Composite CDR-H3.
- [0181] SEQ ID NO:106 sets forth the nucleotide sequence of a murine 2P22VL (kappa).
- [0182] SEQ ID NO:107 sets forth the amino acid sequence of mouse 2P22VL signal peptide.
- [0183] SEQ ID NO:108 sets forth the amino acid sequence of murine Vk_2P22VL.
- [0184] SEQ ID NO:109 sets forth the amino acid sequence of murine 2P22_ Kabat Chothia Composite CDR-L1.
- [0185] SEQ ID NO:110 sets forth the amino acid sequence of murine 2P22_ Kabat Chothia Composite CDR-L2.
- [0186] SEQ ID NO:111 sets forth the amino acid sequence of murine 2P22_ Kabat Chothia Composite CDR-L3.
- [0187] SEQ ID NO:112 sets forth the nucleotide sequence of a murine 6B15VH (IgG1).
- [0188] SEQ ID NO:113 sets forth the amino acid sequence of 6B15VH signal peptide.
- [0189] SEQ ID NO:114 sets forth the amino acid sequence of murine 6B15VH.

[0190] SEQ ID NO:115 sets forth the amino acid sequence of murine 6B15_ Kabat Chothia Composite CDR-H1.

[0191] SEQ ID NO:116 sets forth the amino acid sequence of murine 6B15_ Kabat Chothia Composite CDR-H2.

[0192] SEQ ID NO:117 sets forth the amino acid sequence of murine 6B15_ Kabat Chothia Composite CDR-H3.

[0193] SEQ ID NO:118 sets forth the nucleotide sequence of a murine 6B15VL (kappa).

[0194] SEQ ID NO:119 sets forth the amino acid sequence of 6B15VL signal peptide.

[0195] SEQ ID NO:120 sets forth the amino acid sequence of murine 6B15VL Vk_6B15.

[0196] SEQ ID NO:121 sets forth the amino acid sequence of murine 6B15_ Kabat Chothia Composite CDR-L1.

[0197] SEQ ID NO:122 sets forth the amino acid sequence of murine 6B15_ Kabat Chothia Composite CDR-L2.

[0198] SEQ ID NO:123 sets forth the amino acid sequence of murine 6B15_ Kabat Chothia Composite CDR-L3.

[0199] SEQ ID NO:124 sets forth the nucleotide sequence of a murine 2C14VH (IgG1).

[0200] SEQ ID NO:125 sets forth the amino acid sequence of mouse 2C14VH signal peptide.

[0201] SEQ ID NO:126 sets forth the amino acid sequence of murine 2C14VH.

[0202] SEQ ID NO:127 sets forth the amino acid sequence of murine 2C14_ Kabat Chothia Composite CDR-H1.

[0203] SEQ ID NO:128 sets forth the amino acid sequence of murine 2C14_ Kabat Chothia Composite CDR-H2.

[0204] SEQ ID NO:129 sets forth the amino acid sequence of murine 2C14_ Kabat Chothia Composite CDR-H3.

[0205] SEQ ID NO:130 sets forth the nucleotide sequence of a murine 2C14VL (kappa).

- [0206] SEQ ID NO:131 sets forth the amino acid sequence of mouse 2C14VL signal peptide.
- [0207] SEQ ID NO:132 sets forth the amino acid sequence of murine 2C14VL Vk_2C14.
- [0208] SEQ ID NO:133 sets forth the amino acid sequence of murine 2C14_ Kabat Chothia Composite CDR-L1.
- [0209] SEQ ID NO:134 sets forth the amino acid sequence of murine 2C14_ Kabat Chothia Composite CDR-L2.
- [0210] SEQ ID NO:135 sets forth the amino acid sequence of murine 2C14_ Kabat Chothia Composite CDR-L3.
- [0211] SEQ ID NO:136 sets forth the nucleotide sequence of a murine 9N18VH (IgG2b).
- [0212] SEQ ID NO:137 sets forth the amino acid sequence of mouse 9N18VH signal peptide.
- [0213] SEQ ID NO:138 sets forth the amino acid sequence of murine 9N18VH.
- [0214] SEQ ID NO:139 sets forth the amino acid sequence of murine 9N18_ Kabat Chothia Composite CDR-H1.
- [0215] SEQ ID NO:140 sets forth the amino acid sequence of murine 9N18_ Kabat Chothia Composite CDR-H2.
- [0216] SEQ ID NO:141 sets forth the amino acid sequence of murine 9N18_ Kabat Chothia Composite CDR-H3.
- [0217] SEQ ID NO:142 sets forth the nucleotide sequence of a murine 9N18VL (kappa).
- [0218] SEQ ID NO:143 sets forth the amino acid sequence of mouse 9N18VL signal peptide.
- [0219] SEQ ID NO:144 sets forth the amino acid sequence of murine 9N18VL Vk_9N18.
- [0220] SEQ ID NO:145 sets forth the amino acid sequence of murine 9N18_ Kabat Chothia Composite CDR-L1.
- [0221] SEQ ID NO:146 sets forth the amino acid sequence of murine 9N18_ Kabat Chothia Composite CDR-L2.
- [0222] SEQ ID NO:147 sets forth the amino acid sequence of murine 9N18_ Kabat Chothia Composite CDR-L3.

- [0223] SEQ ID NO:148 sets forth the nucleotide sequence of a murine 4N2VH (IgG3).
- [0224] SEQ ID NO:149 sets forth the amino acid sequence of murine 4N2VH signal peptide.
- [0225] SEQ ID NO:150 sets forth the amino acid sequence of murine 4N2VH Vh_4N2.
- [0226] SEQ ID NO:151 sets forth the amino acid sequence of murine 4N2_ Kabat Chothia Composite CDR-H1.
- [0227] SEQ ID NO:152 sets forth the amino acid sequence of murine 4N2_ Kabat Chothia Composite CDR-H2.
- [0228] SEQ ID NO:153 sets forth the amino acid sequence of murine 4N2_ Kabat Chothia Composite CDR-H3.
- [0229] SEQ ID NO:154 sets forth the nucleotide sequence of a murine 4N2VL (kappa).
- [0230] SEQ ID NO:155 sets forth the amino acid sequence of murine 4N2VL signal peptide.
- [0231] SEQ ID NO:156 sets forth the amino acid sequence of murine 4N2VL Vk_4N2.
- [0232] SEQ ID NO:157 sets forth the amino acid sequence of murine 4N2_ Kabat Chothia Composite CDR-L1.
- [0233] SEQ ID NO:158 sets forth the amino acid sequence of murine 4N2_ Kabat Chothia Composite CDR-L2.
- [0234] SEQ ID NO:159 sets forth the amino acid sequence of murine 4N2_ Kabat Chothia Composite CDR-L3.
- [0235] SEQ ID NO:160 sets forth the amino acid sequence of 3V6F-VH_mSt.
- [0236] SEQ ID NO:161 sets forth the amino acid sequence of AEX29086-VH_huFrwk.
- [0237] SEQ ID NO:162 sets forth the amino acid sequence of IGHV3-21*01.
- [0238] SEQ ID NO:163 sets forth the amino acid sequence of h5E20VHv1.
- [0239] SEQ ID NO:164 sets forth the amino acid sequence of h5E20VHv2.
- [0240] SEQ ID NO:165 sets forth the amino acid sequence of h5E20VHv3.
- [0241] SEQ ID NO:166 sets forth the amino acid sequence of h5E20VHv4.

- [0242] SEQ ID NO:167 sets forth the amino acid sequence of h5E20VHv5.
- [0243] SEQ ID NO:168 sets forth the amino acid sequence of h5E20VHv6.
- [0244] SEQ ID NO:169 sets forth the amino acid sequence of h5E20VHv7.
- [0245] SEQ ID NO:170 sets forth the amino acid sequence of 3V6F-VL_mSt.
- [0246] SEQ ID NO:171 sets forth the amino acid sequence of BAH04687-VL_huFrwk.
- [0247] SEQ ID NO:172 sets forth the amino acid sequence of IGKV1-12*01.
- [0248] SEQ ID NO:173 sets forth the amino acid sequence of h5E20VLv1.
- [0249] SEQ ID NO:174 sets forth the amino acid sequence of h5E20VL v2.
- [0250] SEQ ID NO:175 sets forth the amino acid sequence of h5E20VLv3.
- [0251] SEQ ID NO:176 sets forth the amino acid sequence of h5E20VLv4.
- [0252] SEQ ID NO:177 sets forth the amino acid sequence of 1MRC-VH_mSt.
- [0253] SEQ ID NO:178 sets forth the amino acid sequence of AAC51714-VH_huFrwk.
- [0254] SEQ ID NO:179 sets forth the amino acid sequence of IGHV1-69*08_IGHJ1*01.
- [0255] SEQ ID NO:180 sets forth the amino acid sequence of h8H24VHv1.
- [0256] SEQ ID NO:181 sets forth the amino acid sequence of h8H24VHv2.
- [0257] SEQ ID NO:182 sets forth the amino acid sequence of 1MRC -VL_mSt.
- [0258] SEQ ID NO:183 sets forth the amino acid sequence of ABC66914-VL_huFrwk.
- [0259] SEQ ID NO:184 sets forth the amino acid sequence of IGKV2-40*01.
- [0260] SEQ ID NO:185 sets forth the amino acid sequence of h8H24VLv1.
- [0261] SEQ ID NO:186 sets forth the amino acid sequence of h8H24VLv2.
- [0262] SEQ ID NO:187 sets forth the amino acid sequence of 1MQK-VH_mSt.
- [0263] SEQ ID NO:188 sets forth the amino acid sequence of ACS96198-VH_huFrwk.
- [0264] SEQ ID NO:189 sets forth the amino acid sequence of IGHV3-48*03.

- [0265] SEQ ID NO:190 sets forth the amino acid sequence of h11M14VHv1b.
- [0266] SEQ ID NO:191 sets forth the amino acid sequence of h11M14VHv2b.
- [0267] SEQ ID NO:192 sets forth the amino acid sequence of h11M14VHv3b.
- [0268] SEQ ID NO:193 sets forth the amino acid sequence of 1MQK -VL_mSt.
- [0269] SEQ ID NO:194 sets forth the amino acid sequence of CBZ39892-VL_huFrwk.
- [0270] SEQ ID NO:195 sets forth the amino acid sequence of IGKV1-39*01.
- [0271] SEQ ID NO:196 sets forth the amino acid sequence of h11M14VLv1b.
- [0272] SEQ ID NO:197 sets forth the amino acid sequence of h11M14VLv2b.
- [0273] SEQ ID NO:198 sets forth the amino acid sequence of h11M14VLv3b.
- [0274] SEQ ID NO:199 sets forth the amino acid sequence of h11M14VLv4b.
- [0275] SEQ ID NO:200 sets forth the amino acid sequence of HA peptide.
- [0276] SEQ ID NO:201 sets forth the amino acid sequence of c-Myc peptide.
- [0277] SEQ ID NO:202 sets forth the amino acid sequence of consensus motif of a peptide bound by antibody 5E20.
- [0278] SEQ ID NO:203 sets forth the amino acid sequence of consensus motif of a peptide bound by antibody 5E20.
- [0279] SEQ ID NO:204 sets forth the amino acid sequence of peptide bound by antibody 5E20.
- [0280] SEQ ID NO:205 sets forth the amino acid sequence of peptide bound by antibody 5E20.
- [0281] SEQ ID NO:206 sets forth the amino acid sequence of sequence motif bound by antibody 5E20.
- [0282] SEQ ID NO:207 sets forth the amino acid sequence of a linker.
- [0283] SEQ ID NO:208 sets forth the amino acid sequence of chimeric 11M14 heavy chain.
- [0284] SEQ ID NO:209 sets forth the amino acid sequence of chimeric 11M14 light chain.

- [0285] SEQ ID NO:210 sets forth the amino acid sequence of residues 523-610 of sortilin.
- [0286] SEQ ID NO:211 sets forth the amino acid sequence of a sortilin peptide.
- [0287] SEQ ID NO:212 sets forth the amino acid sequence of a sortilin peptide.
- [0288] SEQ ID NO:213 sets forth the amino acid sequence of a peptide bound by antibody 8H24.
- [0289] SEQ ID NO:214 sets forth the amino acid sequence of a peptide bound by antibody 11M14.
- [0290] SEQ ID NO:215; sets forth the amino acid sequence of human sortilin extracellular domain minus signal peptide.
- [0291] SEQ ID NO:216 sets forth the amino acid sequence of hSORT1_ECD_Emut1.
- [0292] SEQ ID NO:217 sets forth the amino acid sequence of hSORT1_ECD_Emut2a.
- [0293] SEQ ID NO:218 sets forth the amino acid sequence of hSORT1_ECD_Emut2b.
- [0294] SEQ ID NO:219 sets forth the amino acid sequence of hSORT1_ECD_Emut3.
- [0295] SEQ ID NO:220 sets forth the amino acid sequence of hSORT1_ECD_Emut4.
- [0296] SEQ ID NO:221 sets forth the amino acid sequence of hSORT1_ECD_Emut5.
- [0297] SEQ ID NO:222 sets forth the amino acid sequence of hSORT1_ECD_Emut6N.
- [0298] SEQ ID NO:223 sets forth the amino acid sequence of hSORT1_ECD_Emut8N.
- [0299] SEQ ID NO:224 sets forth the amino acid sequence of hSORT1_ECD_Emut11N.
- [0300] SEQ ID NO:225 sets forth the amino acid sequence of hSORT1_ECD_Emut14N.
- [0301] SEQ ID NO:226 sets forth the amino acid sequence of hSORT1_ECD_Emut16.
- [0302] SEQ ID NO:227 sets forth the amino acid sequence of hSORT1_ECD_Emut17.
- [0303] SEQ ID NO:228 sets forth the amino acid sequence of hSORT1_ECD_Emut18.
- [0304] SEQ ID NO:229 sets forth the amino acid sequence of hSORT1_ECD_Emut19.
- [0305] SEQ ID NO:230 sets forth the amino acid sequence of hSORT1_ECD_Emut20.
- [0306] SEQ ID NO:231 sets forth the amino acid sequence of hSORT1_ECD_Emut21.

- [0307] SEQ ID NO:232 sets forth the amino acid sequence of hSORT1_ECD_Emut22.
- [0308] SEQ ID NO : 233 sets forth the amino acid sequence of hSORT1_ECD_Emut23.
- [0309] SEQ ID NO:234 sets forth the amino acid sequence of hSORT1_ECD_Emut24.
- [0310] SEQ ID NO: 235 sets forth the amino acid sequence of hSORT1_ECD_Emut25.
- [0311] SEQ ID NO: 236 sets forth the amino acid sequence of hSORT1_ECD_Emut26.
- [0312] SEQ ID NO:237 sets forth the amino acid sequence of hSORT1_ECD_Emut27.
- [0313] SEQ ID NO:238 sets forth the amino acid sequence of hSORT1_ECD_Emut28.
- [0314] SEQ ID NO:239 sets forth the amino acid sequence of hSORT1_ECD_Emut29.
- [0315] SEQ ID NO:240 sets forth the amino acid sequence of hSORT1_ECD_Emut30.
- [0316] SEQ ID NO:241 sets forth the amino acid sequence of hSORT1_ECD_Emut31.
- [0317] SEQ ID NO:242 sets forth the amino acid sequence of hSORT1_ECD_Emut32.
- [0318] SEQ ID NO: 243 sets forth the amino acid sequence of hSORT1_ECD_Emut33.
- [0319] SEQ ID NO:244 sets forth the amino acid sequence of hu11M14_H1b_IgG1 LALA_YTE – heavy chain.
- [0320] SEQ ID NO:245 sets forth the amino acid sequence of hu11M14_L3b– light chain.
- [0321] SEQ ID NO:246 sets forth the amino acid sequence of hu8H24_H1_IgG1 LALA_YTE – heavy chain.
- [0322] SEQ ID NO:247 sets forth the amino acid sequence of hu8H24_L2_light chain.
- [0323] SEQ ID NO:248 sets forth the amino acid sequence of hu5E20_H7_IgG1 LALA_YTE – heavy chain.
- [0324] SEQ ID NO:249 sets forth the amino acid sequence of hu5E20_L4_light chain.
- [0325] SEQ ID NO:250 sets forth the amino acid sequence of hu11M14_H1b_IgG1 LALA_– heavy chain.
- [0326] SEQ ID NO:251 sets forth the amino acid sequence of hu8H24_H1_IgG1 LALA– heavy chain.

[0327] SEQ ID NO:252 sets forth the amino acid sequence of hu5E20_H7_IgG1 LALA_ heavy chain.

DEFINITIONS

[0328] Monoclonal antibodies or other biological entities are typically provided in isolated form. This means that an antibody or other biological entity is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the monoclonal antibody is combined with an excess of pharmaceutically acceptable carrier(s) or other vehicle intended to facilitate its use. Sometimes monoclonal antibodies are at least 60%, 70%, 80%, 90%, 95% or 99% w/w pure of interfering proteins and contaminants from production or purification. Often an isolated monoclonal antibody or other biological entity is the predominant macromolecular species remaining after its purification.

[0329] Specific binding of an antibody to its target antigen means an affinity and/or avidity of at least 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , or 10^{12} M^{-1} . Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (*e.g.*, lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that an antibody binds one and only one target.

[0330] The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region means a light chain variable region without the light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

[0331] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. *See generally, Fundamental Immunology*, Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989, Ch. 7 (incorporated by reference in its entirety for all purposes).

[0332] An immunoglobulin light or heavy chain variable region (also referred to herein as a "light chain variable domain" ("VL domain") or "heavy chain variable domain" ("VH domain"), respectively) consists of a "framework" region interrupted by three "complementarity determining regions" or "CDRs." The framework regions serve to align the CDRs for specific binding to an epitope of an antigen. The CDRs include the amino acid residues of an antibody that are primarily responsible for antigen binding. From amino-terminus to carboxyl-terminus, both VL and VH domains comprise the following framework (FR) and CDR regions: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. CDRs 1, 2, and 3 of a VL domain are also referred to herein, respectively, as CDR-L1, CDR-L2, and CDR-L3; CDRs 1, 2, and 3 of a VH domain are also referred to herein, respectively, as CDR-H1, CDR-H2, and CDR-H3. When the application discloses a VL sequence with R as the C-terminal residue, the R can alternatively be considered as being the N-terminal residue of the light chain constant region. Thus, the application should also be understood as disclosing the VL sequence without the C-terminal R.

[0333] The assignment of amino acids to each VL and VH domain is in accordance with any conventional definition of CDRs. Conventional definitions include, the Kabat definition (Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991), the Chothia definition (Chothia & Lesk, *J. Mol. Biol.* 196:901-917, 1987; Chothia *et al.*, *Nature* 342:878-883, 1989); a composite of Chothia Kabat CDR in which CDR-H1 is a composite of Chothia and Kabat CDRs; the AbM definition used by Oxford Molecular's antibody modelling software; and, the contact definition of Martin *et al* (bioinfo.org.uk/abs) (*see* Table 1). Kabat provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number. When an antibody is said to comprise CDRs by a certain definition of CDRs (e.g., Kabat) that definition specifies the minimum number of CDR residues present in

the antibody (*i.e.*, the Kabat CDRs). It does not exclude that other residues falling within another conventional CDR definition but outside the specified definition are also present. For example, an antibody comprising CDRs defined by Kabat includes among other possibilities, an antibody in which the CDRs contain Kabat CDR residues and no other CDR residues, and an antibody in which CDR H1 is a composite Chothia-Kabat CDR H1 and other CDRs contain Kabat CDR residues and no additional CDR residues based on other definitions.

Table 1
Conventional Definitions of CDRs Using Kabat Numbering

Loop	Kabat	Chothia	Composite of Chothia & Kabat	AbM	Contact
L1	L24--L34	L24--L34	L24--L34	L24--L34	L30--L36
L2	L50--L56	L50--L56	L50--L56	L50--L56	L46--L55
L3	L89--L97	L89--L97	L89--L97	L89--L97	L89--L96
H1	H31--H35B	H26--H32..H34*	H26--H35B*	H26--H35B	H30--H35B
H2	H50--H65	H52--H56	H50--H65	H50--H58	H47--H58
H3	H95--H102	H95--H102	H95--H102	H95--H102	H93--H101

*CDR-H1 by Chothia can end at H32, H33, or H34 (depending on the length of the loop). This is because the Kabat numbering scheme places insertions of extra residues at 35A and 35B, whereas Chothia numbering places them at 31A and 31B. If neither H35A nor H35B (Kabat numbering) is present, the Chothia CDR-H1 loop ends at H32. If only H35A is present, it ends at H33. If both H35A and H35B are present, it ends at H34.

[0334] The term “antibody” includes intact antibodies and binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', F(ab')₂, F(ab)_c, Dabs, nanobodies, and Fv. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins. The term “antibody” also includes a bispecific antibody and/or a humanized antibody. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites (*see, e.g.*, Songsivilai and Lachmann, *Clin. Exp. Immunol.*, 79:315-321 (1990); Kostelny *et*

al., *J. Immunol.*, 148:1547-53 (1992)). In some bispecific antibodies, the two different heavy/light chain pairs include a humanized 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 heavy chain/light chain pair and a heavy chain/light chain pair specific for a different epitope on sortilin than that bound by 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2.

[0335] In some bispecific antibodies, one heavy chain/light chain pair is a humanized 5E20 antibody, humanized 8H24 antibody, humanized 11M14 antibody, humanized 5M13 antibody, humanized 2F18 antibody, humanized 2P22 antibody, humanized 6B15 antibody, humanized 2C14 antibody, humanized 9N18 antibody, or humanized 4N2 antibody as further disclosed below and the other heavy chain/light chain pair is from an antibody that binds to a receptor expressed on the blood brain barrier, such as an insulin receptor, an insulin-like growth factor (IGF) receptor, a leptin receptor, or a lipoprotein receptor, or a transferrin receptor (Friden *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4771-4775, 1991; Friden *et al.*, *Science* 259:373-377, 1993). Such a bispecific antibody can be transferred cross the blood brain barrier by receptor-mediated transcytosis. Brain uptake of the bispecific antibody can be further enhanced by engineering the bi-specific antibody to reduce its affinity to the blood brain barrier receptor. Reduced affinity for the receptor resulted in a broader distribution in the brain (*see, e.g.*, Atwal *et al.*, *Sci. Trans. Med.* 3, 84ra43, 2011; Yu *et al.*, *Sci. Trans. Med.* 3, 84ra44, 2011).

[0336] Exemplary bispecific antibodies can also be: (1) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu *et al.*, Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-IgTM) Molecule, In: Antibody Engineering, Springer Berlin Heidelberg (2010)); (2) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (3) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (4) a so-called "dock and lock" molecule, based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; or (5) a so-called Scorpion molecule, comprising, *e.g.*, two scFvs fused to both termini of a human Fc-region. Examples of platforms useful for preparing bispecific antibodies include BiTE (Micromet),

DART (MacroGenics), Fcab and Mab2 (F-star), Fc-engineered IgG1 (Xencor) or DuoBody (based on Fab arm exchange, Genmab).

[0337] The term “epitope” refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids (also known as linear epitopes) are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding (also known as conformational epitopes) are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. *See, e.g.,* Epitope Mapping Protocols, in *Methods in Molecular Biology*, Vol. 66, Glenn E. Morris, Ed. (1996).

[0338] Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined by X-ray crystallography of the antibody bound to its antigen to identify contact residues. Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0339] Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody to a common antigen (*see, e.g.,* Junghans *et al., Cancer Res.* 50:1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (*e.g.,* at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50% as measured in a competitive binding assay. Some test antibodies inhibit binding of the reference antibody by at least 75%, 90% or 99%. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[0340] The term “pharmaceutically acceptable” means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

[0341] The term “patient” includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0342] An individual is at increased risk of a disease if the subject has at least one known risk-factor (*e.g.*, genetic, biochemical, family history, and situational exposure) placing individuals with that risk factor at a statistically significant greater risk of developing the disease than individuals without the risk factor.

[0343] The term “biological sample” refers to a sample of biological material within or obtainable from a biological source, for example a human or mammalian subject. Such samples can be organs, organelles, tissues, sections of tissues, bodily fluids, peripheral blood, blood plasma, blood serum, cells, molecules such as proteins and peptides, and any parts or combinations derived therefrom. The term biological sample can also encompass any material derived by processing the sample. Derived material can include cells or their progeny. Processing of the biological sample may involve one or more of filtration, distillation, extraction, concentration, fixation, inactivation of interfering components, and the like.

[0344] The term “control sample” refers to a biological sample not known or suspected to include progranulin-related disease-affected tissue, or at least not known or suspect to include diseased tissues of a given type. Control samples can be obtained from individuals not afflicted with the progranulin-related disease. Alternatively, control samples can be obtained from patients afflicted with the progranulin-related disease. Such samples can be obtained at the same time as a biological sample thought to comprise the progranulin-related disease or on a different occasion. A biological sample and a control sample can both be obtained from the same tissue. Preferably, control samples consist essentially or entirely of normal, healthy tissues and can be used in comparison to a biological sample thought to comprise progranulin-related disease - affected regions. Preferably, the tissue in the control sample is the same type as the tissue in the biological sample. Preferably, the progranulin-related disease -affected cells thought to be in the biological sample arise from the same cell type (*e.g.*, neurons or glia) as the type of cells in the control sample.

[0345] The term “disease” refers to any abnormal condition that impairs physiological function. The term is used broadly to encompass any disorder, illness, abnormality, pathology, sickness, condition, or syndrome in which physiological function is impaired, irrespective of the nature of the etiology.

[0346] The term “symptom” refers to a subjective evidence of a disease, such as altered gait, as perceived by the subject. A “sign” refers to objective evidence of a disease as observed by a physician.

[0347] The term “positive response to treatment” refers to a more favorable response in an individual patient or average response in a population of patients relative to an average response in a control population not receiving treatment.

[0348] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0349] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (*e.g.*, the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

[0350] Compositions or methods “comprising” or “including” one or more recited elements may include other elements not specifically recited. For example, a composition that “comprises” or “includes” an antibody may contain the antibody alone or in combination with other ingredients.

[0351] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[0352] Unless otherwise apparent from the context, the term “about” encompasses insubstantial variations, such as values within a standard margin of error of measurement (*e.g.*, SEM) of a stated value.

[0353] Statistical significance means $p \leq 0.05$.

[0354] The singular forms of the articles “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a compound” or “at least one compound” can include a plurality of compounds, including mixtures thereof.

DETAILED DESCRIPTION

I. General

[0355] The invention provides antibodies that bind to sortilin.

[0356] Exemplary antibodies of the invention are 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2. Some antibodies of the invention serve to treat, inhibit progression, or delay diseases or disorders associated with changes in progranulin levels. Although an understanding of mechanism is not required for practice of the invention, an increase in extracellular progranulin levels may occur as a result of the antibody binding sortilin, among other mechanisms. The antibodies of the invention or agents that induce such antibodies can be used in methods of treating or effecting prophylaxis of frontotemporal dementia and other diseases and disorders associated with changes in progranulin levels, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, Batten Disease (a type of neuronal ceroid lipofuscinosis (NCL), neurodegenerative disorders, and neurodegenerative disorders associated with aging. Neurodegenerative disorders associated with aging tend to progress in an irreversible manner and are typically associated with one or more biological hallmarks of aging: genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, cellular senescence, deregulated nutrient sensing, stem cell exhaustion and altered intercellular communication (See for example Hou, Y. et al., 2019, Nature Reviews Neurology 15, pages 565–581).

II. Target Molecules

[0357] Unless otherwise apparent from the context, reference to sortilin means a natural human form of sortilin including all isoforms, including soluble forms, irrespective of whether posttranslational modification (*e.g.*, phosphorylation, glycation, or acetylation) is present. The amino acid sequence of the extracellular domain of sortilin is indicated below. The 33 amino acid signal peptide is indicated by boldface.

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRAAAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVG DSTGVILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENS GKVVLTAEVSGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWVSKNFGGKWEEIHKAVCLAKWGS DNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFASVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEQFY SILAANDDMVFMHVDEPGDTGFGTIFTSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRKPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAV GIVIAHGSVGD AISVMVPDVYISDDGGYSWTKMLEGPHYYTILDSGGIIVAIEHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGLASEPGARSMNISIWGFTESFLT SQWVSYTIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILGYKEQFLRLRKSSVCQNGRDYVVT KQPSICLCSLEDFLCDFGYR PENDSKCVEQPELKGHDLEFCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNS (SEQ ID NO:1; UniProtKB/Swiss-Prot: Q99523)

[0358] The amino acid sequence of the extracellular domain of sortilin without the 33- amino acid signal peptide is indicated below.

QDRLDAPPPPAAPLPRWSGPIGVSWGLRAAAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVG DSTGVILVLTTFHVPLVIMTFGQSKLYRSEDY GKNFKDITDLINNTFIRTEFGMAIGPENS GKVVLTAEVSGGSRGGRIFRSSDFAKNFVQTD LPFHPLTQMMYSPQNSDYLLALSTENGLWVSKNFGGKWEEIHKAVCLAKWGS DNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFASVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEQFY SILAANDDMVFMHVDEPGDTGFGTIFTSDDRGIVY SKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRKPENSE CDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAV GIVIAHGSVGD AISVMVPDVYISDDGGYSWTKMLEGPHYYTILDSGGIIVAIEHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGLASEPGARSMNISIWGFTESFLT SQWVSYTIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILGYKEQFLRLRKSSVCQNGRDYVVT KQPSICLCSLEDFLCDFGYR PENDSKCVEQPELKGHDLEFCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNS (SEQ ID NO:215)

[0359] Reference to sortilin includes known natural variations which are listed in the Swiss-Prot database and permutations thereof, as well as mutations associated with pathologies.

[0360] Additionally, reference to sortilin includes sortilin with known post-translational modifications. Examples of known post-translational modifications are listed in the UniProtKB/Swiss-Prot database.

[0361] Unless otherwise apparent from context, reference to sortilin, or its fragments includes the natural human amino acid sequences including isoforms, mutants, and allelic variants thereof.

III. Antibodies

A. Binding Specificity and Functional Properties

[0362] The invention provides antibodies that specifically bind to sortilin. The examples describe isolation of eight mouse monoclonal antibodies against human sortilin. The epitope specificities of three of these antibodies have been mapped.

[0363] From a primary screening the epitope specificity of antibody 5E20 was mapped to being within approximately residues 555-561 of human sortilin ECD of SEQ ID NO:215 (FTESFLT, SEQ ID NO:202). On further screening the epitope was mapped to residues E557, S558, F559, L560 P510, and Y535 of the sortilin ECD of SEQ ID NO:215.

[0364] From a primary screening the epitope specificity of antibody 8H24 was mapped to being within approximately residues 134-143 of human sortilin ECD of SEQ ID NO:215 (RTEFGMAIGP, SEQ ID NO:213). On further screening the epitope was mapped to residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215.

[0365] From a primary screening the epitope specificity of antibody 11M14 was mapped to being within approximately residues 553-562 of human sortilin ECD of SEQ ID NO:215. On further screening the epitope was mapped to residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215.

[0366] Some antibodies specifically bind to an epitope within E(S/Q/D)FL (SEQ ID NO:206). Some antibodies of the invention specifically bind to a peptide comprising or consisting of residues 588-594 of sortilin ECD of SEQ ID NO:1 (corresponding to residues 555-561 of sortilin ECD of SEQ ID NO:215), namely residues FTESFLT (SEQ ID NO:202). Some antibodies of the invention specifically bind to a peptide comprising or consisting of residues 590-593 of sortilin ECD of SEQ ID NO:1 (corresponding to residues 557-560 of sortilin ECD of SEQ ID

NO:215), namely ESFL (SEQ ID NO:203). Some antibodies of the invention specifically bind to a peptide comprising or consisting of residues 632-643 of sortilin ECD of SEQ ID NO:1 (corresponding to residues 599-610 of SEQ ID NO:215), namely DGCILGYKEQFL (SEQ ID NO:204). Some antibodies of the invention specifically bind to a peptide comprising or consisting of residues 663-674 of sortilin ECD of SEQ ID NO:1 (corresponding to residues 630-641 of sortilin ECD of SEQ ID NO:15), namely PSICLCSLEDL (SEQ ID NO:205). Some antibodies of the invention specifically bind to a peptide comprising or consisting of the consensus motif E(S/Q/D)FL (SEQ ID NO:206).

[0367] Some antibodies bind within the amino acid sequence
 HYYTILDSGGIIVAIEHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGLASEPGARSMNISI
 WGFTEFLTSQWVSYTIDFKDILER (SEQ ID NO:210) (corresponding to residues 523-610 of sortilin ECD of SEQ ID NO:1). Some antibodies specifically bind to a peptide within the preceding sequence. Peptides within this sequence include amino acid sequences that comprise or consist of TGL, FTESFLTSQW (SEQ ID NO:211), or LTSQW (SEQ ID NO:212).

[0368] Some antibodies specifically bind to a peptide comprising or consisting of amino acid sequence RTEFGMAIGP (SEQ ID NO:213, corresponding to residues 167-176 of the sortilin ECD of SEQ ID NO:1, corresponding to residues 134-143 of sortilin ECD of SEQ ID NO:215). Some antibodies specifically bind to a peptide comprising or consisting of amino acid sequence WGFTEFLTS (SEQ ID NO:214, corresponding to residues 586-595 of the sortilin ECD of SEQ ID NO:1, corresponding to residues 553-562 of sortilin ECD of SEQ ID NO:215). Some antibodies specifically bind residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215. Some antibodies specifically bind residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215. Some antibodies specifically bind residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215. That is, binding of the antibody to the sortilin ECD can be reduced by mutations of any of the residues specified as forming an epitope.

[0369] These antibodies can be obtained by immunizing with a sortilin polypeptide purified from a natural source or recombinantly expressed. The invention also provides antibodies binding to the same epitope as any of the foregoing antibodies, such as, for example, the epitope of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. Also included are

antibodies competing for binding to sortilin with any of the foregoing antibodies, such as, for example, competing with 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. In an embodiment, antibodies binding to the same epitope as a reference antibody such as 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 or competing with the reference antibody share one or more of its functional properties, such as capacity to inhibit progranulin internalization into cells. Optionally, such property is possessed to the same extent within experimental error, or greater than that of the reference antibody. Some antibodies specifically binding to sortilin increase progranulin levels without inhibiting binding of other SORT1 ligands, such as neurotensin, to sortilin. Some antibodies specifically binding to sortilin do so without inducing internalization of sortilin.

[0370] The above-mentioned antibodies can be generated de novo by immunizing with a sortilin peptide comprising or consisting of amino acid sequence FTESFLT (SEQ ID NO:202), comprising or consisting of amino acid sequence ESFL (SEQ ID NO:203), comprising or consisting of amino acid sequence DGCILGYKEQFL (SEQ ID NO:204), comprising or consisting of amino acid sequence PSICLCSLEDL (SEQ ID NO:205), comprising or consisting of amino acid sequence E(S/Q/D)FL (SEQ ID NO:206), comprising or consisting of amino acid sequence RTEFGMAIGP (SEQ ID NO:213), comprising or consisting of amino acid sequence WGFTEFLTS (SEQ ID NO:214), or by immunizing with a full length sortilin ECD polypeptide or fragment thereof comprising such residues and screening for specific binding to a peptide including such residues. Antibodies against conformational epitopes such as an epitope comprising or consisting of residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, comprising or consisting of residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, or comprising or consisting of residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215 can be generated by immunizing with a full length ECD or fragment thereof including the residues of the epitope. Such sortilin peptides are preferably attached to a heterologous conjugate molecule that helps elicit an antibody response to the peptide. Attachment can be direct or via a spacer peptide or amino acid. Cysteine is used as a spacer amino acid because its free SH group facilitates attachment of a carrier molecule. A polyglycine linker (*e.g.*, 2-6 glycines), with or without a cysteine residue between the glycines and the peptide can also be used. The carrier molecule serves to provide a T-cell epitope that helps elicit an antibody

response against the peptide. Several carriers are commonly used particularly keyhole limpet hemocyanin (KLH), ovalbumin and bovine serum albumin (BSA). Peptide spacers can be added to peptide immunogen as part of solid phase peptide synthesis. Carriers are typically added by chemical cross-linking. Some examples of chemical crosslinkers that can be used include cross-N-maleimido-6-aminocaproyl ester or m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) (see for example, Harlow, E. et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. 1988; Sinigaglia et al., *Nature*, 336:778-780 (1988); Chicz et al., *J. Exp. Med.*, 178:27-47 (1993); Hammer et al., *Cell* 74:197-203 (1993); Falk K. et al., *Immunogenetics*, 39:230-242 (1994); WO 98/23635; and, Southwood et al. *J. Immunology*, 160:3363-3373 (1998)). The carrier and spacer if present can be attached to either end of the immunogen.

[0371] A peptide with optional spacer and carrier can be used to immunize laboratory animals or B-cells as described in more detail below. Hybridoma supernatants can be tested for ability to bind a sortilin peptide comprising or consisting of amino acid sequence FTESFLT (SEQ ID NO:202), comprising or consisting of amino acid sequence ESFL (SEQ ID NO:203), comprising or consisting of amino acid sequence DGCILGYKEQFL (SEQ ID NO:204), comprising or consisting of amino acid sequence PSICLCSLEDL (SEQ ID NO:205), comprising or consisting of amino acid sequence E(S/Q/D)FL (SEQ ID NO:206), comprising or consisting of amino acid sequence RTEFGMAIGP (SEQ ID NO:213), comprising or consisting of amino acid sequence WGFTEFLTS (SEQ ID NO:214). For conformational epitopes, antibodies binding to the extracellular domain of sortilin can be screened for competition with a reference antibody and/or to determine that binding is reduced by mutation of some or all residues within the epitope. When several residues of a conformational epitope are clustered within the length of a typical linear epitope (e.g., up to about 15 residues), a peptide comprising or consisting of these residues can be used to elicit an antibody. Thus, for example for an epitope defined by residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, a peptide comprising or consisting of residues 557-560 of the sortilin ECD of SEQ ID NO:215 can be used to elicit an antibody. For an epitope defined by residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, a peptide comprising or consist of residues 557-561 of the sortilin ECD of SEQ ID NO:215 can be used to elicit an antibody. For an epitope defined by residues E557, S558, F559, L560, P510, and Y535 of the

sortilin ECD of SEQ ID NO:215], a peptide comprising or consisting of residues 557-560 of the sortilin ECD of SEQ ID NO:215 can be used to elicit an antibody. The peptide can be attached to a carrier or other tag to facilitate the screening assay. In this case, the carrier or tag is preferentially different than the combination of spacer and carrier molecule used for immunization to eliminate antibodies specific for the spacer or carrier rather than the sortilin peptide.

[0372] An antibody designated 5E20 is an exemplary antibody specifically binding to sortilin. 5E20 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:4 and SEQ ID NO:10 respectively. Unless otherwise apparent from context, reference to 5E20 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. The antibody has been deposited as [DEPOSIT NUMBER]. This antibody specifically binds a peptide comprising or consisting of amino acid sequence FTESFLT (SEQ ID NO:202), comprising or consisting of amino acid sequence ESFL (SEQ ID NO:203), comprising or consisting of amino acid sequence DGCILGYKEQFL (SEQ ID NO:204), comprising or consisting of amino acid sequence PSICLCSLEDFL (SEQ ID NO:205), or comprising or consisting of amino acid sequence E(S/Q/D)FL (SEQ ID NO:206), or specifically binds residues E557, S558, F559, L560 and P510, and Y535 of the sortilin ECD of SEQ ID NO:215. Kabat/Chothia Composite CDRs of the heavy chain of 5E20 are designated SEQ ID NOS:5-7, respectively, and Kabat-Chothia Composite CDRs of the light chain of 5E20 are designated SEQ ID NOS:11-13, respectively. An exemplary signal peptide sequence for mouse 5E20 variable heavy chain is SEQ ID NO:3. An exemplary signal peptide sequence for mouse 5E20 variable light chain is SEQ ID NO:9.

[0373] An antibody designated 8H24 is another exemplary antibody specifically binding to sortilin. 8H24 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:28 and SEQ ID NO:34 respectively. Unless otherwise apparent from the context, reference to 8H24 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 8H24 has been deposited as [DEPOSIT NUMBER]. This antibody specifically binds a peptide comprising or consisting of amino acid sequence RTEFGMAIGP (SEQ ID NO:213) or specifically binds residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215. Kabat/Chothia Composite CDRs of the heavy chain of 8H24 are designated SEQ ID NOS:29-31, respectively,

and Kabat/Chothia Composite CDRs of the light chain of 8H24 are designated SEQ ID NOS:35-37, respectively. An exemplary signal peptide sequence for mouse 8H24 variable heavy chain is SEQ ID NO:27. An exemplary signal peptide sequence for mouse 8H24 variable light chain is SEQ ID NO:33.

[0374] An antibody designated 11M14 is another exemplary antibody specifically binding to sortilin. 11M14 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:52 and SEQ ID NO:58 respectively. Unless otherwise apparent from the context, reference to 11M14 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 11M14 has been deposited as [DEPOSIT NUMBER]. This antibody specifically binds a peptide comprising or consisting of amino acid sequence WGFTEFLTS (SEQ ID NO:214) or specifically binds residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215. Kabat/Chothia Composite CDRs of the heavy chain of 11M14 are designated SEQ ID NOS:53-55, respectively, and Kabat/Chothia Composite CDRs of the light chain of 11M14 are designated SEQ ID NOS:59-61, respectively. An exemplary signal peptide sequence for mouse 11M14 variable heavy chain is SEQ ID NO:51. An exemplary signal peptide sequence for mouse 11M14 variable light chain is SEQ ID NO:57.

[0375] An antibody designated 5M13 is another exemplary antibody specifically binding to sortilin. 5M13 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:78 and SEQ ID NO:84 respectively. Unless otherwise apparent from the context, reference to 5M13 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 5M13 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 5M13 are designated SEQ ID NOS:79-81, respectively, and Kabat/Chothia Composite CDRs of the light chain of 5M13 are designated SEQ ID NOS:85-87, respectively. An exemplary signal peptide sequence for mouse 5M13 variable heavy chain is SEQ ID NO:77. An exemplary signal peptide sequence for mouse 5M13 variable light chain is SEQ ID NO:83.

[0376] An antibody designated 2F18 is another exemplary antibody specifically binding to sortilin. 2F18 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:90 and SEQ ID NO:96 respectively. Unless otherwise apparent

from the context, reference to 2F18 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 2F18 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 2F18 are designated SEQ ID NOS:91-93, respectively, and Kabat/Chothia Composite CDRs of the light chain of 2F18 are designated SEQ ID NOS:97-99, respectively. An exemplary signal peptide sequence for mouse 2F18 variable heavy chain is SEQ ID NO:89. An exemplary signal peptide sequence for mouse 2F18 variable light chain is SEQ ID NO:95.

[0377] An antibody designated 2P22 is another exemplary antibody specifically binding to sortilin. 2P22 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:102 and SEQ ID NO:108 respectively. Unless otherwise apparent from the context, reference to 2P22 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 2P22 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 2P22 are designated SEQ ID NOS:103-105, respectively, and Kabat/Chothia Composite CDRs of the light chain of 2P22 are designated SEQ ID NOS:109-111, respectively. An exemplary signal peptide sequence for mouse 2P22 variable heavy chain is SEQ ID NO:101. An exemplary signal peptide sequence for mouse 2P22 variable light chain is SEQ ID NO:107.

[0378] An antibody designated 6B15 is another exemplary antibody specifically binding to sortilin. 6B15 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:114 and SEQ ID NO:120 respectively. Unless otherwise apparent from the context, reference to 6B15 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 6B15 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 6B15 are designated SEQ ID NOS:115-117, respectively, and Kabat/Chothia Composite CDRs of the light chain of 6B15 are designated SEQ ID NOS:121-123, respectively. An exemplary signal peptide sequence for mouse 6B15 variable heavy chain is SEQ ID NO:113. An exemplary signal peptide sequence for mouse 6B15 variable light chain is SEQ ID NO:119.

[0379] An antibody designated 2C14 is another exemplary antibody specifically binding to sortilin. 2C14 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:126 and SEQ ID NO:132 respectively. Unless otherwise apparent

from the context, reference to 2C14 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 2C14 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 2C14 are designated SEQ ID NOS:127-129, respectively, and Kabat/Chothia Composite CDRs of the light chain of 2C14 are designated SEQ ID NOS:133-135, respectively. An exemplary signal peptide sequence for mouse 2C14 variable heavy chain is SEQ ID NO:125. An exemplary signal peptide sequence for mouse 2C14 variable light chain is SEQ ID NO:131.

[0380] An antibody designated 9N18 is another exemplary antibody specifically binding to sortilin. 9N18 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:138 and SEQ ID NO:144 respectively. Unless otherwise apparent from the context, reference to 9N18 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 9N18 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 9N18 are designated SEQ ID NOS:139-141, respectively, and Kabat/Chothia Composite CDRs of the light chain of 9N18 are designated SEQ ID NOS:145-147, respectively. An exemplary signal peptide sequence for mouse 9N18 variable heavy chain is SEQ ID NO:137. An exemplary signal peptide sequence for mouse 9N18 variable light chain is SEQ ID NO:143.

[0381] An antibody designated 4N2 is another exemplary antibody specifically binding to sortilin. 4N2 has mature variable heavy and light regions (after cleavage of signal peptide) characterized by SEQ ID NO:150 and SEQ ID NO:156 respectively. Unless otherwise apparent from the context, reference to 4N2 should be understood as referring to any of the mouse, chimeric, veneered, and humanized forms of this antibody. 4N2 has been deposited as [DEPOSIT NUMBER]. Kabat/Chothia Composite CDRs of the heavy chain of 4N2 are designated SEQ ID NOS:151-153, respectively, and Kabat/Chothia Composite CDRs of the light chain of 4N2 are designated SEQ ID NOS:157-159, respectively. An exemplary signal peptide sequence for mouse 4N2 variable heavy chain is SEQ ID NO:149. An exemplary signal peptide sequence for mouse 4N2 variable light chain is SEQ ID NO:155.

[0382] Some antibodies of the invention bind to the same or overlapping epitope as an antibody designated 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. Other antibodies having such a binding specificity can be produced by immunizing mice with sortilin

or a portion thereof including the desired epitope (e.g. a sortilin peptide comprising or consisting of the amino acid sequence FTESFLT (SEQ ID NO:202), comprising or consisting of amino acid sequence ESFL (SEQ ID NO:203), comprising or consisting of amino acid sequence DGCILGYKEQFL (SEQ ID NO:204), comprising or consisting of amino acid sequence PSICLCSLEDFL (SEQ ID NO:205), or comprising or consisting of amino acid sequence E(S/Q/D)FL (SEQ ID NO:206), comprising or consisting of amino acid sequence RTEFGMAIGP (SEQ ID NO:213), comprising or consisting of amino acid sequence WGFTEFLTS (SEQ ID NO:214). To elicit antibodies binding to a conformational epitope comprising or consisting of residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, comprising or consisting of residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, or an epitope comprising or consisting of residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215, a full length ECD or fragment thereof including the residues of the peptides can be used. Resulting antibodies can be screened for binding to sortilin optionally in competition with an antibody having the variable regions of mouse 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. Fragments of sortilin including the desired epitope can be linked to a carrier that helps elicit an antibody response to the fragment and/or be combined with an adjuvant that helps elicit such a response. Such antibodies can be screened for differential binding to sortilin or a fragment thereof compared with mutants of specified residues. Screening against such mutants more precisely defines the binding specificity to allow identification of antibodies whose binding is inhibited by mutagenesis of particular residues and which are likely to share the functional properties of other exemplified antibodies. The mutations can be systematic replacement substitution with alanine (or serine if an alanine is present already) one residue at a time, or more broadly spaced intervals, throughout the target or throughout a section thereof in which an epitope is known to reside. If the same set of mutations significantly reduces the binding of two antibodies, the two antibodies bind the same epitope.

[0383] Antibodies having the binding specificity of a selected murine antibody (e.g., 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2) can also be produced using a variant of the phage display method. *See* Winter, WO 92/20791. This method is particularly suitable for producing human antibodies. In this method, either the heavy or light chain variable region of the selected murine antibody is used as a starting material. If, for example, a light

chain variable region is selected as the starting material, a phage library is constructed in which members display the same light chain variable region (*i.e.*, the murine starting material) and a different heavy chain variable region. The heavy chain variable regions can for example be obtained from a library of rearranged human heavy chain variable regions. A phage showing strong specific binding for sortilin or a fragment thereof (*e.g.*, at least 10^8 and preferably at least 10^9 M⁻¹) is selected. The heavy chain variable region from this phage then serves as a starting material for constructing a further phage library. In this library, each phage displays the same heavy chain variable region (*i.e.*, the region identified from the first display library) and a different light chain variable region. The light chain variable regions can be obtained for example from a library of rearranged human variable light chain regions. Again, phage showing strong specific binding for sortilin or a fragment thereof are selected. The resulting antibodies usually have the same or similar epitope specificity as the murine starting material.

[0384] Kabat/Chothia Composite CDRs of the heavy chain of 5E20 are designated SEQ ID NOS:5-7, respectively, and Kabat/Chothia Composite CDRs of the light chain of 5E20 are designated SEQ ID NOS:11-13, respectively.

[0385] Table 2 indicates the 5E20 CDRs as defined by Kabat, Chothia, Composite of Chothia and Kabat (also referred to herein as “Kabat/Chothia Composite”), AbM, and Contact.

Table 2: 5E20 CDRs as defined by Kabat, Chothia, Composite of Chothia and Kabat, AbM, and Contact

Loop	Kabat	Chothia	Composite of Chothia & Kabat	AbM	Contact
L1	L24--L34 SEQ ID NO:11	L24--L34 SEQ ID NO:11	L24--L34 SEQ ID NO:11	L24--L34 SEQ ID NO:11	L30--L36 SEQ ID NO:21
L2	L50--L56 SEQ ID NO:12	L50--L56 SEQ ID NO:12	L50--L56 SEQ ID NO:12	L50--L56 SEQ ID NO:12	L46--L55 SEQ ID NO:22
L3	L89--L97 SEQ ID NO:13	L89--L97 SEQ ID NO:13	L89--L97 SEQ ID NO:13	L89--L97 SEQ ID NO:13	L89--L96 SEQ ID NO:23
H1	H31--H35B SEQ ID NO:14	H26--H32 SEQ ID NO:15	H26--H35B SEQ ID NO:5	H26--H35B SEQ ID NO:5	H30--H35B SEQ ID NO:18
H2	H50--H65 SEQ ID NO:6	H52--H56 SEQ ID NO:16	H50--H65 SEQ ID NO:6	H50--H58 SEQ ID NO:17	H47--H58 SEQ ID NO:19
H3	H95--H102 SEQ ID NO:7	H95--H102 SEQ ID NO:7	H95--H102 SEQ ID NO:7	H95--H102 SEQ ID NO:7	H93--H101 SEQ ID NO:20

[0386] Kabat/Chothia Composite CDRs of the heavy chain of 8H24 are designated SEQ ID NOS:29-31, respectively, and Kabat/Chothia Composite CDRs of the light chain of 8H24 are designated SEQ ID NOS:35-37, respectively.

[0387] Table 3 indicates the 8H24 CDRs as defined by Kabat, Chothia, Composite of Chothia and Kabat (also referred to herein as “Kabat/Chothia Composite”), AbM, and Contact.

Table 3: 8H24 CDRs as defined by Kabat, Chothia, Composite of Chothia and Kabat, AbM, and Contact

Loop	Kabat	Chothia	Composite of Chothia & Kabat	AbM	Contact
L1	L24--L34 SEQ ID NO:35	L24--L34 SEQ ID NO:35	L24--L34 SEQ ID NO:35	L24--L34 SEQ ID NO:35	L30--L36 SEQ ID NO:45
L2	L50--L56 SEQ ID NO:36	L50--L56 SEQ ID NO:36	L50--L56 SEQ ID NO:36	L50--L56 SEQ ID NO:36	L46--L55 SEQ ID NO:46
L3	L89--L97 SEQ ID NO:37	L89--L97 SEQ ID NO:37	L89--L97 SEQ ID NO:37	L89--L97 SEQ ID NO:37	L89--L96 SEQ ID NO:47
H1	H31--H35B SEQ ID NO:38	H26--H32 SEQ ID NO:39	H26--H35B SEQ ID NO:29	H26--H35B SEQ ID NO:29	H30--H35B SEQ ID NO:42
H2	H50--H65 SEQ ID NO:30	H52--H56 SEQ ID NO:40	H50--H65 SEQ ID NO:30	H50--H58 SEQ ID NO:41	H47--H58 SEQ ID NO:43
H3	H95--H102 SEQ ID NO:31	H95--H102 SEQ ID NO:31	H95--H102 SEQ ID NO:31	H95--H102 SEQ ID NO:31	H93--H101 SEQ ID NO:44

[0388] Kabat/Chothia Composite CDRs of the heavy chain of 11M14 are designated SEQ ID NOS:53-55, respectively, and Kabat/Chothia Composite CDRs of the light chain of 11M14 are designated SEQ ID NOS:59-61 respectively.

[0389] Table 4 indicates the 11M14 CDRs as defined by Kabat, Chothia, Composite of Chothia and Kabat (also referred to herein as “Kabat/Chothia Composite”), AbM, and Contact.

Table 4: 11M14 CDRs as defined by Kabat, Chothia, Composite of Chothia and Kabat, AbM, and Contact

Loop	Kabat	Chothia	Composite of Chothia & Kabat	AbM	Contact
L1	L24--L34 SEQ ID NO:59	L24--L34 SEQ ID NO:59	L24--L34 SEQ ID NO:59	L24--L34 SEQ ID NO:59	L30--L36 SEQ ID NO:69
L2	L50--L56 SEQ ID NO:60	L50--L56 SEQ ID NO:60	L50--L56 SEQ ID NO:60	L50--L56 SEQ ID NO:60	L46--L55 SEQ ID NO:70
L3	L89--L97 SEQ ID NO:61	L89--L97 SEQ ID NO:61	L89--L97 SEQ ID NO:61	L89--L97 SEQ ID NO:61	L89--L96 SEQ ID NO:71
H1	H31--H35B SEQ ID NO:62	H26--H32 SEQ ID NO:63	H26--H35B SEQ ID NO:53	H26--H35B SEQ ID NO:53	H30--H35B SEQ ID NO:66
H2	H50--H65 SEQ ID NO:54	H52--H56 SEQ ID NO:64	H50--H65 SEQ ID NO:54	H50--H58 SEQ ID NO:65	H47--H58 SEQ ID NO:67
H3	H95--H102 SEQ ID NO:55	H95--H102 SEQ ID NO:55	H95--H102 SEQ ID NO:55	H95--H102 SEQ ID NO:55	H93--H101 SEQ ID NO:68

[0390] Kabat/Chothia Composite CDRs of the heavy chain of 5M13 are designated SEQ ID NOS:79-81, respectively, and Kabat/Chothia Composite CDRs of the light chain of 5M13 are designated SEQ ID NOS:85-87, respectively.

[0391] Kabat/Chothia Composite CDRs of the heavy chain of 2F18 are designated SEQ ID NOS:91-93, respectively, and Kabat/Chothia Composite CDRs of the light chain of 2F18 are designated SEQ ID NOS:97-99, respectively.

[0392] Kabat/Chothia Composite CDRs of the heavy chain of 2P22 are designated SEQ ID NOS:103-105, respectively, and Kabat/Chothia Composite CDRs of the light chain of 2P22 are designated SEQ ID NOS:109-111, respectively.

[0393] Kabat/Chothia Composite CDRs of the heavy chain of 6B15 are designated SEQ ID NOS:115-117, respectively, and Kabat/Chothia Composite CDRs of the light chain of 6B15 are designated SEQ ID NOS:121-123, respectively.

[0394] Kabat/Chothia Composite CDRs of the heavy chain of 2C14 are designated SEQ ID NOS:127-129, respectively, and Kabat/Chothia Composite CDRs of the light chain of 2C14 are designated SEQ ID NOS:133-135, respectively.

[0395] Kabat/Chothia Composite CDRs of the heavy chain of 9N18 are designated SEQ ID NOS:139-141, respectively, and Kabat/Chothia Composite CDRs of the light chain of 9N18 are designated SEQ ID NOS:145-147, respectively.

[0396] Kabat/Chothia Composite CDRs of the heavy chain of 4N2 are designated SEQ ID NOS:151-153, respectively, and Kabat/Chothia Composite CDRs of the light chain of 4N2 are designated SEQ ID NOS:157-159, respectively.

[0397] Other antibodies can be obtained by mutagenesis of cDNA encoding the heavy and light chains of an exemplary antibody, such as 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. Monoclonal antibodies that are at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 in amino acid sequence of the mature heavy and/or light chain variable regions and maintain its functional properties, and/or which differ from the respective antibody by a small number of functionally inconsequential amino acid substitutions (*e.g.*, conservative substitutions), deletions, or insertions are also included in the invention. Monoclonal antibodies having at least one or all six CDR(s) as defined by any conventional definition, but preferably Kabat, that are 90%, 95%, 99% or 100% identical to corresponding CDRs of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 are also included.

[0398] The invention also provides antibodies having some or all (*e.g.*, 3, 4, 5, and 6) CDRs entirely or substantially from 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. Such antibodies can include a heavy chain variable region that has at least two, and usually all three, CDRs entirely or substantially from the heavy chain variable region of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 and/or a light chain variable region having at least two, and usually all three, CDRs entirely or substantially from the light chain variable region of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. The antibodies can include both heavy and light chains. A CDR is substantially from a corresponding 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 CDR when it contains no more than 4, 3, 2, or 1 substitutions, insertions, or deletions, except that CDR-H2

(when defined by Kabat) can have no more than 6, 5, 4, 3, 2, or 1 substitutions, insertions, or deletions. Such antibodies can have at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identity to 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 in the amino acid sequence of the mature heavy and/or light chain variable regions and maintain their functional properties, and/or differ from 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 by a small number of functionally inconsequential amino acid substitutions (*e.g.*, conservative substitutions), deletions, or insertions.

[0399] Some antibodies identified by such assays can bind to sortilin.

[0400] The invention further provides a means for specifically binding to a peptide consisting of residues E(S/Q/D)FL (SEQ ID NO:206), residues FTESFLT (SEQ ID NO:202), residues ESFL(SEQ ID NO:203), residues DGCILGYKEQFL (SEQ ID NO:204), or residues PSICLCSLEDFL (SEQ ID NO:205), or specifically binds residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215.. An exemplary means is an antibody comprising the heavy chain CDRs of SEQ ID NOS:5-7 and light chain CDRs of SEQ ID NOS.:11-13.

[0401] The invention further provides a means for specifically binding to a peptide consisting of residues RTEFGMAIGP (SEQ ID NO:213) or specifically binding residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215. An exemplary means is an antibody comprising the heavy chain CDRs of SEQ ID NOS: 29-31 and light chain CDRs of SEQ ID NOS. 35-37.

[0402] The invention further provides a means for specifically binding to a peptide consisting of residues WGFTESFLTS (SEQ ID NO:214) or specifically binding residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215. An exemplary means is an antibody comprising the heavy chain CDRs of SEQ ID NOS: 53-55 and light chain CDRs of SEQ ID NOS. 59-61.

B. Non-Human Antibodies

[0403] The production of other non-human antibodies, *e.g.*, murine, guinea pig, primate, rabbit or rat, against sortilin or a fragment thereof (*e.g.*, a peptide comprising an amino acid sequence of FTESFLT (SEQ ID NO:202), ESFL(SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), or PSICLCSLEDFL (SEQ ID NO:205), E(S/Q/D)FL (SEQ ID NO:206), RTEFGMAIGP) (SEQ ID NO:213), or WGFTEFLTS (SEQ ID NO:214) can be accomplished by, for example, immunizing the animal with sortilin or a fragment thereof. *See* Harlow & Lane, *Antibodies, A Laboratory Manual* (CSHP NY, 1988) (incorporated by reference for all purposes). Antibodies against conformational epitopes such as an epitope comprising, residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, residues K110, Y535, E557, T561, and Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, or residues E557, S558, F559, L560 and P510, and Y535 of the sortilin ECD of SEQ ID NO:215 can be elicited using a full-length ECD or sufficient of the ECD to span the epitope residues. Optionally, the immunogen can be an extracellular domain (ECD) of recombinant human Sortilin with a C-terminal HIS tag (ECD-huSortilin-HIS). Optionally, the animal is immunized with a sortilin fragment comprising a peptide represented by E(S/Q/D)FL (SEQ ID NO:206), linked to a carrier. Optionally, the peptide is FTESFLT (SEQ ID NO:202), ESFL(SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), RTEFGMAIGP (SEQ ID NO:213), or WGFTEFLTS (SEQ ID NO:214). Such an immunogen can be obtained from a natural source, by peptide synthesis, or by recombinant expression. Optionally, the immunogen can be administered fused or otherwise complexed with a carrier protein. Optionally, the immunogen can be administered with an adjuvant. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant can be used for immunization of laboratory animals. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Mice are typically used for making monoclonal antibodies. Antibodies are screened for specific binding to sortilin or an epitope within sortilin (*e.g.*, FTESFLT (SEQ ID NO:202), ESFL(SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), E(S/Q/D)FL (SEQ ID NO:206), RTEFGMAIGP (SEQ ID NO:213), WGFTEFLTS (SEQ ID NO:214), or to an epitope defined by residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, or to an epitope defined by residues K110, Y535, E557, T561, Q563, D74, P510, S558,

F559, and L560 of the sortilin ECD of SEQ ID NO:215, or to an epitope defined by residues E557, S558, F559, L560 and P510, and Y535 of the sortilin ECD of SEQ ID NO:215).

Optionally, the screening can be performed against 15 amino acid peptides comprising FTESFLT (SEQ ID NO:202), ESFL (SEQ ID NO:203), DGCILGYKEQFL (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), RTEFGMAIGP (SEQ ID NO:213), or WGFTESFLTS (SEQ ID NO:214), or a consensus motif represented by E(S/Q/D)FL (SEQ ID NO:206).

Optionally, the peptides comprise or consist of FTESFLT (SEQ ID NO:202), ESFL (SEQ ID NO:203), DGCILGYKEQFL (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), RTEFGMAIGP (SEQ ID NO:213), or WGFTESFLTS (SEQ ID NO:214). Such screening can be accomplished by determining binding of an antibody to a collection of sortilin variants, such as sortilin variants of SEQ ID NOs: 216-243, or such as sortilin variants comprising or consisting of amino acid residues 588-594 or 590-593 or 632-643 or 663-674 or 167-176 or 586-595 of SEQ ID NO:1), or mutations within these residues, and determining which sortilin variants bind to the antibody. Binding can be assessed, for example, by Western blot, FACS or ELISA.

C. Humanized Antibodies

[0404] A humanized antibody is a genetically engineered antibody in which CDRs from a non-human “donor” antibody are grafted into human “acceptor” antibody sequences (see, e.g., Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205; and Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. Thus, a humanized antibody is an antibody having at least three, four, five or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light

chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 85%, 90%, 95% or 100% of corresponding residues (as defined by any conventional definition but preferably defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85%, 90%, 95% or 100% of corresponding residues defined by Kabat are identical. To be classified as humanized under the 2014 World Health Organization (WHO) International non-proprietary names (INN) definition of humanized antibodies, an antibody must have at least 85% identity to human germline antibody sequences (i.e., prior to somatic hypermutation). Mixed antibodies are antibodies for which one antibody chain (e.g., heavy chain) meets the threshold but the other chain (e.g., light chain) does not meet the threshold. An antibody is classified as chimeric if neither chain meets the threshold, even though the variable framework regions for both chains were substantially human with some murine backmutations. See, Jones et al. (2016) The INNs and outs of antibody nonproprietary names, mAbs 8:1, 1-9, DOI: 10.1080/19420862.2015.1114320. See also “WHO-INN: International nonproprietary names (INN) for biological and biotechnological substances (a review)” (Internet) 2014. Available from: <http://www.who.int/medicines/services/inn/BioRev2014.pdf>), incorporated herein by reference. For the avoidance of doubt, the term “humanized” as used herein is not intended to be limited to the 2014 WHO INN definition of humanized antibodies. Some of the humanized antibodies provided herein have at least 85% sequence identity to human germline sequences and some of the humanized antibodies provided herein have less than 85% sequence identity to human germline sequences. Some of the heavy chains of the humanized antibodies provided herein have from about 60% to 100% sequence identity to human germ line sequences, such as, for example, in the range of about 60% to 69%, 70% to 79%, 80% to 84%, or 85% to 89%. Some heavy chains fall below the 2014 WHO INN definition and have, for example, about 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, or 82%, 83%, or 84% sequence identity to human germ line sequences, while other heavy chains meet the 2014 WHO INN definition and have about 85%, 86%, 87%, 88%, 89% or greater sequence

identity to human germ line sequences. Some of the light chains of the humanized antibodies provided herein have from about 60% to 100% sequence identity to human germ line sequences, such as, for example, in the range of about 80% to 84% or 85% to 89%. Some light chains fall below the 2014 WHO INN definition and have, for example, about 81%, 82%, 83% or 84% sequence identity to human germ line sequences, while other light chains meet the 2014 WHO INN definition and have about 85%, 86%, 87%, 88%, 89% or greater sequence identity to human germ line sequences. Some humanized antibodies provided herein that are "chimeric" under the 2014 WHO INN definition have heavy chains with less than 85% identity to human germ line sequences paired with light chains having less than 85% identity to human germ line sequences. Some humanized antibodies provided herein are "mixed" under the 2014 WHO INN definition, for example, having a heavy chain with at least 85% sequence identity to human germ line sequences paired with a light chain having less than 85% sequence identity to human germ line sequences, or vice versa. Some humanized antibodies provided herein meet the 2014 WHO INN definition of "humanized" and have a heavy chain with at least 85% sequence identity to human germ line sequences paired with a light chain having at least 85% sequence identity to human germ line sequences. Some humanized antibodies provided herein meet the 2014 WHO INN definition of "mixed." Exemplary 5E20 antibodies that meet the 2014 WHO INN definition of "mixed" include antibodies having a mature heavy chain with the amino acid sequence of any of SEQ ID NOS:163-166 and SEQ ID NO:168 paired with a mature light chain sequence having an amino acid sequence of any of SEQ ID NO:173-176. Exemplary 8H24 antibodies that meet the 2014 WHO INN definition of "mixed" include antibodies having a mature heavy chain with the amino acid sequence of SEQ ID NO:180 or SEQ ID NO:181 paired with a mature light chain sequence having an amino acid sequence of SEQ ID NO:185 or SEQ ID NO:186. Exemplary 11M14 antibodies that meet the 2014 WHO INN definition of "mixed" include antibodies having a mature heavy chain with the amino acid sequence of any of SEQ ID NOS:190-192 paired with a mature light chain sequence having an amino acid sequence of any of SEQ ID NOS:196-199. Additional humanized 5E20 antibodies of the invention include antibodies having a mature heavy chain having an amino acid sequence of SEQ ID NOS:167 or SEQ ID NO:169 paired with a mature light chain having an amino acid sequence of any of SEQ ID NOS:173-176.

[0405] Although humanized antibodies often incorporate all six CDRs (defined by any conventional definition but preferably as defined by Kabat) from a mouse antibody, they can also

be made with less than all CDRs (*e.g.*, at least 3, 4, or 5 CDRs) from a mouse antibody (*e.g.*, Pascalis *et al.*, *J. Immunol.* 169:3076, 2002; Vajdos *et al.*, *J. of Mol. Biol.*, 320: 415-428, 2002; Iwahashi *et al.*, *Mol. Immunol.* 36:1079-1091, 1999; Tamura *et al.*, *J. Immunol.*, 164:1432-1441, 2000).

[0406] In some antibodies only part of the CDRs, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (Chothia, *J. Mol. Biol.* 196:901, 1987), by molecular modeling and/or empirically, or as described in Gonzales *et al.*, *Mol. Immunol.* 41: 863, 2004. In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor CDR is omitted, the amino acid occupying the position can be an amino acid occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of mouse amino acids in a humanized antibody and consequently decreasing potential immunogenicity and/or for meeting the WHO INN definition of “humanized”. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

[0407] The human acceptor antibody sequences can optionally be selected from among the many known human antibody sequences to provide a high degree of sequence identity (*e.g.*, 65-85% identity) between a human acceptor sequence variable region frameworks and corresponding variable region frameworks of a donor antibody chain.

[0408] Some humanized antibodies have the same (within experimental error) or improved functional properties, *e.g.*, binding affinity for human sortilin, increasing extracellular progranulin in plasma while minimizing decrease in surface sortilin as described in the examples as a murine antibody from which they were derived. For example, some humanized antibodies have a binding affinity within a factor of 3, 2 or 1 of the murine antibody from which they were derived or an affinity indistinguishable within experimental error. Some humanized antibodies

increase extracellular progranulin in plasma while minimizing decrease in surface sortilin as described in the examples within a factor of 3, 2 or 1 of the murine antibody from which they were derived or inhibit the same within experimental error as the mouse antibody from which they were derived.

[0409] An example of an acceptor sequence for the 5E20 heavy chain is the human mature heavy chain variable region of human antibody AEX29086 VH (AEX29086-VH_huFrwk; SEQ ID NO:161). The heavy chain variable domains of 5E20 and AEX29086 VH share identical lengths for the CDR-H1, H2 loops. An example of an acceptor sequence for the 5E20 heavy chain is the human mature heavy chain variable region IMGT# IGHV3-21*01 (SEQ ID NO:162). An example of an acceptor sequence for the 5E20 light chain is the human mature light chain variable region human antibody BAH04687 VL (BAH04687-VL_huFrwk; SEQ ID NO:171). The variable light domain of 5E20 and BAH04687 antibody share identical lengths for the CDR-L1, L2 and L3 loops. An example of an acceptor sequence for the 5E20 light chain is the human mature light chain variable region IGKV1-12*01 (SEQ ID NO:172).

[0410] An example of an acceptor sequence for the 8H24 heavy chain is the human antibody AAC51714 VH (GenBank Acc. # AAC51714-VH_huFrwk; SEQ ID NO:178). The variable heavy domains of 8H24 and AAC51714 also share identical lengths for the CDR-H1, H2 loops. An example of an acceptor sequence for the 8H24 heavy chain is the human mature heavy chain variable region IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179). An example of an acceptor sequence for the 8H24 light chain is the human mature light chain variable region human antibody ABC66914 VL (GenBank Acc. # ABC66914-VL_huFrwk; SEQ ID NO:183). The variable light domain of 8H24 and ABC66914 antibody also share identical lengths for the CDR-L1, L2 and L3 loops. An example of an acceptor sequence for the 8H24 light chain is the human mature light chain variable region IMGT# IGKV2-40*01 (SEQ ID NO:184).

[0411] An example of an acceptor sequence for the 11M14 heavy chain is the human mature heavy chain variable region of human Ig heavy chain ACS96198 (GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188). human Ig heavy chain ACS96198 has the same canonical classes as 11M14 heavy chain variable region CDRs. An example of an acceptor sequence for the 11M14 heavy chain is the human mature heavy chain variable region IMGT# IGHV3-48*03 (SEQ ID NO:189). An example of an acceptor sequence for the 11M14 light chain is the a

human kappa light chain with NCBI accession code CBZ39892 (GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194). This has the same canonical classes for CDR-L1 & L2. An example of an acceptor sequence for the 11M14 light chain is the human mature light chain variable region with IMGT# IGKV1-39*01 (SEQ ID NO:195).

[0412] If more than one human acceptor antibody sequence is selected, a composite or hybrid of those acceptors can be used, and the amino acids used at different positions in the humanized light chain and heavy chain variable regions can be taken from any of the human acceptor antibody sequences used.

[0413] Certain amino acids from the human variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids.

[0414] For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid can be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

- (1) noncovalently binds antigen directly;
- (2) is adjacent to a CDR region or within a CDR as defined by Chothia but not Kabat;
- (3) otherwise interacts with a CDR region (*e.g.*, is within about 6 Å of a CDR region), (*e.g.*, identified by modeling the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); or
- (4) is a residue participating in the VL-VH interface.

[0415] In an embodiment, humanized sequences are generated using a two-stage PCR protocol that allows introduction of multiple mutations, deletions, and insertions using QuikChange site-directed mutagenesis [Wang, W. and Malcolm, B.A. (1999) *BioTechniques* 26:680-682].

[0416] Framework residues from classes (1) through (3) as defined by Queen, US 5,530,101, are sometimes alternately referred to as canonical and vernier residues. Framework residues that help define the conformation of a CDR loop are sometimes referred to as canonical residues (Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Thornton & Martin, *J. Mol. Biol.* 263:800-815 (1996)). Framework residues that support antigen-binding loop conformations and play a

role in fine-tuning the fit of an antibody to antigen are sometimes referred to as vernier residues (Foote & Winter, *J. Mol. Biol* 224:487-499 (1992)).

[0417] Other framework residues that are candidates for substitution are residues creating a potential glycosylation site. Still other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins.

[0418] Other framework residues that are candidates for substitution are N-terminal glutamine residues (Q) that may be replaced with glutamic acid (E) to minimize potential for pyroglutamate conversion [Y. Diana Liu, et al., 2011, *J. Biol. Chem.*, 286: 11211–11217]. Glutamic acid (E) conversion to pyroglutamate (pE) occurs more slowly than from glutamine (Q). Because of the loss of a primary amine in the glutamine to pE conversion, antibodies become more acidic. Incomplete conversion produces heterogeneity in the antibody that can be observed as multiple peaks using charge-based analytical methods. Heterogeneity differences may indicate a lack of process control.

[0419] Exemplary humanized antibodies include humanized forms of mouse 5E20, designated Hu5E20.

[0420] The mouse antibody 5E20 comprises mature heavy and light chain variable regions having amino acid sequences comprising SEQ ID NO:4 and SEQ ID NO:10, respectively. The invention provides 7 exemplified humanized mature heavy chain variable regions: hu5E20VHv1, hu5E20VHv2, hu5E20VHv3, hu5E20VHv4, hu5E20VHv5, hu5E20VHv6, and hu5E20VHv7. The invention further provides 4 exemplified mature light chain variable regions hu5E20VLv1, hu5E20VLv2, hu5E20VLv3, and hu5E20VLv4. Figures 1 and 2 show alignments of the heavy chain variable region and light chain variable region, respectively, of murine 5E20 and various humanized antibodies.

[0421] For reasons such as possible influence on CDR conformation and/or binding to antigen, mediating interaction between heavy and light chains, interaction with the constant region, being a site for desired or undesired post-translational modification, being an unusual residue for its position in a human variable region sequence and therefore potentially immunogenic, reducing

aggregation potential, and other reasons, the following 18 variable region framework positions were considered as candidates for substitutions in the 4 exemplified human mature light chain variable regions and the 7 exemplified human mature heavy chain variable regions, as further specified in the examples: L11 (L11V), L36 (Y36L), L44 (P44F), L46 (L46G), L69 (T69A), L85 (T85D), L87 (Y87F), L100 (G100Q), L106 (I106K), H5 (L5V), H40 (A40T), H42 (G42D), H44 (G44R), H49 (S49A), H77 (T77S), H83 (R83K), H93 (A93S), H94 (K94R).

[0422] Here, as elsewhere, the first-mentioned residue is the residue of a humanized antibody formed by grafting Kabat CDRs or a composite Chothia-Kabat CDR in the case of CDR-H1 into a human acceptor framework, and the second-mentioned residue is a residue being considered for replacing such residue. Thus, within variable region frameworks, the first mentioned residue is human, and within CDRs, the first mentioned residue is mouse.

[0423] Exemplified antibodies include any permutations or combinations of the exemplified mature heavy and light chain variable regions hu5E20VHv1/ hu5E20VLv1, hu5E20VHv1/ hu5E20VLv2, hu5E20VHv1/ hu5E20VLv3, hu5E20VHv1/ hu5E20VLv4, hu5E20VHv2/ hu5E20VLv1, hu5E20VHv2/ hu5E20VLv2, hu5E20VHv2/ hu5E20VLv3, hu5E20VHv2/ hu5E20VLv4, hu5E20VHv3/ hu5E20VLv1, hu5E20VHv3/ hu5E20VLv2, hu5E20VHv3/ hu5E20VLv3, hu5E20VHv3/ hu5E20VLv4, hu5E20VHv4/ hu5E20VLv1, hu5E20VHv4/ hu5E20VLv2, hu5E20VHv4/ hu5E20VLv3, hu5E20VHv4/ hu5E20VLv4, hu5E20VHv5/ hu5E20VLv1, hu5E20VHv5/ hu5E20VLv2, hu5E20VHv5/ hu5E20VLv3, hu5E20VHv5/ hu5E20VLv4, hu5E20VHv6/ hu5E20VLv1, hu5E20VHv6/ hu5E20VLv2, hu5E20VHv6/ hu5E20VLv3, hu5E20VHv6/ hu5E20VLv4, hu5E20VHv7/ hu5E20VLv1, hu5E20VHv7/ hu5E20VLv2, hu5E20VHv7/ hu5E20VLv3, hu5E20VHv7/ hu5E20VLv4.

[0424] Exemplified antibodies include any permutations or combinations of the exemplified mature heavy chain variable regions hu5E20VHv1 (SEQ ID NO:163), hu5E20VHv2 (SEQ ID NO:164), hu5E20VHv3 (SEQ ID NO:165), hu5E20VHv4 (SEQ ID NO:166), hu5E20VHv5 (SEQ ID NO:167), hu5E20VHv6 (SEQ ID NO:168), and hu5E20VHv7 (SEQ ID NO:169), with any of the exemplified mature light chain variable regions hu5E20VLv1 (SEQ ID NO:173), hu5E20VLv2 (SEQ ID NO:174), hu5E20VLv3 (SEQ ID NO:175), and hu5E20VLv4 (SEQ ID NO:176).

[0425] The invention provides variants of the 5E20 humanized antibody in which the humanized mature heavy chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to hu5E20VHv1 (SEQ ID NO:163), hu5E20VHv2 (SEQ ID NO:164), hu5E20VHv3 (SEQ ID NO:165), hu5E20VHv4 (SEQ ID NO:166), hu5E20VHv5 (SEQ ID NO:167), hu5E20VHv6 (SEQ ID NO:168), or hu5E20VHv7 (SEQ ID NO:169), and the humanized mature light chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to hu5E20VLv1 (SEQ ID NO:173), hu5E20VLv2 (SEQ ID NO:174), hu5E20VLv3 (SEQ ID NO:175), or hu5E20VLv4 (SEQ ID NO:176). In some such antibodies at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, or all 18 of the backmutations or other mutations in SEQ ID NOS:163-169, and SEQ ID NOS:173-176 are retained.

[0426] In some humanized 5E20 antibodies, at least one of the following positions in the VH region is occupied by the amino acid as specified: H5 is occupied by L or V, H40 is occupied by A or T, H42 is occupied by G or D, H44 is occupied by G or R, H49 is occupied by A, H77 is occupied by T or S, H83 is occupied by R or K, H93 is occupied by S, H94 is occupied by R.

[0427] In some humanized 5E20 antibodies, positions H49, H93, and H94 in the VH region are occupied by A, S, and R, respectively, as in hu5E20VHv1. In some humanized 5E20 antibodies, positions H5, H49, H77, H93, and H94 in the VH region are occupied by V, A, S, S, and R, respectively, as in hu5E20VHv2. In some humanized 5E20 antibodies, positions H5, H44, H49, H77, H93, and H94 in the VH region are occupied by V, R, A, S, S, and R, respectively, as in hu5E20VHv3. In some humanized 5E20 antibodies, positions H5, H42, H44, H49, H77, H93, and H94 in the VH region are occupied by V, D, R, A, S, S, and R, respectively, as in hu5E20VHv4. In some humanized 5E20 antibodies, positions H5, H42, H44, H49, H77, H83, H93, and H94 in the VH region are occupied by V, D, R, A, S, K, S, and R, respectively, as in hu5E20VHv5. In some humanized 5E20 antibodies, positions H5, H40, H44, H49, H77, H93, and H94 in the VH region are occupied by V, T, R, A, S, S, and R, respectively, as in hu5E20VHv6. In some humanized 5E20 antibodies, positions H5, H40, H42, H44, H49, H77, H93, and H94 in the VH region are occupied by V, T, D, R, A, S, S, and R, respectively, as in hu5E20VHv7.

[0428] In some humanized 5E20 antibodies, at least one of the following positions in the VL region is occupied by the amino acid as specified: L11 is L or V, L36 is L, L44 is F, L46 is G,

L69 is A, L85 is T or D, L87 is F, L100 is G or Q, L106 is I or K. In some humanized 5E20 antibodies, positions L36, L44, L46, L69, and L87 in the VL region are occupied by L, F, G, A, and F, respectively, as in hu5E20VLv1. In some humanized 5E20 antibodies, positions L11, L36, L44, L46, L69, and L87 in the VL region are occupied by V, L, F, G, A, and F, respectively, as in 5E20VLv2. In some humanized 5E20 antibodies, positions L11, L36, L44, L46, L69, L87, L100, and L106 in the VL region are occupied by V, L, F, G, A, F, Q, and K, respectively, as in hu5E20VLv3. In some humanized 5E20 antibodies, positions L11, L36, L44, L46, L69, L85, L87, L100, and L106 in the VL region are occupied by V, L, F, G, A, D, F, Q, and K, respectively, as in hu5E20VLv4.

[0429] In some humanized 5E20 antibodies, the variable heavy chain has $\geq 85\%$ identity to human sequence. In some humanized 5E20 antibodies, the variable light chain has $\geq 85\%$ identity to human sequence. In some humanized 5E20 antibodies, each of the variable heavy chain and variable light chain has $\geq 85\%$ identity to human germline sequence. In some humanized 5E20 antibodies, the three heavy chain CDRs are as defined by Kabat/Chothia Composite (SEQ ID NOS:5-7) and the three light chain CDRs are as defined by Kabat/Chothia Composite (SEQ ID NOS:11-13).

[0430] Exemplary humanized antibodies include humanized forms of mouse 8H24, designated Hu8H24.

[0431] The mouse antibody 8H24 comprises mature heavy and light chain variable regions having amino acid sequences comprising SEQ ID NO:28 and SEQ ID NO:34, respectively. The invention provides 2 exemplified humanized mature heavy chain variable regions: hu8H24VHv1 and hu8H24VHv2. The invention further provides 2 exemplified mature light chain variable regions hu8H24VLv1 and hu8H24VLv2. Figures 3 and 4 show alignments of the heavy chain variable region and light chain variable region, respectively, of murine 8H24 and various humanized antibodies.

[0432] For reasons such as possible influence on CDR conformation and/or binding to antigen, mediating interaction between heavy and light chains, interaction with the constant region, being a site for desired or undesired post-translational modification, being an unusual residue for its position in a human variable region sequence and therefore potentially immunogenic, reducing aggregation potential, and other reasons, the following 10 variable region framework positions

were considered as candidates for substitutions in the 2 exemplified human mature light chain variable regions and the 2 exemplified human mature heavy chain variable regions, as further specified in the examples: L2 (I2V), L9 (L9S), L74 (K74T), H2 (V2A), H12 (K12V), H48 (M48I), H67 (V67A), H71 (A71V), H91 (Y91F), H108 (L108T).

[0433] Here, as elsewhere, the first-mentioned residue is the residue of a humanized antibody formed by grafting Kabat CDRs or a composite Chothia-Kabat CDR in the case of CDR-H1 into a human acceptor framework, and the second-mentioned residue is a residue being considered for replacing such residue. Thus, within variable region frameworks, the first mentioned residue is human, and within CDRs, the first mentioned residue is mouse.

[0434] Exemplified antibodies include any permutations or combinations of the exemplified mature heavy and light chain variable regions hu8H24VHv1/ hu8H24VLv1, hu8H24VHv1/ hu8H24VLv2, hu8H24VHv2/ hu8H24VLv1, hu8H24VHv2/ hu8H24VLv2.

[0435] Exemplified antibodies include any permutations or combinations of the exemplified mature heavy chain variable regions hu8H24VHv1 (SEQ ID NO:180) and hu8H24VHv2 (SEQ ID NO:181), with any of the exemplified mature light chain variable regions hu8H24VLv1 (SEQ ID NO:185) and hu8H24VLv2 (SEQ ID NO:186).

[0436] The invention provides variants of the 8H24 humanized antibody in which the humanized mature heavy chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to hu8H24VHv1 (SEQ ID NO:180) or hu8H24VHv2 (SEQ ID NO:181) and the humanized mature light chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to hu8H24VLv1 (SEQ ID NO:185) or hu8H24VLv2 (SEQ ID NO:186). In some such antibodies at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the backmutations or other mutations in SEQ ID NOS:180-181, and SEQ ID NOS:185-186 are retained.

[0437] In some humanized 8H24 antibodies, at least one of the following positions in the VH region is occupied by the amino acid as specified: H2 is occupied by A, H12 is occupied by K or V, H48 is occupied by I, H67 is occupied by A, H71 is occupied by V, H91 is occupied by F, H108 is occupied by T. In some humanized 8H24 antibodies, positions H2, H48, H67, H71, H91, and H108 in the VH region are occupied by A, I, A, V, F, and T, respectively, as in hu8H24VHv1. In some humanized 8H24 antibodies, positions H2, H12, H48, H67, H71, H91,

and H108 in the VH region are occupied by A, V, I, A, V, F, and T, respectively, as in hu8H24VHv2.

[0438] In some humanized 8H24 antibodies, at least one of the following positions in the VL region is occupied by the amino acid as specified: L2 is V, L9 is L or S, L74 is K or T. In some humanized 8H24 antibodies, position L2 in the VL region are occupied by V, as in hu8H24VLv1. In some humanized 8H24 antibodies, positions L2, L9, and L74 in the VL region are occupied by V, S, and T, respectively, as in hu8H24VLv2.

[0439] In some humanized 8H24 antibodies, the variable heavy chain has $\geq 85\%$ identity to human sequence. In some humanized 8H24 antibodies, the variable light chain has $\geq 85\%$ identity to human sequence. In some humanized 8H24 antibodies, each of the variable heavy chain and variable light chain has $\geq 85\%$ identity to human germline sequence. In some humanized 8H24 antibodies, the three heavy chain CDRs are as defined by Kabat/Chothia Composite (SEQ ID NOS:29-31) and the three light chain CDRs are as defined by Kabat/Chothia Composite (SEQ ID NOS:35-37).

[0440] Exemplary humanized antibodies include humanized forms of mouse 11M14, designated Hu11M14.

[0441] The mouse antibody 11M14 comprises mature heavy and light chain variable regions having amino acid sequences comprising SEQ ID NO:52 and SEQ ID NO:58, respectively. The invention provides 3 exemplified humanized mature heavy chain variable regions: hu11M14VHv1b, hu11M14VHv2b, and hu11M14VHv3b. The invention further provides 4 exemplified mature light chain variable regions hu11M14VLv1b, hu11M14VLv2b, hu11M14VLv3b, and hu11M14VLv4b. Figures 5 and 6 show alignments of the heavy chain variable region and light chain variable region, respectively, of murine 11M14 and various humanized antibodies.

[0442] For reasons such as possible influence on CDR conformation and/or binding to antigen, mediating interaction between heavy and light chains, interaction with the constant region, being a site for desired or undesired post-translational modification, being an unusual residue for its position in a human variable region sequence and therefore potentially immunogenic, getting aggregation potential, and other reasons, the following 7 variable region framework positions were considered as candidates for substitutions in the 4 exemplified human mature light chain

variable regions and the 3 exemplified human mature heavy chain variable regions, as further specified in the examples: L43 (A43S), L48 (I48V), L71 (F71Y), L76 (N76S), H49 (S49A), H80 (L80G), H82c (L82cG).

[0443] The following variable region CDR position was considered as a candidate for substitution in the 4 exemplified human mature light chain variable regions, as further specified in the examples: L54 (L54G, L54I). In some humanized 11M14 antibodies, Kabat-Chothia Composite CDR-L2 has an amino acid sequence comprising SEQ ID NO:72. In some humanized 11M14 antibodies, Kabat-Chothia Composite CDR-L2 has an amino acid sequence comprising SEQ ID NO:73.

[0444] Here, as elsewhere, the first-mentioned residue is the residue of a humanized antibody formed by grafting Kabat CDRs or a composite Chothia-Kabat CDR in the case of CDR-H1 into a human acceptor framework, and the second-mentioned residue is a residue being considered for replacing such residue. Thus, within variable region frameworks, the first mentioned residue is human, and within CDRs, the first mentioned residue is mouse.

[0445] Exemplified antibodies include any permutations or combinations of the exemplified mature heavy and light chain variable regions hu11M14VHv1b/ hu11M14VLv1b, hu11M14VHv1b/ hu11M14VLv2b, hu11M14VHv1b/ hu11M14VLv3b, hu11M14VHv1b/ hu11M14VLv4b, hu11M14VHv2b/ hu11M14VLv1b, hu11M14VHv2b/ hu11M14VLv2b, hu11M14VHv2b/ hu11M14VLv3b, hu11M14VHv2b/ hu11M14VLv4b, hu11M14VHv3b/ hu11M14VLv1b, hu11M14VHv3b/ hu11M14VLv2b, hu11M14VHv3b/ hu11M14VLv3b, hu11M14VHv3b/ hu11M14VLv4b.

[0446] Exemplified antibodies include any permutations or combinations of the exemplified mature heavy chain variable regions hu11M14VHv1b (SEQ ID NO:190), hu11M14VHv2b (SEQ ID NO:191), and hu11M14VHv3b (SEQ ID NO:192) with any of the exemplified mature light chain variable regions hu11M14VLv1b (SEQ ID NO:196), hu11M14VLv2b (SEQ ID NO:197), hu11M14VLv3b (SEQ ID NO:198), and hu11M14VLv4b (SEQ ID NO:199).

[0447] The invention provides variants of the 11M14 humanized antibody in which the humanized mature heavy chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to hu11M14VHv1b (SEQ ID NO:190), hu11M14VHv2b (SEQ ID NO:191), or hu11M14VHv3b (SEQ ID NO:192) and the humanized mature light chain variable region shows

at least 90%, 95%, 96%, 97%, 98%, or 99% identity to hu11M14VLv1b (SEQ ID NO:196), hu11M14VLv2b (SEQ ID NO:197), hu11M14VLv3b (SEQ ID NO:198), or hu11M14VLv4b (SEQ ID NO:199). In some such antibodies at least 1, 2, 3, 4, 5, 6, 7, or all 8 of the positions subject to backmutations or other mutations in SEQ ID NOS:190-192, and SEQ ID NOS:196-199 are likewise backmutated or otherwise mutated.

[0448] In some humanized 11M14 antibodies, at least one of the following positions in the VH region is occupied by the amino acid as specified: H49 is occupied by A, H80 is occupied by L or G, H82c is occupied by L or G. In some humanized 11M14 antibodies, position H49 in the VH region is occupied by A, as in hu11M14VHv1b. In some humanized 11M14 antibodies, positions H49 and H82c in the VH region are occupied by A and G, respectively, as in hu11M14VHv2b. In some humanized 11M14 antibodies, positions H49 and H80 in the VH region are occupied by A and G, respectively, as in hu11M14VHv3b.

[0449] In some humanized 11M14 antibodies, at least one of the following positions in the VL region is occupied by the amino acid as specified: L43 is A or S, L48 is V, L54 is L, G, or I, L71 is Y, L76 is N or S. In some humanized 11M14 antibodies, positions L48 and L71 in the VL region are occupied by V and Y respectively, as in hu11M14VLv1b. In some humanized 11M14 antibodies, positions L43, L48, L71, and L76 in the VL region are occupied by S, V, Y, and S, respectively, as in hu11M14VLv2b. In some humanized 11M14 antibodies, positions L43, L48, L54, L71, and L76 in the VL region are occupied by S, V, G, Y, and S, respectively, as in hu11M14VLv3b. In some humanized 11M14 antibodies, positions L43, L48, L54, L71, and L76 in the VL region are occupied by S, V, I, Y, and S, respectively, as in hu11M14VLv4b.

[0450] The heavy chain variable region of any of the above referenced antibodies can be modified to further reduce immunogenicity. For example, in some of the humanized antibodies position H80 in the VH region is occupied by G. For example, in some of the humanized antibodies, position H82c in the VH region is occupied by G. The light chain variable region of any of the above referenced antibodies can be modified to further reduce immunogenicity. For example, in some of the humanized antibodies position L54 in the VL region is occupied by G or I.

[0451] In some of the humanized antibodies, position H82c in the VH region is occupied by G, as in hu11M14VHv3b. In some of the humanized antibodies, position L54 in the VL region is

occupied by G, as in hu11M14VLv3b. In some of the humanized antibodies, position L54 in the VL region is occupied by I, as in hu11M14VLv4b.

[0452] In some humanized 11M14 antibodies, Kabat-Chothia Composite CDR-L2 has an amino acid sequence comprising SEQ ID NO:72. In some humanized 11M14 antibodies, Kabat-Chothia Composite CDR-L2 has an amino acid sequence comprising SEQ ID NO:73.

[0453] In some humanized 11M14 antibodies, the variable heavy chain has $\geq 85\%$ identity to human sequence. In some humanized 11M14 antibodies, the variable light chain has $\geq 85\%$ identity to human sequence. In some humanized 11M14 antibodies, each of the variable heavy chain and variable light chain has $\geq 85\%$ identity to human germline sequence. In some humanized 11M14 antibodies, the three heavy chain CDRs are as defined by Kabat/Chothia Composite (SEQ ID NOS:53-55) and the three light chain CDRs are as defined by Kabat/Chothia Composite (SEQ ID NOS:59-61), except that position L54 can be L, G, or I.

[0454] The CDR regions of such humanized 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2 antibodies can be identical or substantially identical to the CDR regions of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2. The CDR regions can be defined by any conventional definition (e.g., Chothia, or composite of Chothia and Kabat) but are preferably as defined by Kabat.

[0455] Variable regions framework positions are in accordance with Kabat numbering unless otherwise stated. Other such variants typically differ from the sequences of the exemplified Hu5E20, Hu8H24, or Hu11M14 heavy and light chains by a small number (*e.g.*, typically no more than 1, 2, 3, 5, 10, or 15) of replacements, deletions or insertions. Such differences are usually in the framework but can also occur in the CDRs.

[0456] A possibility for additional variation in humanized 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2 variants is additional backmutations in the variable region frameworks. Many of the framework residues not in contact with the CDRs in the humanized mAb can accommodate substitutions of amino acids from the corresponding positions of the donor mouse mAb or other mouse or human antibodies, and even many potential CDR-contact residues are also amenable to substitution. Even amino acids within the CDRs may be altered, for example, with residues found at the corresponding position of the human acceptor sequence used to supply variable region frameworks. In addition, alternate human acceptor sequences can

be used, for example, for the heavy and/or light chain. If different acceptor sequences are used, one or more of the backmutations recommended above may not be performed because the corresponding donor and acceptor residues are already the same without backmutations.

[0457] Preferably, replacements or backmutations in humanized 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2 variants (whether or not conservative) have no substantial effect on the binding affinity or potency of the humanized mAb, that is, its ability to bind to sortilin.

[0458] Humanized variants hu11M14 H1bL3b_IgG1 LALA, hu11M14 H1bL3b_IgG1 LALA_YTE, hu8H24 H1L2_IgG1_LALA, and hu5E20 H7L4_IgG1_LALA increase plasma and CSF progranulin levels in non-human primates (Examples 21-22, Figures 15-16 and 19-20). Humanized variants hu11M14 H1bL3b_IgG1 LALA_ and hu11M14 H1bL3b_IgG1 LALA_YTE reduce sortilin levels in PBMCs from non-human primates (Example 23, Figure 21). hu11M14 H1bL3b_IgG1 LALA_-YTE shows improved pharmacokinetics and plasma pharmacodynamics profile over hu11M14 H1bL3b_IgG1 LALA (Example 23, Figures 17-18).

[0459] Hu11M14VHv1bVLv3b is also referred to as hu11M14H1bL3b.

Hu5E20VHv7VLv4 is also referred to as hu5E20H7L4. Hu8H24VHv1VLv2 is also referred to as hu8H24H1L2. **D. Chimeric and Veneered Antibodies**

[0460] The invention further provides chimeric and veneered forms of non-human antibodies, particularly the 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2 antibodies of the examples.

[0461] A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (*e.g.*, a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody, and are about two-thirds human sequence.

[0462] A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, *Mol. Immunol.* 28:489, 1991) with residues from the corresponding positions of a human antibody sequence. The result is an antibody in

which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions.

Veneered forms of the 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2 antibodies are included in the invention.

E. Human Antibodies

[0463] Human antibodies specifically binding to sortilin or a fragment thereof (e.g., a peptide comprising or consisting of amino acid sequence FTESFLT (SEQ ID NO:202), ESFL (SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), E(S/Q/D)FL (SEQ ID NO:206), RTEFGMAIGP) (SEQ ID NO:213), WGFTEFLTS (SEQ ID NO:214), or an epitope defined by residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, or to an epitope defined by residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, or to an epitope defined by residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215, are provided by a variety of techniques described below. Some human antibodies are selected by competitive binding experiments, by the phage display method of Winter, above, or otherwise, to have the same epitope specificity as a particular mouse antibody, such as one of the mouse monoclonal antibodies described in the examples. Human antibodies can also be screened for a particular epitope specificity by using only a fragment of sortilin, such as a sortilin fragment comprising or consisting of an amino acid sequence of FTESFLT (SEQ ID NO:202), ESFL (SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), E(S/Q/D)FL (SEQ ID NO:206), RTEFGMAIGP) (SEQ ID NO:213), or WGFTEFLTS (SEQ ID NO:214), as the target antigen, and/or by screening antibodies against a collection of sortilin variants, such as sortilin variants of SEQ ID NOs: 216-243, or such as sortilin variants containing various mutations within amino acid residues 588-594 or 590-593 or 632-643 or 663-674 or 167-176 or 586-595 of SEQ ID NO:1.

[0464] Methods for producing human antibodies include the trioma method of Oestberg *et al.*, *Hybridoma* 2:361-367 (1983); Oestberg, U.S. Patent No. 4,634,664; and Engleman *et al.*, US Patent 4,634,666, use of transgenic mice including human immunoglobulin genes (*see, e.g.*, Lonberg *et al.*, WO93/12227 (1993); US 5,877,397; US 5,874,299; US 5,814,318; US 5,789,650; US 5,770,429; US 5,661,016; US 5,633,425; US 5,625,126; US 5,569,825; US 5,545,806;

Neuberger, *Nat. Biotechnol.* 14:826 (1996); and Kucherlapati, WO 91/10741 (1991)) phage display methods (*see, e.g.*, Dower *et al.*, WO 91/17271; McCafferty *et al.*, WO 92/01047; US 5,877,218; US 5,871,907; US 5,858,657; US 5,837,242; US 5,733,743; and US 5,565,332); and methods described in WO 2008/081008 (e.g., immortalizing memory B cells isolated from humans, e.g., with EBV, screening for desired properties, and cloning and expressing recombinant forms).

F. Selection of Constant Region

[0465] The heavy and light chain variable regions of chimeric, veneered or humanized antibodies can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent cell-mediated cytotoxicity, antibody dependent cellular phagocytosis and/or complement dependent cytotoxicity are desired. For example, human isotypes IgG1 and IgG3 have complement-dependent cytotoxicity and human isotypes IgG2 and IgG4 do not. Human IgG1 and IgG3 also induce stronger cell mediated effector functions than human IgG2 and IgG4. Light chain constant regions can be lambda or kappa. Numbering conventions for constant regions include EU numbering (Edelman, G.M. et al., *Proc. Natl. Acad. USA*, 63, 78-85 (1969)), Kabat numbering (Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1991, IMGT unique numbering (Lefranc M.-P. et al., IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains, *Dev. Comp. Immunol.*, 29, 185-203 (2005), and IMGT exon numbering (Lefranc, *supra*).

[0466] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (*see, e.g.*, Winter et al., US Patent No. 5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al., *Proc. Natl. Acad. Sci. USA* 103:4005, 2006), or to prolong half-life in humans (*see, e.g.*, Hinton et al., *J. Biol. Chem.* 279:6213, 2004). Exemplary substitutions include a Gln at position 250 and/or a Leu at position 428 (EU numbering is used in this paragraph for the constant region) for increasing the half-life of an antibody. Substitution at any or all of positions 234, 235, 236 and/or 237 reduce affinity for Fc γ receptors, particularly Fc γ RI receptor (*see, e.g.*, US

6,624,821). An alanine substitution at positions 234, 235, and 237 of human IgG1 can be used for reducing effector functions. Some antibodies have alanine substitution at positions 234, 235 and 237 of human IgG1 for reducing effector functions. Optionally, positions 234, 236 and/or 237 in human IgG2 are substituted with alanine and position 235 with glutamine (see, e.g., US 5,624,821). In some antibodies, a mutation at one or more of positions 241, 264, 265, 270, 296, 297, 322, 329, and 331 by EU numbering of human IgG1 is used. In some antibodies, a mutation at one or more of positions 318, 320, and 322 by EU numbering of human IgG1 is used. In some antibodies, positions 234 and/or 235 are substituted with alanine and/or position 329 is substituted with glycine. In some antibodies, positions 234 and 235 are substituted with alanine. In some antibodies, the isotype is human IgG2 or IgG4.

[0467] In some antibodies the 'LALA' double mutation (Leu234Ala together with Leu235Ala) is used for diminished effector functions (Lund, J., et al. (1992) *Mol. Immunol.*, 29,53-59; Tamm and Schmidt, 1997, *Int Rev Immunol* 16(1-2):57-85). Fc variant L234A/L235A (LALA) (numbering according to EU nomenclature) mutant has been shown to have either eliminated or reduced interaction with FcγRs (FcγRIa, FcγRIIa, FcγRIIIa and FcγRIIIa) and exhibit significantly reduced effector function. Additionally, Fc LALA mutants have also been shown to have diminished interaction with complement proteins resulting in reduced complement-dependent cytotoxicity (CDC). Exemplary antibody heavy chain sequences incorporating the LALA mutations are SEQ ID NO:244, SEQ ID NO:246, SEQ ID NO:248, SEQ ID NO:250, SEQ ID NO:251, and SEQ ID NO:252.

[0468] In some antibodies, the "YTE" (M252T/S254T/T256E) (numbering according to EU nomenclature) mutations in the Fc region are used (W.F. Dall'Acqua *et al.* 2006 *J. Biol. Chem.* 281:23514-23). YTE mutant has been shown to have enhanced binding/interaction to the neonatal Fc receptor (FcRn); thereby leading to antibody half-life extension in circulation. In some antibodies, 'LALA' double mutation and YTE mutations are used. Exemplary antibody heavy chain sequences incorporating the LALA and YTE mutations are SEQ ID NO:244, SEQ ID NO:246, and SEQ ID NO:248.

[0469] Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')₂, and Fv, or as single chain antibodies in which heavy and light chain mature variable domains are linked through a spacer.

[0470] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype bind to a non-polymorphic region of a one or more other isotypes. Thus, for example, another heavy chain constant region is of IgG1 G1m3 with or without the C-terminal lysine. Reference to a human constant region includes a constant region with any natural allotype or any permutation of residues occupying positions in natural allotypes.

G. Expression of Recombinant Antibodies

[0471] A number of methods are known for producing chimeric and humanized antibodies using an antibody-expressing cell line (*e.g.*, hybridoma). For example, the immunoglobulin variable regions of antibodies can be cloned and sequenced using well known methods. In one method, the heavy chain variable VH region is cloned by RT-PCR using mRNA prepared from hybridoma cells. Consensus primers are employed to the VH region leader peptide encompassing the translation initiation codon as the 5' primer and a g2b constant regions specific 3' primer. Exemplary primers are described in U.S. patent publication US 2005/0009150 by Schenk *et al.* (hereinafter "Schenk"). The sequences from multiple, independently derived clones can be compared to ensure no changes are introduced during amplification. The sequence of the VH region can also be determined or confirmed by sequencing a VH fragment obtained by 5' RACE RT-PCR methodology and the 3' g2b specific primer.

[0472] The light chain variable VL region can be cloned in an analogous manner. In one approach, a consensus primer set is designed for amplification of VL regions using a 5' primer designed to hybridize to the VL region encompassing the translation initiation codon and a 3' primer specific for the Ck region downstream of the V-J joining region. In a second approach, 5'RACE RT-PCR methodology is employed to clone a VL encoding cDNA. Exemplary primers are described in Schenk, *supra*. The cloned sequences are then combined with sequences encoding human (or other non-human species) constant regions.

[0473] In one approach, the heavy and light chain variable regions are re-engineered to encode splice donor sequences downstream of the respective VDJ or VJ junctions and are cloned into a mammalian expression vector, such as pCMV-hy1 for the heavy chain and pCMV-Mcl for the light chain. These vectors encode human $\gamma 1$ and Ck constant regions as exonic fragments downstream of the inserted variable region cassette. Following sequence verification, the heavy chain and light chain expression vectors can be co-transfected into CHO cells to produce chimeric antibodies. Conditioned media is collected 48 hours post-transfection and assayed by western blot analysis for antibody production or ELISA for antigen binding. The chimeric antibodies are humanized as described above.

[0474] Chimeric, veneered, humanized, and human antibodies are typically produced by recombinant expression. Recombinant polynucleotide constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally associated or heterologous expression control elements, such as a promoter. The expression control sequences can be promoter systems in vectors capable of transforming or transfecting eukaryotic or prokaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences and the collection and purification of the crossreacting antibodies.

[0475] These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers, *e.g.*, ampicillin resistance or hygromycin resistance, to permit detection of those cells transformed with the desired DNA sequences.

[0476] *E. coli* is one prokaryotic host useful for expressing antibodies, particularly antibody fragments. Microbes, such as yeast, are also useful for expression. *Saccharomyces* is a yeast host with suitable vectors having expression control sequences, an origin of replication, termination sequences, and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

[0477] Mammalian cells can be used for expressing nucleotide segments encoding immunoglobulins or fragments thereof. *See Winnacker, From Genes to Clones, (VCH*

Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed, and include CHO cell lines, various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. The cells can be nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen *et al.*, *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Expression control sequences can include promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See Co *et al.*, *J. Immunol.* 148:1149 (1992).

[0478] Alternatively, antibody coding sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (*see, e.g.*, U.S. Pat. No. 5,741,957; U.S. Pat. No. 5,304,489; and U.S. Pat. No. 5,849,992). Suitable transgenes include coding sequences for light and/or heavy chains operably linked with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

[0479] The vectors containing the DNA segments of interest can be transferred into the host cell by methods depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics, or viral-based transfection can be used for other cellular hosts. Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes or can be incorporated into the genome of embryonic stem cells or induced pluripotent stem cells (iPSCs), and the nuclei of such cells transferred into enucleated oocytes.

[0480] Having introduced vector(s) encoding antibody heavy and light chains into cell culture, cell pools can be screened for growth productivity and product quality in serum-free media. Top-producing cell pools can then be subjected of FACS-based single-cell cloning to generate monoclonal lines. Specific productivities above 50 pg or 100 pg per cell per day, which correspond to product titers of greater than 7.5 g/L culture, can be used. Antibodies produced by

single cell clones can also be tested for turbidity, filtration properties, PAGE, IEF, UV scan, HP-SEC, carbohydrate-oligosaccharide mapping, mass spectrometry, and binding assay, such as ELISA or Biacore. A selected clone can then be banked in multiple vials and stored frozen for subsequent use.

[0481] Once expressed, antibodies can be purified according to standard procedures of the art, including protein A capture, HPLC purification, column chromatography, gel electrophoresis and the like (*see generally*, Scopes, *Protein Purification* (Springer-Verlag, NY, 1982)).

[0482] Methodology for commercial production of antibodies can be employed, including codon optimization, selection of promoters, selection of transcription elements, selection of terminators, serum-free single cell cloning, cell banking, use of selection markers for amplification of copy number, CHO terminator, or improvement of protein titers (*see, e.g.*, US 5,786,464; US 6,114,148; US 6,063,598; US 7,569,339; W02004/050884; W02008/012142; W02008/012142; W02005/019442; W02008/107388; W02009/027471; and US 5,888,809).

IV. Active Immunogens

[0483] An agent used for active immunization serves to induce in a patient the same types of antibody described in connection with passive immunization above. Agents used for active immunization can be the same types of immunogens used for generating monoclonal antibodies in laboratory animals, *e.g.*, a peptide of 3-15 or 3-12 or 5-12, or 5-8 contiguous amino acids from a region of sortilin corresponding to residues 588-594 or 590-593 or 632-643 or 663-674 or 167-176 of 586-595 of SEQ ID NO:1, such as, for example, a sortilin peptide including or consisting of residues 588-594 or 590-593 or 632-643 or 663-674 or 167-176 or 586-595 of SEQ ID NO:1) or a sortilin peptide comprising or consisting of amino acid sequence FTESFLT (SEQ ID NO:202), a sortilin peptide comprising or consisting of amino acid sequence ESFL (SEQ ID NO:203), a sortilin peptide comprising or consisting of amino acid sequence DGCILGYKEQFL (SEQ ID NO:204), or a sortilin peptide comprising or consisting of amino acid sequence PSICLCSLEDL (SEQ ID NO:205), or a sortilin peptide comprising or consisting of amino acid sequence E(S/Q/D)FL (SEQ ID NO:206), a sortilin peptide comprising or consisting of amino acid sequence RTEFGMAIGP (SEQ ID NO:213), a sortilin peptide comprising or consisting of amino acid sequence WGFTESFLTS (SEQ ID NO:214), a sortilin peptide comprising or consisting of some or all of the amino acid residues D74, R76, F97, K110, Y535, L560, and

E557 of the sortilin ECD of SEQ ID NO:215, a sortilin peptide comprising or consisting of some or all of amino acid residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, or a sortilin peptide comprising or consisting of some or all of the amino acid residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215. For conformational epitopes with widely separated residues, peptides can be selected against some of the residues sufficiently proximate within one another to form a linear epitope. For inducing antibodies binding to the same or overlapping epitope as 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2, the epitope specificity of these antibodies can be mapped (*e.g.*, by testing binding to a series of overlapping peptides spanning sortilin). A fragment of sortilin consisting of or including or overlapping the epitope can then be used as an immunogen.

[0484] The heterologous carrier and adjuvant, if used may be the same as used for generating monoclonal antibody, but may also be selected for better pharmaceutical suitability for use in humans. Suitable carriers include serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, or a toxoid from other pathogenic bacteria, such as diphtheria (*e.g.*, CRM197), *E. coli*, cholera, or *H. pylori*, or an attenuated toxin derivative. T cell epitopes are also suitable carrier molecules. Some conjugates can be formed by linking agents of the invention to an immunostimulatory polymer molecule (*e.g.*, tripalmitoyl-S-glycerine cysteine (Pam₃Cys), mannan (a mannose polymer), or glucan (a β 1→2 polymer)), cytokines (*e.g.*, IL-1, IL-1 alpha and β peptides, IL-2, γ -INF, IL-10, GM-CSF), and chemokines (*e.g.*, MIP1- α and β , and RANTES). Immunogens may be linked to the carriers with or without spacers amino acids (*e.g.*, gly-gly). Additional carriers include virus-like particles. Virus-like particles (VLPs), also called pseudovirions or virus-derived particles, represent subunit structures composed of multiple copies of a viral capsid and/or envelope protein capable of self-assembly into VLPs of defined spherical symmetry *in vivo*. (Powilleit, et al., (2007) PLoS ONE 2(5):e415.) Alternatively, peptide immunogens can be linked to at least one artificial T-cell epitope capable of binding a large proportion of MHC Class II molecules., such as the pan DR epitope ("PADRE"). PADRE is described in US 5,736,142, WO 95/07707, and Alexander J et al, Immunity, 1:751-761 (1994). Active immunogens can be presented in multimeric form in which multiple copies of an immunogen and/or its carrier are presented as a single covalent molecule.

[0485] Fragments are often administered with pharmaceutically acceptable adjuvants. The adjuvant increases the titer of induced antibodies and/or the binding affinity of induced antibodies relative to the situation if the peptide were used alone. A variety of adjuvants can be used in combination with an immunogenic fragment of sortilin to elicit an immune response. Some adjuvants augment the intrinsic response to an immunogen without causing conformational changes in the immunogen that affect the qualitative form of the response. Some adjuvants include aluminum salts, such as aluminum hydroxide and aluminum phosphate, 3 De-O-acylated monophosphoryl lipid A (MPLTM) (*see* GB 2220211 (RIBI ImmunoChem Research Inc., Hamilton, Montana, now part of Corixa). StimulonTM QS-21 is a triterpene glycoside or saponin isolated from the bark of the Quillaja Saponaria Molina tree found in South America (*see* Kensil *et al.*, in *Vaccine Design: The Subunit and Adjuvant Approach* (eds. Powell & Newman, Plenum Press, NY, 1995); US 5,057,540), (Aquila BioPharmaceuticals, Framingham, MA; now Antigenics, Inc., New York, NY). Other adjuvants are oil in water emulsions (such as squalene or peanut oil), optionally in combination with immune stimulants, such as monophosphoryl lipid A (*see* Stoute *et al.*, *N. Engl. J. Med.* 336, 86-91 (1997)), pluronic polymers, and killed mycobacteria. Ribi adjuvants are oil-in-water emulsions. Ribi contains a metabolizable oil (squalene) emulsified with saline containing Tween 80. Ribi also contains refined mycobacterial products which act as immunostimulants and bacterial monophosphoryl lipid A. Another adjuvant is CpG (WO 98/40100). Adjuvants can be administered as a component of a therapeutic composition with an active agent or can be administered separately, before, concurrently with, or after administration of the therapeutic agent.

[0486] Analogs of natural fragments of sortilin that induce antibodies against sortilin can also be used. For example, one or more or all L-amino acids can be substituted with D amino acids in such peptides. Also the order of amino acids can be reversed (retro peptide). Optionally a peptide includes all D-amino acids in reverse order (retro-inverso peptide). Peptides and other compounds that do not necessarily have a significant amino acid sequence similarity with sortilin peptides but nevertheless serve as mimetics of sortilin peptides and induce a similar immune response. Anti- idiotypic antibodies against monoclonal antibodies to sortilin as described above can also be used. Such anti-Id antibodies mimic the antigen and generate an immune response to it (*see* Essential Immunology, Roit ed., Blackwell Scientific Publications, Palo Alto, CA 6th ed., p. 181).

[0487] Peptides (and optionally a carrier fused to the peptide) can also be administered in the form of a nucleic acid encoding the peptide and expressed in situ in a patient. A nucleic acid segment encoding an immunogen is typically linked to regulatory elements, such as a promoter and enhancer that allow expression of the DNA segment in the intended target cells of a patient. For expression in blood cells, as is desirable for induction of an immune response, promoter and enhancer elements from light or heavy chain immunoglobulin genes or the CMV major intermediate early promoter and enhancer are suitable to direct expression. The linked regulatory elements and coding sequences are often cloned into a vector. Antibodies can also be administered in the form of nucleic acids encoding the antibody heavy and/or light chains. If both heavy and light chains are present, the chains are preferably linked as a single chain antibody. Antibodies for passive administration can also be prepared *e.g.*, by affinity chromatography from sera of patients treated with peptide immunogens.

[0488] The DNA can be delivered in naked form (*i.e.*, without colloidal or encapsulating materials). Alternatively a number of viral vector systems can be used including retroviral systems (see, *e.g.*, Lawrie and Tumin, *Cur. Opin. Genet. Develop.* 3, 102-109 (1993)) including retrovirus derived vectors such as MMLV, HIV-1, and ALV; adenoviral vectors {see, *e.g.*, Bett et al., *J. Virol.* 67, 591-1 (1993)); adeno-associated virus vectors {see, *e.g.*, Zhou et al., *J. Exp. Med.* 179, 1867 (1994)), lentiviral vectors such as those based on HIV or FIV gag sequences, viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses (see, *e.g.*, Dubensky et al., *J. Virol.* 70, 508-519 (1996)), Venezuelan equine encephalitis virus (see US 5,643,576) and rhabdoviruses, such as vesicular stomatitis virus (see WO 96/34625) and papillomaviruses (Ohe et al., *Human Gene Therapy* 6, 325-333 (1995); Woo et al., WO 94/12629 and Xiao & Brandsma, *Nucleic Acids. Res.* 24, 2630-2622 (1996)).

[0489] DNA encoding an immunogen, or encoding the antibody heavy and/or light chains, or a vector containing the same, can be packaged into liposomes. Suitable lipids and related analogs are described by US 5,208,036, US 5,264,618, US 5,279,833, and US 5,283,185. Vectors and DNA encoding an immunogen or encoding the antibody heavy and/or light chains can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate polymers and polylactides and poly(lactide-co-glycolides), (see, *e.g.*, McGee et al., *J. Micro Encap.* 1996).

[0594] Vectors or segments therefrom encoding the antibody heavy and/or light chains can be incorporated in cells ex vivo, for example to cells explanted from an individual patient (e.g., lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have incorporated the transgenes. (see, e.g., WO 2017/091512). Exemplary patient-derived cells include patient derived induced pluripotent stem cells (iPSCs) or other types of stem cells (embryonic, hematopoietic, neural, or mesenchymal).

[0594] A vector or segment therefrom encoding the antibody heavy and/or light chains can be introduced into any region of interest in cells ex vivo, such as an albumin gene or other safe harbor gene. Cells incorporating the vector can be implanted with or without prior differentiation. Cells can be implanted into a specific tissue, such as a secretory tissue or a location of pathology, or systemically, such as by infusion into the blood. For example, cells can be implanted into a secretory tissue of a patient, such as the liver, optionally with prior differentiation to cells present in that tissue, such as hepatocytes in the case of a liver. Expression of the antibody in the liver results in secretion of the antibody to the blood.

H. Antibody Screening Assays

[0490] Antibodies can be initially screened for the intended binding specificity as described above. Active immunogens can likewise be screened for capacity to induce antibodies with such binding specificity. In this case, an active immunogen is used to immunize a laboratory animal and the resulting sera tested for the appropriate binding specificity.

[0491] Antibodies having the desired binding specificity can then be tested in cellular and animal models. An exemplary cellular model are HEK 293 cells expressing recombinant huSortilin on their surface. Another exemplary cellular model are U251MG cells (human glioblastoma cells line) which endogenously express sortilin on their cell surface. Human cellular models include iPSC-derived cortical neurons from or microglia expressing FTD-GRN mutations. Exemplary mouse cellular models are primary neurons from GRN^{+/-} and/or GRN^{-/-} transgenic mice + AAV-TDP43CT-GFP (Chang, M.C. et al., J. Exp. Med. 2017; 214 (9): 2611–2628). TDP43CT forms cytoplasmic clusters and GRN^{-/-} neurons accumulated about 40% higher levels of TDP43CT than WT neuron. This phenotype was rescued with addition of recombinant PRGN (10 μ M).

[0492] The activity of antibodies or active agents can be assessed by various criteria including increase in extracellular progranulin and inhibition or delay or behavioral deficits. Active immunogens can also be tested for induction of antibodies in the sera. Both passive and active immunogens can be tested for passage of antibodies across the blood brain barrier into the brain of a wild type or transgenic animal. Antibodies or fragments inducing an antibody can also be tested in non-human primates without disease or that naturally or through induction develop symptoms of diseases characterized by changes in progranulin levels; for example as in Examples 21-22 and Figures 14-21. Tests on an antibody or active agent are usually performed in conjunction with a control in which a parallel experiment is conducted except that the antibody or active agent is absent (*e.g.*, replaced by vehicle). Reduction, delay or inhibition of signs or symptoms of disease attributable to an antibody or active agent under test can then be assessed relative to the control.

V. Patients Amenable to Treatment

[0493] Changes in progranulin levels have been found in several diseases including frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, neurodegenerative disorders, and neurodegenerative disorders associated with aging. The present regimen can also be used in treatment or prophylaxis of any of these diseases. Because of the widespread association between neurological diseases and conditions and changes in progranulin levels, the present regimen can be used in treatment or prophylaxis of any subject showing reduced levels of progranulin (*e.g.*, in the plasma or CSF) compared with a mean value in individuals without neurological disease. The present regimen can also be used in treatment or prophylaxis of neurological disease in individuals having a mutation in progranulin associated with neurological disease. The present methods are particularly suitable for treatment or prophylaxis of frontotemporal dementia. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8.

[0494] Patients amenable to treatment include individuals at risk of disease but not showing symptoms, as well as patients presently showing symptoms. Patients at risk of disease include those having a known genetic risk of disease. Such individuals include those having relatives who have experienced this disease, and those whose risk is determined by analysis of genetic or

biochemical markers. Genetic markers of risk include mutations in progranulin, such as FTD-*GRN* as discussed above. Individuals presently suffering from frontotemporal dementia can be recognized by PET imaging, from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have frontotemporal dementia. These include measurement of CSF and plasma progranulin levels.

[0495] In asymptomatic patients, treatment can begin at any age (*e.g.*, 10, 20, 30). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70 years of age. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying antibody levels over time. If the response falls, a booster dosage is indicated.

I. Nucleic Acids

[0496] The invention further provides nucleic acids encoding any of the heavy and light chains described above (*e.g.*, SEQ ID NOS:4, 10, 24-25, 28, 34, 48-49, 52, 58, 78, 84, 90, 96, 102, 108, 114, 120, 126, 132, 138, 144, 150, 156, 163-169, 173-176, 180-181, 185-186, 190-192, 196-199). Exemplary nucleotide sequences include SEQ ID NOS:2, 8, 26, 32, 50, 56, 76, 82, 88, 94, 100, 106, 112, 118, 124, 130, 136, 142, 148, and 154. Optionally, such nucleic acids further encode a signal peptide and can be expressed with the signal peptide linked to the variable region. Coding sequences of nucleic acids can be operably linked with regulatory sequences to ensure expression of the coding sequences, such as a promoter, enhancer, ribosome binding site, transcription termination signal, and the like. The regulatory sequences can include a promoter, for example, a prokaryotic promoter or a eukaryotic promoter. The nucleic acids encoding heavy or light chains can be codon-optimized for expression in a host cell. The nucleic acids encoding heavy and light chains can encode a selectable gene. The nucleic acids encoding heavy and light chains can occur in isolated form or can be cloned into one or more vectors. The nucleic acids can be synthesized by, for example, solid state synthesis or PCR of overlapping oligonucleotides. Nucleic acids encoding heavy and light chains can be joined as one contiguous nucleic acid, *e.g.*, within an expression vector, or can be separate, *e.g.*, each cloned into its own expression vector.

J. Conjugated Antibodies

[0497] Conjugated antibodies that specifically bind to antigens, such as sortilin, are useful in detecting the presence of sortilin; monitoring and evaluating the efficacy of therapeutic agents being used to treat patients diagnosed with a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorders associated with aging; increasing extracellular progranulin levels,; or treating or effecting prophylaxis of disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging in a patient. For example, such antibodies can be conjugated with other therapeutic moieties, other proteins, other antibodies, and/or detectable labels. *See* WO 03/057838; US 8,455,622. Such therapeutic moieties can be any agent that can be used to treat, combat, ameliorate, prevent, or improve an unwanted condition or disease in a patient, such as disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8.

[0498] Conjugated therapeutic moieties can include, neurotrophic agents, neuroprotective agents, radiotherapeutic agents, radioactive (radiopharmaceuticals), fluorescent, paramagnetic tracers, ultrasound contrast agents, immunomodulators, or any biologically active agents that facilitate or enhance the activity of the antibody, or modify bioavailability, and distribution in the body or within organs. A neurotrophic agent can be any agent, including chemical or proteinaceous agents, that promotes neuron maintenance, growth, or differentiation. A neuroprotective agent can be agent, including chemical or proteinaceous agents, that protects neurons from acute insult or degenerative processes. An immunomodulator can be any agent that stimulates or inhibits the development or maintenance of an immunologic response. A radiotherapeutic agent can be any molecule or compound that emits radiation. If such therapeutic moieties are coupled to a sortilin-specific antibody, such as the antibodies described herein, the coupled therapeutic moieties will have a specific affinity for sortilin- and progranulin

-related disease-affected cells over normal cells. In addition, smaller quantities of the therapeutic moieties can be used.

[0499] Some such antibodies can be modified to act as immunotoxins. *See, e.g.*, U.S. Patent No. 5,194,594. For example, ricin, a cellular toxin derived from plants, can be coupled to antibodies by using the bifunctional reagents S-acetylmercaptosuccinic anhydride for the antibody and succinimidyl 3-(2-pyridyldithio)propionate for ricin. *See Pietersz et al., Cancer Res.* 48(16):4469-4476 (1998). The coupling results in loss of B-chain binding activity of ricin, while impairing neither the toxic potential of the A-chain of ricin nor the activity of the antibody. Similarly, saporin, an inhibitor of ribosomal assembly, can be coupled to antibodies via a disulfide bond between chemically inserted sulfhydryl groups. *See Polito et al., Leukemia* 18:1215-1222 (2004).

[0500] Some such antibodies can be linked to radioisotopes. Examples of radioisotopes include, for example, yttrium⁹⁰ (90Y), indium¹¹¹ (111In), ¹³¹I, ^{99m}Tc, radiosilver-111, radiosilver-199, and Bismuth²¹³. Linkage of radioisotopes to antibodies may be performed with conventional bifunction chelates. For radiosilver-111 and radiosilver-199 linkage, sulfur-based linkers may be used. *See Hazra et al., Cell Biophys.* 24-25:1-7 (1994). Linkage of silver radioisotopes may involve reducing the immunoglobulin with ascorbic acid. For radioisotopes such as 111In and 90Y, ibritumomab tiuxetan can be used and will react with such isotopes to form 111In-ibritumomab tiuxetan and 90Y-ibritumomab tiuxetan, respectively. *See Witzig, Cancer Chemother. Pharmacol.*, 48 Suppl 1:S91-S95 (2001).

[0501] Some such antibodies can be linked to other therapeutic moieties. Such therapeutic moieties can be, for example, cytotoxic, cytostatic, neurotrophic, or neuroprotective. For example, antibodies can be conjugated with toxic chemotherapeutic drugs such as maytansine, geldanamycin, tubulin inhibitors such as tubulin binding agents (*e.g.*, auristatins), or minor groove binding agents such as calicheamicin. Other representative therapeutic moieties include agents known to be useful for treatment, management, or amelioration of a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging.

[0502] Antibodies can also be coupled with other proteins. For example, antibodies can be coupled with Fynomers. Fynomers are small binding proteins (*e.g.*, 7 kDa) derived from the human Fyn SH3 domain. They can be stable and soluble, and they can lack cysteine residues and disulfide bonds. Fynomers can be engineered to bind to target molecules with the same affinity and specificity as antibodies. They are suitable for creating multi-specific fusion proteins based on antibodies. For example, Fynomers can be fused to N-terminal and/or C-terminal ends of antibodies to create bi- and tri-specific FynomAbs with different architectures. Fynomers can be selected using Fynomer libraries through screening technologies using FACS, Biacore, and cell-based assays that allow efficient selection of Fynomers with optimal properties. Examples of Fynomers are disclosed in Grabulovski *et al.*, *J. Biol. Chem.* 282:3196-3204 (2007); Bertschinger *et al.*, *Protein Eng. Des. Sel.* 20:57-68 (2007); Schlatter *et al.*, *MAbs.* 4:497-508 (2011); Banner *et al.*, *Acta. Crystallogr. D. Biol. Crystallogr.* 69(Pt6):1124-1137 (2013); and Brack *et al.*, *Mol. Cancer Ther.* 13:2030-2039 (2014).

[0503] The antibodies disclosed herein can also be coupled or conjugated to one or more other antibodies (*e.g.*, to form antibody heteroconjugates). Such other antibodies can bind to different epitopes within sortilin or can bind to a different target antigen.

[0504] Antibodies can also be coupled with a detectable label. Such antibodies can be used, for example, for detect sortilin in a subject having or being treated for a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging, and/or for assessing efficacy of treatment. Such antibodies are particularly useful for performing such determinations in subjects having or being susceptible to of a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging, or in appropriate biological samples obtained from such subjects. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8. Representative detectable labels that may be coupled or linked to an antibody include various enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such streptavidin/biotin and avidin/biotin; fluorescent materials, such as umbelliferone, fluorescein,

fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as luminol; bioluminescent materials, such as luciferase, luciferin, and aequorin; radioactive materials, such as radiosilver-111, radiosilver-199, Bismuth²¹³, iodine (¹³¹I, ¹²⁵I, ¹²³I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹⁵In, ¹¹³In, ¹¹²In, ¹¹¹In), technetium (⁹⁹Tc), thallium (²⁰¹Tl), gallium (⁶⁸Ga, ⁶⁷Ga), palladium (¹⁰³Pd), molybdenum (⁹⁹Mo), xenon (¹³³Xe), fluorine (¹⁸F), ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, and ¹¹⁷Tm; positron emitting metals using various positron emission tomographies; nonradioactive paramagnetic metal ions; and molecules that are radiolabelled or conjugated to specific radioisotopes.

[0505] Linkage of radioisotopes to antibodies may be performed with conventional bifunction chelates. For radiosilver-111 and radiosilver-199 linkage, sulfur-based linkers may be used. *See Hazra et al., Cell Biophys.* 24-25:1-7 (1994). Linkage of silver radioisotopes may involve reducing the immunoglobulin with ascorbic acid. For radioisotopes such as 111In and 90Y, ibritumomab tiuxetan can be used and will react with such isotopes to form 111In-ibritumomab tiuxetan and 90Y-ibritumomab tiuxetan, respectively. *See Witzig, Cancer Chemother. Pharmacol.*, 48 Suppl 1:S91-S95 (2001).

[0506] Therapeutic moieties, other proteins, other antibodies, and/or detectable labels may be coupled or conjugated, directly or indirectly through an intermediate (*e.g.*, a linker), to an antibody of the invention. *See e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery," in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies 84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy," in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985); and Thorpe *et al.*, *Immunol. Rev.*, 62:119-58 (1982). Suitable linkers include, for example, cleavable and non-cleavable linkers. Different linkers that release the coupled therapeutic moieties, proteins,

antibodies, and/or detectable labels under acidic or reducing conditions, on exposure to specific proteases, or under other defined conditions can be employed.

VI. Pharmaceutical Compositions and Methods of Use

[0507] In prophylactic applications, an antibody or agent for inducing an antibody or a pharmaceutical composition the same is administered to a patient susceptible to, or otherwise at risk of a disease (*e.g.*, frontotemporal dementia) in regimen (dose, frequency and route of administration) effective to reduce the risk, lessen the severity, or delay the onset of at least one sign or symptom of the disease. In particular, the regimen is preferably effective to increase extracellular progranulin levels in the brain, or plasma and/or inhibit/or delay development of behavioral deficits. In therapeutic applications, an antibody or agent to induce an antibody is administered to a patient suspected of, or already suffering from a disease (*e.g.*, frontotemporal dementia) in a regimen (dose, frequency and route of administration) effective to ameliorate or at least inhibit further deterioration of at least one sign or symptom of the disease. In particular, the regimen is preferably effective to increase or at least normalize levels of progranulin and/or behavioral deficits.

[0508] A regimen is considered therapeutically or prophylactically effective if an individual treated patient achieves an outcome more favorable than the mean outcome in a control population of comparable patients not treated by methods of the invention, or if a more favorable outcome is demonstrated in treated patients versus control patients in a controlled clinical trial (*e.g.*, a phase II, phase II/III or phase III trial) at the $p < 0.05$ or 0.01 or even 0.001 level.

[0509] Effective doses of vary depending on many different factors, such as means of administration, target site, physiological state of the patient, whether the patient is a FTD-GRN carrier, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic.

[0510] Exemplary dosage ranges for antibodies are from about 0.01 to 60 mg/kg, or from about 0.1 to 3 mg/kg or 0.15-2 mg/kg or 0.15-1.5 mg/kg, of patient body weight. Antibody can be administered such doses daily, on alternative days, weekly, fortnightly, monthly, quarterly, or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six

months. Additional exemplary treatment regimen entail administration once per every two weeks or once a month or once every 3 to 6 months.

[0511] The amount of an agent for active administration varies from 0.1-500 µg per patient and more usually from 1-100 or 1-10 µg per injection for human administration. The timing of injections can vary significantly from once a day, to once a year, to once a decade. A typical regimen consists of an immunization followed by booster injections at time intervals, such as 6 week intervals or two months. Another regimen consists of an immunization followed by booster injections 1, 2 and 12 months later. Another regimen entails an injection every two months for life. Alternatively, booster injections can be on an irregular basis as indicated by monitoring of immune response. Exemplary doses and dosing regimens for non-human primates are in Examples 21-22, and Figures 14-21.

[0512] Antibodies or agents for inducing antibodies are preferably administered via a peripheral route (*i.e.*, one in which an administered or induced antibody crosses the blood brain barrier to reach an intended site in the brain.) Routes of administration include topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intrathecal, intraperitoneal, intranasal, intraocular, intradermal, or intramuscular. Some routes for administration of antibodies are intravenous and subcutaneous. Some routes for active immunization are subcutaneous and intramuscular. This type of injection is most typically performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example intracranial injection.

[0513] Pharmaceutical compositions for parenteral administration are preferably sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (*i.e.*, the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. For injection, antibodies can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfort at the site of injection). The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Alternatively antibodies can be in lyophilized form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

[0514] The present regimen can be administered in combination with another agent effective in treatment or prophylaxis of the disease being treated. For example, in the case of frontotemporal dementia, the present regimen can be combined with an antidepressant, such as trazodone and selective serotonin reuptake inhibitors (*e.g.*, citalopram (Celexa), paroxetine (Paxil) or sertraline (Zoloft)); and/or an antipsychotic medication (*e.g.*, such as olanzapine (Zyprexa) or quetiapine (Seroquel), Alector's AL001, or antibodies as described in published US patent application US20170267761 A1). In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8.

[0515] Antibodies are administered in an effective regimen meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a disorder being treated. If a patient is already suffering from a disorder, the regimen can be referred to as a therapeutically effective regimen. If the patient is at elevated risk of the disorder relative to the general population but is not yet experiencing symptoms, the regimen can be referred to as a prophylactically effective regimen. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients.

[0516] Exemplary dosages for an antibody are 0.1-60 mg/kg (*e.g.*, 0.5, 3, 10, 30, or 60 mg/kg), or 0.5-5 mg/kg body weight (*e.g.*, 0.5, 1, 2, 3, 4 or 5 mg/kg) or 10-4000 mg or 10-1500 mg as a fixed dosage. The dosage depends on the condition of the patient and response to prior treatment, if any, whether the treatment is prophylactic or therapeutic and whether the disorder is acute or chronic, among other factors.

[0517] Administration can be parenteral, intravenous, oral, subcutaneous, intra-arterial, intracranial, intrathecal, intraperitoneal, topical, intranasal or intramuscular. Some antibodies can be administered into the systemic circulation by intravenous or subcutaneous administration. Intravenous administration can be, for example, by infusion over a period such as 30-90 min.

[0518] The frequency of administration depends on the half-life of the antibody in the circulation, the condition of the patient and the route of administration among other factors. The frequency can be daily, weekly, monthly, quarterly, or at irregular intervals in response to changes in the patient's condition or progression of the disorder being treated. An exemplary frequency for intravenous administration is between weekly and quarterly over a continuous course of treatment, although more or less frequent dosing is also possible. For subcutaneous administration, an exemplary dosing frequency is daily to monthly, although more or less frequent dosing is also possible.

[0519] The number of dosages administered depends on whether the disorder is acute or chronic and the response of the disorder to the treatment. For acute disorders or acute exacerbations of a chronic disorder, between 1 and 10 doses are often sufficient. Sometimes a single bolus dose, optionally in divided form, is sufficient for an acute disorder or acute exacerbation of a chronic disorder. Treatment can be repeated for recurrence of an acute disorder or acute exacerbation. For chronic disorders, an antibody can be administered at regular intervals, *e.g.*, weekly, fortnightly, monthly, quarterly, every six months for at least 1, 5 or 10 years, or the life of the patient.

A. Diagnostics and Monitoring Methods

[0520] Also provided are methods of detecting sortilin in a subject, for example, by measuring sortilin in a sample from a subject or by *in vivo* imaging of sortilin in a subject. Such methods are useful to diagnose or confirm diagnosis of diseases associated with sortilin or progranulin or susceptibility thereto. The methods can also be used on asymptomatic subjects. . The methods are also useful for monitoring disease progression and/or response to treatment in subjects who have been previously diagnosed with a disease associated with sortilin and progranulin, such as, for example, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8.

[0521] The methods work by administering a reagent, such as any of the antibodies that binds sortilin described in this application (*e.g.*, a mouse, humanized, chimeric or veneered 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, and 4N2 antibody), to the subject and

then detecting the agent after it has bound. Antibodies specifically binding to sortilin at an epitope within amino acid residues FTESFLT (SEQ ID NO:202), ESFL(SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), or E(S/Q/D)FL (SEQ ID NO:207), RTEFGMAIGP (SEQ ID NO:213), or WGFTESFLTS (SEQ ID NO:214), or to an epitope defined by residues D74, R76, F97, K110, Y535, L560, and E557 of the sortilin ECD of SEQ ID NO:215, or to an epitope defined by residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of the sortilin ECD of SEQ ID NO:215, or to an epitope defined by residues E557, S558, F559, L560, P510, and Y535 of the sortilin ECD of SEQ ID NO:215. can be used. In some methods, the antibody binds to a peptide consisting of an epitope within amino acid residues FTESFLT (SEQ ID NO:202), ESFL(SEQ ID NO:203), DGCILGYKEQFL) (SEQ ID NO:204), PSICLCSLEDFL (SEQ ID NO:205), E(S/Q/D)FL (SEQ ID NO:207), RTEFGMAIGP (SEQ ID NO:213), or WGFTESFLTS (SEQ ID NO:214). Antibodies typically bind to an epitope of sortilin. If desired, the clearing response can be avoided by using antibody fragments lacking a full length constant region, such as Fabs. In some methods, the same antibody can serve as both a treatment and diagnostic reagent.

[0522] Diagnostic reagents can be administered by intravenous injection into the body of the patient, or directly into the brain by intracranial injection or by drilling a hole through the skull. The dosage of reagent should be within the same ranges as for treatment methods. Typically, the reagent is labeled, although in some methods, the primary reagent with affinity for sortilin is unlabeled and a secondary labeling agent is used to bind to the primary reagent. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical detection. Use of paramagnetic labels is suitable for tomographic detection without surgical intervention. Radioactive labels can also be detected using positron emission tomography (PET) or single-photon emission computed tomography (SPECT).

[0523] Diagnosis is performed by comparing the number, size, and/or intensity of labeled loci to corresponding base line values. The base line values can represent the mean levels in a population of undiseased individuals. Base line values can also represent previous levels determined in the same subject. For example, base line values can be determined in a subject before beginning treatment, and measured values thereafter compared with the base line values.

If the patient has reductions in cell surface sortilin, then the therapy may be adjusted to increase cell surface sortilin to normal levels.

[0524] The methods of in vivo imaging of sortilin are useful to monitor cell surface sortilin in patients being treated with agents to increase extracellular progranulin, for example in patients being treated for a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging, or susceptibility to such a disease. For example, the methods can be used on a patient being treated for frontotemporal dementia. If the patient has reductions in cell surface sortilin, then the therapy may be adjusted to increase cell surface sortilin to normal levels. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8.

[0525] In some patients, diagnosis of a disease or disorder associated with changes in progranulin levels may be aided by performing a PET scan. A PET scan can be performed using, for example, a conventional PET imager and auxiliary equipment. The scan typically includes one or more regions of the brain known in general to be associated with a disease or disorder associated with changes in progranulin levels and one or more regions in which to serve as controls.

[0526] The signal detected in a PET scan can be represented as a multidimensional image. The multidimensional image can be in two dimensions representing a cross-section through the brain, in three dimensions, representing the three dimensional brain, or in four dimensions representing changes in the three dimensional brain over time. A color scale can be used with different colors indicating different amounts of label and, inferentially, sortilin protein detected. The results of the scan can also be presented numerically, with numbers relating to the amount of label detected and consequently amount of sortilin. The label present in a region of the brain known to be associated with a particular disease or disorder associated with changes in progranulin levels (*e.g.*, frontotemporal dementia) can be compared with the label present in a region known not to be associated with the disease or disorder to provide a ratio indicative of the extent of deposits within the former region. For the same radiolabeled ligand, such ratios provide a comparable measure of sortilin and changes thereof between different patients.

[0527] In some methods, a PET scan is performed concurrent with or in the same patient visit as an MRI or CAT scan. An MRI or CAT scan provides more anatomical detail of the brain than a PET scan. However, the image from a PET scan can be superimposed on an MRI or CAT scan image more precisely indicating the location of PET ligand and inferentially sortilin relative to anatomical structures in the brain. Some machines can perform both PET scanning and MRI or CAT scanning without the patient changing positions between the scans facilitating superimposition of images.

[0528] Suitable PET ligands include radiolabeled antibodies of the invention (*e.g.*, a mouse, humanized, chimeric or veneered 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 antibody. The radioisotope used can be, for example, C¹¹, N¹³, O¹⁵, F¹⁸, or I¹²³. The interval between administering the PET ligand and performing the scan can depend on the PET ligand and particularly its rate of uptake and clearing into the brain, and the half-life of its radiolabel.

[0529] PET scans can also be performed as a prophylactic measure in asymptomatic patients or in patients who have symptoms of mild cognitive impairment but have not yet been diagnosed with a disease or disorder associated with changes in progranulin levels but are at elevated risk of developing a disease or disorder associated with changes in progranulin levels. For asymptomatic patients, scans are particularly useful for individuals considered at elevated risk of a disease or disorder associated with changes in progranulin levels because of a family history, genetic or biochemical risk factors, or mature age. Prophylactic scans can commence for example, at a patient age between 45 and 75 years. In some patients, a first scan is performed at age 50 years.

[0530] Prophylactic scans can be performed at intervals of for example, between six months and ten years, preferably between 1-5 years. In some patients, prophylactic scans are performed annually.

[0531] The foregoing description of diagnosing, monitoring, and adjusting treatment for diseases and disorders associated with sortilin and progranulin has been largely focused on using PET scans. However, any other technique for visualizing and/or measuring sortilin that is amenable to the use of sortilin antibodies of the invention (*e.g.*, a mouse, humanized, chimeric or

veneered 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 antibody) can be used in place of PET scans to perform such methods.

[0532] Also provided are methods of detecting an immune response against sortilin in a patient suffering from or susceptible to diseases and disorders associated with sortilin and progranulin. The methods can be used to monitor a course of therapeutic and prophylactic treatment with the agents provided herein. The antibody profile following passive immunization typically shows an immediate peak in antibody concentration followed by an exponential decay. Without a further dose, the decay approaches pretreatment levels within a period of days to months depending on the half-life of the antibody administered. For example, the half-life of some human antibodies is of the order of 20 days.

[0533] In some methods, a baseline measurement of antibody to sortilin in the subject is made before administration, a second measurement is made soon thereafter to determine the peak antibody level, and one or more further measurements are made at intervals to monitor decay of antibody levels. When the level of antibody has declined to baseline or a predetermined percentage of the peak less baseline (*e.g.*, 50%, 25% or 10%), administration of a further dose of antibody is administered. In some methods, peak or subsequent measured levels less background are compared with reference levels previously determined to constitute a beneficial prophylactic or therapeutic treatment regimen in other subjects. If the measured antibody level is significantly less than a reference level (*e.g.*, less than the mean minus one or, preferably, two standard deviations of the reference value in a population of subjects benefiting from treatment) administration of an additional dose of antibody is indicated.

[0534] Biological samples obtained from a subject having, suspected of having, or at risk of having a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging can be contacted with the antibodies disclosed herein to assess the presence of sortilin. For example, levels of sortilin in such subjects may be compared to those present in healthy subjects. Alternatively, levels of sortilin in such subjects receiving treatment for the disease may be compared to those of subjects who have not been treated for a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's

disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging. Some such tests involve a biopsy of tissue obtained from such subjects. ELISA assays may also be useful methods, for example, for assessing sortilin in fluid samples.

VII. Kits

[0535] The invention further provides kits (*e.g.*, containers) comprising an antibody disclosed herein and related materials, such as instructions for use (*e.g.*, package insert). The instructions for use may contain, for example, instructions for administration of the antibody and optionally one or more additional agents. The containers of antibody may be unit doses, bulk packages (*e.g.*, multi-dose packages), or sub-unit doses.

[0536] Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products

[0537] Kits can also include a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It can also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

VIII. Other Applications

[0538] The antibodies can be used for detecting sortilin, or fragments thereof, in the context of clinical diagnosis or treatment or in research. For example, the antibodies can be used to detect the presence of sortilin in a biological sample. Binding of the antibodies to the biological sample can be compared to binding of the antibodies to a control sample. The control sample and the biological sample can comprise cells of the same tissue origin. Control samples and biological samples can be obtained from the same individual or different individuals and on the same occasion or on different occasions. If desired, multiple biological samples and multiple control samples are evaluated on multiple occasions to protect against random variation independent of the differences between the samples. A direct comparison can then be made between the biological sample(s) and the control sample(s) to determine whether antibody binding (*i.e.*, the

presence of sortilin) to the biological sample(s) is increased, decreased, or the same relative to antibody binding to the control sample(s). Increased binding of the antibody to the biological sample(s) relative to the control sample(s) indicates the presence of sortilin in the biological sample(s). In some instances, the increased antibody binding is statistically significant. Optionally, antibody binding to the biological sample is at least 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, or 100-fold higher than antibody binding to the control sample.

[0539] In addition, the antibodies can be used to detect the presence of the sortilin in a biological sample to monitor and evaluate the efficacy of a therapeutic agent being used to treat a patient diagnosed with a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8. A biological sample from a patient diagnosed with a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging is evaluated to establish a baseline for the binding of the antibodies to the sample (*i.e.*, a baseline for the presence of the sortilin in the sample) before commencing therapy with the therapeutic agent. In some instances, multiple biological samples from the patient are evaluated on multiple occasions to establish both a baseline and measure of random variation independent of treatment. A therapeutic agent is then administered in a regimen. The regimen may include multiple administrations of the agent over a period of time. Optionally, binding of the antibodies (*i.e.*, presence of sortilin) is evaluated on multiple occasions in multiple biological samples from the patient, both to establish a measure of random variation and to show a trend in response to immunotherapy. The various assessments of antibody binding to the biological samples are then compared. If only two assessments are made, a direct comparison can be made between the two assessments to determine whether antibody binding (*i.e.*, presence of sortilin) has increased, decreased, or remained the same between the two assessments. If more than two measurements are made, the measurements can be analyzed as a time course starting before treatment with the therapeutic agent and proceeding through the course of therapy. In patients for whom antibody binding to biological samples has

decreased (*i.e.*, reduced cell surface sortilin), then the therapy may be adjusted to increase cell surface sortilin to normal levels. Assessment of antibody binding can be made in conjunction with assessing other signs and symptoms of with a disease or disorder associated with changes in progranulin levels, frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging. In some embodiments, the methods further comprise detecting, measuring, and/or monitoring progranulin levels, for example as in Example 8.

[0540] The antibodies can also be used as research reagents for laboratory research in detecting sortilin, or fragments thereof. In such uses, antibodies can be labeled with fluorescent molecules, spin-labeled molecules, enzymes, or radioisotopes, and can be provided in the form of kit with all the necessary reagents to perform the detection assay. The antibodies can also be used to purify sortilin, or binding partners of sortilin, *e.g.*, by affinity chromatography.

[0541] All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

EXAMPLES

[0542] Example 1. Generation of mouse anti-human Sortilin antibodies

[0543] A. Immunizations

[0544] Eight approximately 6-week-old female mice of various stains (2 x Swiss webster, 2 x NZB/w, 2 x AJ, 1 x SJL and 1 x Balb/C) were immunized subcutaneously in the Hock (lateral tarsal region just above the ankle) as in Table 5 below, with the extracellular domain (ECD) of recombinant human Sortilin with a C-terminal HIS tag (ECD-huSortilin-HIS). Immunogens in PBS were mixed 1:1 with the appropriate adjuvants (see table below) for the first 8 injections, then received a final injection without any adjuvant. Mice were then titrated against ECD-huSortilin-HIS (R&D Systems) and an irrelevant HIS tagged protein to ensure specificity to Sortilin on days 17 and 24 of the 28 day immunization schedule.

[0545] Table 5: Immunization Schedule

Day	Adjuvant	Amount	Route of administration
Day 0	CFA (Sigma)	10ug / 60uL per injection	Hock
Day 3	Titermax (Sigma)	10ug / 60uL per injection	Hock
Day 7	Titermax (Sigma)	10ug / 60uL per injection	Hock
Day 9	RIBI (Sigma)	10ug / 60uL per injection	Hock
Day 11	RIBI(Sigma)	10ug / 60uL per injection	Hock
Day 15	GERBU (V-Biognostics)	10ug / 60uL per injection	Hock
Day 18	Titermax (Sigma)	10ug / 60uL per injection	Hock
Day 22	RIBI (Sigma)	10ug / 60uL per injection	Hock
Day 25	None	20ug / 60uL per injection	Hock
Day 17	Bleed -1 st Titers		
Day 24	Bleed -2 nd Titers		

[0546] B. Fusions & Screening

[0547] All mice showed antigen-specific titers and were sacrificed. The popliteal, inguinal and mesenteric lymph nodes and spleens were collected (spleens from all mice were pooled and lymph nodes from all mice were pooled) and processed. Splenocytes were cryopreserved and B cell enrichment (Stem Cell Technologies) was carried out on pooled lymphocytes according to

the manufacturers protocol. Approximately 80 million B cells were fused at a 1:1 ratio with SP2/0 mouse myeloma cells (ATCC) by electrofusion. 1/3 of the fused cells were plated in 11 x 384 well plates in fusion media (80ul/well) and incubated in 37°C-5% CO₂ and the remaining cells were cryopreserved.

[0548] After 8-10 days supernatants were screened by ELISA. For the primary screen, 384-well ELISA plates (chemiluminescence compatible) were coated with 1ug/mL recombinant ECD-huSortilin-HIS (20uL/well) and incubated overnight at 4°C. all plates were blocked with 3% BSA in PBS for 1 hour at room temperature. Plates were then washed with Tris buffered saline containing 0.1% Tween20 (TBST) three times. 20uL of supernatants were added to the analogous wells in the ELISA plates, incubated and then washed as above. Goat anti-mouse IgG specific-HRP (Jackson ImmunoResearch) diluted 1:7000 in 1% BSA in TBST was then added at 20uL per well to react with the bound antibodies and was incubated for 1 hour before being washed as above. Super signal Pico chemiluminescent ELISA substrate (ThermoFisher) was used for detection. 20uL of substrate was added to each well and Relative Light Units (RLU) were measured immediately using the SpectraMax Paradigm (Molecular Devices). The top 576 wells (based on RLU signal) were transferred to 96 well plates and cultured for 2 days. A confirmation screen was then conducted using ECD-huSortilin-HIS and an irrelevant His protein to determine whether the antibodies were Sortilin specific. 105 non-clonal hybridomas were selected from the confirmation screen and cultured further for both cryopreservation and to produce saturated supernatants for further testing. 105 saturated supernatants were tested for blocking Progranulin (ligand of sortilin) binding to sortilin by ELISA (methods as in Example 4). Results are depicted in Figure 7. 29 antibodies blocked the binding of progranulin to sortilin. The 29 blocking hybridomas and 2 non-blocking (negative controls) hybridomas were thawed and cloned to produce monoclonal antibodies.

[0549] Example 2: Cloning and Monoclonal Antibody Purification

[0550] Cloning of hybridomas was done by serial dilutions or using the clonepix 2 (molecular Devices). Hybridomas that were cloned using the Clonepix2 were done so by following the manufacturers protocol. For serial dilution cloning, cells were counted and plated at a density of 0.5 cells per well into ½ of a 384-well plate in cloning media and incubated at 37°C-5% CO₂.

The next day, each well was examined visually under the microscope and wells with a single colony of cells were marked as monoclonal. After 7-8 days of culture supernatants were screened by ELISA for binding to ECD-huSortilin-HIS. 28 monoclonal antibodies were successfully cloned. The 28 hybridomas were expanded for cryopreservation and antibody purification. Antibodies were purified using Protein A chromatography utilizing standard methods. Following purification, the antibodies were exchanged into 1xPBS and protein concentrations were determined by absorbance at 280nm. Purified monoclonal antibodies (mAbs) then moved on for further screening.

[0551] Example 3: Sortilin Binding ELISA

[0552] Purified anti-huSortilin mAb binding to huSortilin was characterized by ELISA (ELISA protocol as in Example 1) using a 12 point titration of mAb concentration. mAbs were diluted 2-fold from a starting concentration of 67nM and then added to ECD- huSortilin-HIS coated plates. Data was analyzed and EC₅₀ (nM) was calculated using graphed Prism. Commercially available goat anti-huSortilin (R&D Biosystems) was used as a positive control. Results are depicted in Figure 8.

[0553] Example 4 Progranulin blocking ELISA

[0554] Blocking ELISA using non-clonal Saturated supernatants

[0555] To select which hybridomas to clone, saturated supernatants were examined for blocking the binding of Progranulin to Sortilin. Untagged human progranulin (huPGRN, Adipogen) was first biotinylated at a ratio of 5:1 using EZ-Link NHS-PEG4-Biotin (Thermo Fisher) and ZebaSpin Columns following manufacturers protocols. Results are depicted in Figure 7.

[0556] 384-well ELISA plates were coated with 2ug/mL recombinant ECD-huSortilin-HIS (20uL/well) and incubated overnight at 4°C. all plates were blocked with 3% BSA in PBS for 1 hour at room temperature. Plates were then washed with TBST three times. Saturated supernatants were diluted 4-fold in 1% BSA in TBST. 20uL of diluted supernatant was added to the wells and incubated for 1 hour at room temperature. Plates were then washed with TBST and 20uL of 2ug/mL huPGRN-Biotin was added to each well and incubated for 1 hour at room temperature and then washed as above. Streptavidin-HRP (GE Life Sciences) diluted 1:10,000 in 1% BSA in TBST was then added at 20uL per well to react with the bound huPGRN-Biotin and

was incubated for 1 hour before being washed and detected using Super signal Pico chemiluminescent ELISA substrate (ThermoFisher) as above. Hybridomas (of corresponding Supernatants) which Blocked the binding of huPGRN to huSortilin by at least 30% compared to no Ab control were selected for cloning.

[0557] Blocking ELISA using Purified mAbs

[0558] 28 purified anti-huSortilin mAbs were further characterized for ability to block PGRN binding to Sortilin. Blocking ELISA was conducted as above using 6 point 3-fold dilutions of mAbs in 1%BSA in TBST starting at 134nM. Data was analyzed and % blocking was calculated using Microsoft Excel and GraphPad Prism. Results are depicted in Figure 8.

[0559] Example 5: Neurotensin Competition Biacore Assays

[0560] PGRN and Neurotensin (NT) are known to bind similar regions on sortilin. Surface Plasmon Resonance (SPR) (Biacore T200 instrument) was used to examine whether anti-huSortilin mAbs block the binding of NT to Sortilin. Briefly, 25nM anti-huSortilin mAb was captured on CM5 anti-mouse Fc chip (60 seconds association). Then 100nM recombinant ECD-huSortilin-HIS pre-incubated with 0nM, 100nM, 500nM or 1000nM NT (Tocris) (60 second association, 30 second dissociation) was flowed across the captured mAb surface. The chip surface was regenerated using 10nM glycine pH 1.5 between cycles. The data was analyzed and graphed using the Biacore T200 evaluation software 2.0.15.12933. 18 anti-Sortilin mAbs did not compete with NT for binding to Sortilin, 6 showed some competition and 3 clearly competed with NT for binding to Sortilin. Results are depicted in Figure 9.

[0561] Example 6: Sortilin binding Cell-Based Assays

[0562] The ability of the purified anti-sortilin mAbs to bind recombinant huSortilin expressed on the surface of HEK 293 cells and to U251MG cells (human glioblastoma cells line) which endogenously express sortilin on their cell surface was then examined. Parental HEK 293 cells were used as a negative control. All 28 purified anti-sortilin mAbs were tested at a single concentration (67nM). Cells were harvested and plated at 20K cells per well in a 96-well plate. mAbs, diluted in PBS + 2% FBS were added to the wells (50uL/well) and cells were incubated on ice for 1 hour. Cells were then washed twice in PBS+2%FBS and incubated for 30 minutes on ice with 50uL per well 5ug/mL Alexa-Fluor647goat-anti-mouse IgG (Jackson ImmunoResearch)

in PBS+2%FBS. Cells were then washed twice as above and resuspended in 100uL PBS+2%FBS. Data was acquired using the BD LSR II and analyzed using FlowJo software version 10.6. Calculation of Median Fluorescent Intensity (MFI) values showed that all 28 anti-sortilin mAbs bound huSortilin expressing HEK293 cells. 3 of which also showed background binding to parental HEK cells. 22 mAbs showed varying degrees of binding to sortilin on the surface of U251MG cells. 6 mAbs showed no binding to U251 cells. Results are depicted in Figure 10.

[0563] Example 7: Progranulin blocking titration cell-based assays

[0564] All 28 purified anti-sortilin mAbs were tested at a single concentration (67nM) to determine their ability to block huPGRN-Biotin binding to cell surface huSortilin expressing HEK293 cells. Cells were cultured using standard methods. Cells were harvested and plated at 20K cells per well in a 96-well plate. mAbs, diluted in PBS + 2% FBS containing 30nM huPGRN-Biotin were added to the wells (50uL/well) and cells were incubated on ice for 1 hour. Cells were then washed twice in PBS+2%FBS and incubated for 30 minutes on ice with 50uL per well 1:200 Steptavidin-APC (BD biosciences) in PBS+2%FBS. Cells were then washed as above and resuspended in 100uL PBS+2%FBS. Data was acquired using the BD LSR II and analyzed using FlowJo software version 10.6 and Microsoft excel. 12 Anti-sortilin mAbs showed % blocking of huPGRN greater than 30% and were selected for further characterization. Results are depicted in Figure 11.

[0565] The cell-based blocking assay was repeated with the top 12 blocking mAbs using a dose titration from 67nM to 0.4nM. Blocking assay was carried out as above. Data was analyzed using FlowJo software version 10.6. and GraphPad Prism software. Results are depicted in Figure 12.

[0566] The 12 mAbs fell into three tiers. Tier 1 comprised mAbs which showed high potency with regards to huPGRN blocking (8H24, 5E20, 2C14), 7 mAbs (tier2) showed medium blocking potency (2F18, 6B15, 11M14, 11H24, 6M23, 2P22 and 9N18). The last 2 mAbs (7A22 and 5L16) showed low huPGRN blocking potency.

[0567] Example 8: Extracellular Progranulin levels and surface sortilin levels

[0568] U251MG cells were seeded in 96-well plates and incubated overnight at 37°C-5% CO₂. The next day, media was removed and fresh media containing 50nM either anti-Sortilin mAbs, commercially available goat-anti-hSortilin (positive blocking antibody, R&D Biosystems) or Isotype control antibodies were added to the wells. Cells were then cultured for 72 hours. Supernatants were then used to determine extracellular huPGRN levels and the cells were harvested to examine cell surface sortilin levels.

[0569] The increase in PGRN levels were measured by plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 96-well MSD plates were coated with 1ug/mL mouse anti-huPGRN (R&D Biosystems) in PBS overnight at 4°C. plates were blocked in 3% MSD buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 50uL cell supernatant was added to the wells and incubated at room temperature for 1 hour. Plates were then washed as above and 50uL of 1ug/mL biotinylated goat anti-huPGRN (R&D Biosystems) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1 hour at room temperature. Plates were washed as above and 05.ug/mL SULFO-TAG-Streptavidin (MSD) in 1% MSD buffer A in PBS was added the wells and plates were incubated for 1 hour at room temperature and then washed as above. 150uL 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and were quantitated against a standard curve using 3-fold serial dilutions starting at 100ng/mL of recombinant huPGRN (R&D Biosystems). Fold PGRN levels over controls were calculated using Microsoft excel. Of the 25 mouse anti-huSortilin mAbs tested, 19 showed a >1.5 fold increase in huPGRN over no mAbs control or isotype control wells. Results are depicted in Figure 13.

[0570] The percent decrease in surface sortilin levels was determined by flow cytometry. U251MG cells were harvested and plated into 96-well untreated plates. 2.5ug/mL goat anti-human sortilin antibody (R&D Biosystems) were added to the wells (50uL/well) and cells were incubated on ice for 1 hour. Cells were then washed twice in PBS+2%FBS and incubated for 30 minutes on ice with 50uL per well 5ug/mL Alexa-fluor647-donkey anti-goat (Jackson ImmunoResearch) secondary antibody in PBS+2%FBS. Cells were then washed twice as above and resuspended in 100uL PBS+2%FBS. Data was acquired using the BD LSR II and analyzed

to determine Median Fluorescent Intensity (MFI) using FlowJo software version 10.6. the Percent of cell surface huSortilin compared to no mAb control wells using MFI values was calculated using Microsoft excel. Most mAbs showed a decrease in cell surface sortilin levels in U251 cells to varying degrees. 13/25 mouse anti-huSortilin mAbs increased extracellular huPGRN levels >1.5 fold and decrease cell surface sortilin by a maximum of 60%. Results are depicted in Figure 13.

[0571] Example 9: Characterization of murine mAbs by BIAcore

[0572] Analysis was performed using a Biacore T200 to compare the binding affinity of murine anti-huSortilin antibodies to recombinant human Sortilin. Anti-mouse antibody was immobilized on sensor chip CM5 (GE Healthcare Life Sciences) via amine coupling, and mouse antibodies (ligand) were captured to a level to ensure a maximum binding of analyte of 50 RU (approximately 100RU of ligand binding). Various concentrations of Sortilin (ranging from 0.1nM to 300nM) were passed over the captured ligand at 50 μ L/min in running buffer (HBS + 0.05% P-20, 1 mg/mL BSA) for 180-300s association time and 300-900s dissociation time. Regeneration of the chip surface was accomplished by 2 short injections of 10mM Glycine-HCl at pH 1.7. Data was blank subtracted to both a sensor not containing ligand and 0nM analyte concentration. Analysis was performed using a global 1:1 fit with Biacore Insight Evaluation software (v2.0) with bulk refractive index set to zero RU. Binding data are shown in Table 6.

[0573] Table 6. Binding data of mAbs toward human Sortilin.

mAb	ka 1/Ms	kd 1/s	K_D nM
5E20	8.77e+4	1.39e-4	1.59
8H24	9.66e+4	4.40e-4	4.56
11M14	9.11e+4	5.69e-4	6.24
5M13	1.79 e+5	4.11 e-4	2.29
2C14	2.08e+5	2.45e-3	11.8
2F18	5.76e+4	1.62e-4	2.81
2P22	1.08e+5	2.37e-4	2.21
6B15	1.27e+5	1.30e-4	1.02
9N18	1.91e+5	1.50e-4	11.7
4N2	1.68e+5	5.56e-4	3.2

[0574] Example 10: Epitope mapping of Murine 5E20

[0575] PEPperMAP linear epitope mapping was carried out by PEPperPrint GmbH. Epitope analysis of 5E20 was performed by peptide microarray analysis. The sequence of the extracellular domain of human sortilin (756 amino acids, SEQ ID NO:1) was elongated with neutral GSGSGSG (SEQ ID NO:207) linkers at the C- and N-terminus to avoid truncated peptides. The linked and elongated antigen sequence was translated into linear 15 amino acid peptides with a peptide-peptide overlap of 14 amino acids. The resulting human sortilin peptide microarrays contained 756 different peptides printed in duplicate (1,512 spots) and were framed by additional HA (YPYDVDPDYAG, SEQ ID NO:200, 46 spots) and c-Myc (EQKLISEEDL, SEQ ID NO:201, 46 spots) control peptides.

[0576] After synthesis, the microarray was blocked to prevent nonspecific binding (Rockland catalog #MB-070). Murine 5E20 was then applied to the microarray at a concentration of 1 µg/mL along with positive control mouse monoclonal anti-HA (12CA5) DyLight800 (0.5 µg/ml) for 16h at 4°C with shaking at 140 rpm. The microarray was washed, and secondary antibody

(Goat anti-mouse IgG (H+L) DyLight680 (0.2 µg/ml) was applied for 45 minutes at room temperature. After further washing, the microarray was imaged using a Licor Odyssey Imaging System.

[0577] Quantification of spot intensities and peptide annotation were based on the 16-bit gray scale tiff files at scanning intensities of 7/7 that exhibit a higher dynamic range than the 24-bit colorized tiff files; microarray image analysis was done with PepSlide® Analyzer. A software algorithm separates fluorescence intensities of each spot into raw, foreground and background signal, and calculates averaged median foreground intensities and spot-to-spot deviations of spot duplicates (see “Raw Data” tabs). Based on averaged median foreground intensities, an intensity map was generated and interactions in the peptide map highlighted by an intensity color code with red for high and white for low spot intensities. A maximum spot-to-spot deviation of 40% was tolerated, otherwise the corresponding intensity value was zeroed.

[0578] A very strong monoclonal antibody response was observed for mouse 5E20 against an epitope-like spot pattern formed by adjacent peptides with the consensus motif FTESFLT (SEQ ID NO:202); we also observed two very weak additional responses against peptides with the consensus motifs DGCILGYKEQFL (SEQ ID NO:204) and PSICLCSLEDFL (SEQ ID NO:205) presumably due to a cross-reaction based on minor sequence similarities (see underlined amino acid positions).

[0579] Example 11: Characterization of humanized mAbs: huSortilin Binding ELISA

[0580] 384-well ELISA plates were coated with 1µg/mL recombinant ECD-huSortilin-HIS (20uL/well) and incubated overnight at 4°C. all plates were blocked with 3% BSA in PBS for 1 hour at room temperature. Plates were then washed with Tris buffered saline containing 0.1% Tween20 (TBST) three times. 20uL of humanized anti-Sortilin mAbs diluted 3-fold from 20ug/mL to 0.3ng/mL in 1% BSA in TBST were added to the wells, incubated for 1 hour at room temperature and then washed as above. Goat anti-human IgG specific-HRP (Jackson ImmunoResearch) diluted 1:7000 in 1% BSA in TBST was then added at 20uL per well to react with the bound antibodies and plates were incubated for 1 hour before being washed as above. Super signal Pico chemiluminescent ELISA substrate (ThermoFisher) was used for detection. 20uL of substrate was added to each well and Relative Light Units (RLU) were measured

immediately using the SpectraMax Paradigm (Molecular Devices). Data was analyzed and EC_{50} was calculated using graphed Prism. Results for selected combinations of humanized 5E20 variants are in Table 7. Results for selected combinations of humanized 8H24 variants are in Table 8. Results for selected combinations of humanized 11M14 variants are in Table 9.

[0581] Table 7: Humanized 5E20 EC₅₀

mAb	EC₅₀ (ug/mL)
5E20H1L1	0.12
5E20H1L2	0.12
5E20H1L3	0.09
5E20H2L1	0.12
5E20H2L2	0.08
5E20H2L3	0.10
5E20H3L3	0.09
5E20H3L4	0.07
5E20H4L3	0.07
5E20H4L4	0.04
5E20H5L3	0.07
5E20H5L4	0.05
5E20H7L3	0.06
5E20H7L4	0.04
chimeric 5E20	0.07

[0582] Table 8: Humanized 8H24 EC₅₀

mAb	EC₅₀ (ug/mL)
8H24H1L1	0.14
8H24H1L2	0.10
8H24H2L1	0.06
8H24H2L2	0.07

[0583] Table 9: Humanized 11M14 EC₅₀

mAb	EC₅₀ (ug/mL)
11M14H1bL2b	0.08
11M14H1bL3b	0.07
11M14H1bL4b	0.08
11M14H2bL2b	0.07
11M14H2bL3b	0.13
11M14H2bL4b	0.10
11M14H3bL2b	0.19
11M14H3bL3b	0.23
11M14H3bL4b	0.10
Chimeric 11M14	0.06

[0584] Example 12: Characterization of humanized mAbs: Functional assays

[0585] U251MG cells were seeded in 96-well plates and incubated overnight at 37°C-5% CO₂. The next day, media was removed and fresh media containing 10-fold dilution from 50nM to 0.5 nM (5E20 round 2 and 11M14 round 2) or 3-fold dilution from 50nM to 5.6 nM (5E20 round 1 and 8H24) humanized, murine and chimeric variants of anti-Sortilin mAbs or Isotype control antibodies were added to the wells. Cells were cultured for 72 hours. Supernatants were then used to determine extracellular huPGRN levels and the cells were harvested to examine cell surface sortilin levels.

[0586] The increase in PGRN levels were measured by plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 96-well MSD plates were coated with 1ug/mL mouse anti-huPGRN (R&D Biosystems) in PBS overnight at 4°C. plates were then blocked in 3% MSD buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 50uL supernatant was added to the wells and incubated at room temperature for 1 hour. Plates were then washed as above and 50uL of 1ug/mL biotinylated goat anti-huPGRN (R&D Biosystems) in 1% MSD buffer A in PBS was added to the wells and plates were

incubated for 1 hour at room temperature. Plates were washed as above and 0.5 µg/mL SULFO-TAG-Streptavidin (MSD) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1 hour at room temperature and then washed as above. 150 µL of 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and were quantitated against a standard curve using 3-fold serial dilutions starting at 100 ng/mL of recombinant huPGRN (R&D Biosystems). Fold PGRN levels over controls were calculated using Microsoft Excel. Results for selected combinations of humanized 5E20 variants are in Table 10. Results for selected combinations of humanized 8H24 variants are in Table 11. Results for selected combinations of humanized 11M14 variants are in Table 12.

[0587] The percent decrease in surface sortilin levels was determined by flow cytometry. U251MG cells were harvested and plated into untreated 96-well plate. 2.5 µg/mL goat anti-human sortilin antibody (R&D Biosystems) were added to the wells (50 µL/well) and cells were incubated on ice for 1 hour. Cells were then washed twice in PBS+2%FBS and incubated for 30 minutes on ice with 50 µL per well 5 µg/mL Alexa-fluor647-donkey anti-goat (Jackson ImmunoResearch) secondary antibody in PBS+2%FBS. Cells were then washed twice as above and resuspended in 100 µL PBS+2%FBS. Data was acquired using the BD LSR II and analyzed to determine Median Fluorescent Intensity (MFI) using FlowJo software version 10.6. The Percent of cell surface huSortilin compared to no mAb control wells using MFI values was calculated using Microsoft Excel. Results for selected combinations of humanized 5E20 variants are in Table 10. Results for selected combinations of humanized 8H24 variants are in Table 11. Results for selected combinations of humanized 11M14 variants are in Table 12.

[0588] Table 10: Humanized 5E20 variants: Increase in PGRN levels and Decrease in surface sortilin levels

5E20 Round1

%Cell Surface huSortilin by median MFI	5E20H1L1	5E20H1L2	5E20H1L3	5E20H2L1	5E20H2L2	5E20H2L3	ms5E20	chimeric 5E20	msIgG1 ctrl	huIgG1 ctrl
50nM (7.5ug/mL)	83	83	94	88	86	82	81	94	116	110
16.7nM (2.5ug/mL)	83	82	94	92	89	84	82	94	114	105
5.6 nM (0.8ug/mL)	85	88	98	98	94	87	84	95	112	104

huPGRN fold over Control	5E20H1L1	5E20H1L2	5E20H1L3	5E20H2L1	5E20H2L2	5E20H2L3	ms5E20	chimeric 5E20	msIgG1 ctrl	huIgG1 ctrl
50nM (7.5ug/mL)	1.4	1.3	1.2	1.1	1.4	1.3	2.0	1.8	1.1	1.0
16.7nM (2.5ug/mL)	1.5	1.5	1.4	1.6	1.4	1.4	2.0	1.8	1.0	1.0
5.6 nM (0.8ug/mL)	1.5	1.6	1.2	1.3	1.3	1.3	1.9	1.9	1.1	1.0

5E20 Round2

huPGRN fold over Control	5E20H3L3	5E20H3L4	5E20H4L3	5E20H4L4	5E20H5L3	5E20H5L4	5E20H7L4	chimeric 5E20	ms5E20new	hulgG1 ctrl
50nM (7.5ug/mL)	0.9	1.2	1.4	1.6	1.6	1.6	1.8	2.1	1.9	1.2
5nM (0.75ug/mL)	0.9	1.3	1.2	1.4	1.3	1.5	1.7	1.8	1.8	1.1
0.5nM (0.08ug/mL)	0.9	0.9	1.1	1.4	1.3	1.1	1.4	1.9	1.6	1.1

%Cell Surface huSortilin by median APC	5E20H3L3	5E20H3L4	5E20H4L3	5E20H4L4	5E20H5L3	5E20H5L4	5E20H7L4	chimeric 5E20	ms5E20	hulgG1 ctrl
50nM (7.5ug/mL)	61	60	63	57	59	60	65	63	50	104
5nM (0.75ug/mL)	67	68	62	64	66	65	67	63	51	105
0.5nM (0.08ug/mL)	90	90	87	87	89	85	85	77	70	106

[0589] Table 11: Humanized 8H24 variants: Increase in PGRN levels and Decrease in surface sortilin levels

8H24

huPGRN fold over Control	8H24H1L1	8H24H1L2	8H24H2L1	8H24H2L2	mu8H24	Chimeric 8H24	msIgG2 ctrl	huIgG1 ctrl
50nM (7.5ug/mL)	1.4	1.5	1.4	1.3	1.4	1.4	0.9	1.0
16.7nM (2.5ug/mL)	1.4	1.5	1.4	1.4	1.6	1.5	1.1	1.0
5.6 nM (0.8ug/mL)	1.2	1.2	1.1	1.0	1.2	1.1	0.9	0.9

%Cell Surface huSortilin by median MFI	8H24H1L1	8H24H1L2	8H24H2L1	8H24H2L2	mu8H24	Chimeric 8H24	msIgG2 ctrl	huIgG1 ctrl
50nM (7.5ug/mL)	49	49	49	50	43	51	105	94
16.7nM (2.5ug/mL)	49	49	49	50	43	51	105	94
5.6 nM (0.8ug/mL)	86	81	82	84	77	76	102	101

[0590] Table 12: Humanized 11M14 variants: Increase in PGRN levels and Decrease in surface sortilin levels

11M14 Round 2

huPGRN fold over Control	11M14 H1bL2b	11M14 H1bL3b	11M14 H1bL4b	11M14 H2bL2b	11M14 H2bL3b	11M14 H2bL4b	11M14 H3bL2b	11M14 H3bL3b	11M14 H3bL4b	Chimeric 11M14	ms11M14	huIgG1 ctrl
50nM (7.5ug/mL)	1.9	1.9	1.7	1.6	1.8	1.8	1.7	1.6	1.7	1.5	1.5	1.2
5nM (0.75ug/mL)	1.6	1.7	1.6	1.7	1.7	1.6	1.5	1.5	1.4	1.5	1.3	1.1
0.5nM (0.08ug/mL)	1.3	1.3	1.2	1.0	1.2	1.2	1.2	1.2	1.2	1.2	1.1	1.1

%Cell Surface huSortilin by median MFI	11M14 H1bL2b	11M14 H1bL3b	11M14 H1bL4b	11M14 H2bL2b	11M14 H2bL3b	11M14 H2bL4b	11M14 H3bL2b	11M14 H3bL3b	11M14 H3bL4b	Chimeric 11M14	ms11M14	huIgG1 ctrl
50nM (7.5ug/mL)	65	67	69	71	72	75	77	78	74	75	58	104
5nM (0.75ug/mL)	68	70	72	76	73	75	79	79	83	76	63	105
0.5nM (0.08ug/mL)	77	76	81	80	82	80	87	90	92	85	75	106

[0591] Example 13: Characterization of humanized mAbs by BIAcore

[0592] Analysis was performed using a Biacore T200 to compare the binding affinity of chimeric and humanized antibodies to recombinant human Sortilin. Anti-Human antibody was immobilized on sensor chip CM5 (GE Healthcare Life Sciences) via amine coupling, and humanized antibodies (ligand) were captured to a level to ensure a maximum binding of analyte

of 50 RU (approximately 100 RU of ligand binding). Various concentrations of Sortilin (ranging from 0.1 nM to 300 nM) were passed over the captured ligand at 50 μ L/min in running buffer (HBS + 0.05% P-20, 1 mg/mL BSA) for 300s association time and 1200s dissociation time. Regeneration of the chip surface was accomplished by 2 short injections of 3M MgCl. Data was blank subtracted to both a sensor not containing ligand and 0 nM analyte concentration. Analysis was performed using a global 1:1 fit with Biacore Insight Evaluation software (v2.0) with bulk refractive index set to zero RU. Binding data for selected combinations of 8H24 humanized variants, 5E20 humanized variants, chimeric 5E20, 11M14 humanized variants, and chimeric 11M14 are shown in Table 13.

[0593] Table 13. Binding data of 8H24, 5E20, and 11M14 humanized variants toward human Sortilin.

mAb	ka 1/Ms	kd 1/s	K _D nM
8H24H1L1	6.37e+5	1.41e-3	2.22
8H24H1L2	4.50e+5	1.34e-3	2.98
8H24H2L1	6.84e+5	2.57e-3	2.29
8H24H2L2	4.66e+5	2.50e-3	3.22
Chimeric 5E20	5.97e+5	3.96e-4	0.66
5E20H3L3	1.83e+5	2.80e-3	15.3
5E20H3L4	1.74e+5	2.57e-3	14.8
5E20H4L3	2.28e+5	1.45e-3	6.36
5E20H4L4	2.09e+5	1.17e-3	5.57
5E20H5L3	2.33e+5	1.40e-3	6.01
5E20H5L4	2.11e+5	1.15e-3	5.46
5E20H7L3	2.59e+5	1.08e-3	4.18
5E20H7L4	2.18e+5	1.10e-3	5.02
Chimeric 11M14	3.84e+05	9.69e-04	2.52
11M14H1bL2b	3.60e+05	1.93e-03	5.37
11M14H1bL3b	3.77e+05	2.00e-03	5.30
11M14H1bL4b	3.57e+05	1.90e-03	5.32
11M14H2bL2b	4.21e+05	1.20e-03	2.86
11M14H2bL3b	3.98e+05	1.19e-03	2.99
11M14H2bL4b	4.13e+05	1.17e-03	2.84
11M14H3bL2b	3.19e+05	2.25e-03	7.07
11M14H3bL3b	3.74e+05	3.43e-03	9.17
11M14H3bL4b	4.35e+05	3.44e-03	7.91

[0594] Example 14. Design of Humanized 5E20 Antibodies

[0595] The starting point or donor antibody for humanization was the mouse antibody 5E20. The heavy chain variable amino acid sequence of mature m5E20 is provided as SEQ ID NO:4. The light chain variable amino acid sequence of mature m5E20 is provided as SEQ ID NO:10. The heavy chain Kabat/Chothia Composite CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:5-7, respectively. The light chain Kabat CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:11-13, respectively. Kabat numbering is used throughout.

[0596] The variable kappa (Vk) of 5E20 belongs to mouse Vk subgroup 1b which corresponds to human Vk subgroup 1 and the variable heavy (Vh) to mouse Vh subgroup 3d which corresponds to human Vh subgroup 1 [Kabat E.A., et al., (1991), Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242]. 11 residue Chothia CDR-L1 is similar to Chothia canonical class 2, 7 residue Chothia CDR-L2 is of Chothia canonical class 1, 9 residue Chothia CDR-L3 is similar to Chothia canonical class 1 [Martin ACR. (2010) Protein sequence and structure analysis of antibody variable domains. In: Kontermann R and Dübel S (eds). *Antibody Engineering*. Heidelberg, Germany: Springer International Publishing AG.. [Martin, 2010]. 10 residue Chothia CDR-H1 is similar to Chothia canonical class 1, 17 residue Chothia CDR-H2 is similar to Chothia canonical class 3 [Martin, 2010]. 5 residue CDR-H3 has no canonical classes. A search was made over the protein sequences in the PDB database [Deshpande N, et al., (2005) Nucleic Acids Res. 33: D233-7] to find structures, which would provide a rough structural model of 5E20. The crystal structure of an antibody fab PDB code 3V6F [Dimattia, M.A. et al., Structure 21: 133-142, 2013] for both Vh and Vk structure since it had good resolution (2.52Å) and overall sequence similarity to 5E20 Vh and Vk, retaining the same canonical structures for the loops.

[0597] The frameworks of 5E20 VH share a high degree of sequence similarity with the corresponding regions of human antibody AEX29086 VH, cloned by Bowers, E. et al. (PLoS ONE 9 (1), E81913 (2014)) The variable heavy domains of 5E20 and AEX29086 also share identical lengths for the CDR-H1, H2 loops. Similarly, the frameworks of 5E20 VL share a high degree of sequence similarity with the corresponding regions of human antibody BAH04687 VL cloned by Kurosawa, Y. et al (Direct submission 2016). The variable light domain of 5E20 and BAH04687 antibody also share identical lengths for the CDR-L1, L2 and L3 loops.

Accordingly, the framework regions of AEX29086 VH and BAH04687 VL were chosen as the acceptor sequences for the CDRs of 5E20. A model of the 5E20 CDRs grafted onto the respective human frameworks for VH and VL was built and used as a guidance for further backmutations.

[0598] Heavy and light chain variant sequences resulting from antibody humanization process were further aligned to human germ line sequences using IMGT Domain GapAlign tool to assess the humanness of the heavy and light chain as outlined by WHO INN committee guidelines. (WHO-INN: International nonproprietary names (INN) for biological and biotechnological substances (a review) (Internet) 2014. Available from: <http://www.who.int/medicines/services/inn/BioRev2014.pdf>) Residues were changed to align with corresponding human germ line sequence, where possible, to enhance humanness and to reduce potential immunogenicity. For humanized VLv2, VLv3, VLv4, and VLv5 variants, mutations were introduced to render the sequences more similar to human germ line gene IGKV1-12*01 (SEQ ID NO:172) For humanized VHv2, VHv3, VHv4, VHv5, VHv6, and VHv7 variants, mutations were introduced to render the sequences more similar to human germ line gene IGHV3-21*01 (SEQ ID NO:162).

[0599] Additional versions of hu5E20-VH and hu5E20-VL were designed to enable assessment of various framework residues for their contributions to antigen binding, thermostability, developability (deamination, oxidation, N-glycosylation, proteolysis and aggregation) and immunogenicity. The positions considered for mutation include those that...

- define the canonical CDR conformations (summarized in Martin),
- are within the Vernier zone (Foote and Winter),
- localize to the VH/VL domain interface (summarized in Léger and Saldanha),
- are susceptible to post-translational modifications, such as glycosylation or pyroglutamination,
- are occupied by residues that are predicted to clash with CDRs, according to the model of 5E20 CDRs grafted onto VH and VL frameworks, or
- are occupied by residues that are rare among sequenced human antibodies, where either the parental mouse 5E20 residue or some other residue is much more prevalent within human antibody repertoire.

[0600] Alignments of the murine 5E20 and various humanized antibodies are shown for the light chain variable regions (Table 15 and Figure 2), and heavy chain variable regions (Table 14 and Figure 1)

[0601] 7 humanized heavy chain variable region variants and 4 humanized light chain variable region variants were constructed containing different permutations of substitutions:

hu5E20VHv1, hu5E20VHv2, hu5E20VHv3, hu5E20VHv4, hu5E20VHv5, hu5E20VHv6, or hu5E20VHv7 (SEQ ID NOS:163-169, respectively); and hu5E20VLv1, hu5E20VLv2, hu5E20VLv3, or hu5E20VLv4 (SEQ ID NOS:173-176, respectively) (Tables 14 and 15). The exemplary humanized Vk and Vh designs, with backmutations and other mutations based on selected human frameworks, are shown in Tables 14 and 15, respectively. The bolded areas in Tables 14 and 15 indicate the CDRs as defined by Kabat/Chothia Composite. A “-” in the columns in Tables 14 and 15 indicates no residue at the indicated position. SEQ ID NOS:163-169, and SEQ ID NOS:173-176 contain backmutations and other mutations as shown in Table 16. The amino acids at positions in hu5E20VHv1, hu5E20VHv2, hu5E20VHv3, hu5E20VHv4, hu5E20VHv5, hu5E20VHv6, and hu5E20VHv7 are listed in Table 17. The amino acids at positions in hu5E20VLv1, hu5E20VLv2, hu5E20VLv3, and hu5E20VLv4 are listed in Table 18.

[0602] The percentage humanness for humanized VH chains hu5E20VHv1, hu5E20VHv2, hu5E20VHv3, hu5E20VHv4, hu5E20VHv5, hu5E20VHv6, and hu5E20VHv7 (SEQ ID NOS:163-169, respectively) with respect to the most similar human germline gene IGHV3-21*01 (SEQ ID NO:162), and for humanized VL chains hu5E20VLv1, hu5E20VLv2, hu5E20VLv3, and hu5E20VLv4 (SEQ ID NOS:173-176, respectively) with respect to the most similar human germline gene IGKV1-12*01 (SEQ ID NO:172) is shown in Table 19.

[0603] Table 14 VH sequences

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VH (SEQ ID NO:4)	AEX29086-VH_huFrwk (SEQ ID NO:161)	IMGT# IGHV3-21*01 (SEQ ID NO:162)	Hu5E20VHv1 (SEQ ID NO:163)	Hu5E20VHv2 (SEQ ID NO:164)	Hu5E20VHv3 (SEQ ID NO:165)	Hu5E20VHv4 (SEQ ID NO:166)	Hu5E20VHv5 (SEQ ID NO:167)	Hu5E20VHv6 (SEQ ID NO:168)	Hu5E20VHv7 (SEQ ID NO:169)
1	1	Fr1	E	E	E	E	E	E	E	E	E	E
2	2	Fr1	V	V	V	V	V	V	V	V	V	V
3	3	Fr1	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
4	4	Fr1	L	L	L	L	L	L	L	L	L	L
5	5	Fr1	V	L	V	L	V	V	V	V	V	V
6	6	Fr1	E	E	E	E	E	E	E	E	E	E
7	7	Fr1	S	S	S	S	S	S	S	S	S	S
8	8	Fr1	G	G	G	G	G	G	G	G	G	G
9	9	Fr1	G	G	G	G	G	G	G	G	G	G
10	10	Fr1	D	G	G	G	G	G	G	G	G	G
11	11	Fr1	L	L	L	L	L	L	L	L	L	L
12	12	Fr1	V	V	V	V	V	V	V	V	V	V
13	13	Fr1	K	Q	K	Q	Q	Q	Q	Q	Q	Q
14	14	Fr1	P	P	P	P	P	P	P	P	P	P
15	15	Fr1	G	G	G	G	G	G	G	G	G	G
16	16	Fr1	G	G	G	G	G	G	G	G	G	G
17	17	Fr1	S	S	S	S	S	S	S	S	S	S
18	18	Fr1	L	L	L	L	L	L	L	L	L	L
19	19	Fr1	K	R	R	R	R	R	R	R	R	R
20	20	Fr1	L	L	L	L	L	L	L	L	L	L
21	21	Fr1	S	S	S	S	S	S	S	S	S	S
22	22	Fr1	C	C	C	C	C	C	C	C	C	C
23	23	Fr1	A	A	A	A	A	A	A	A	A	A
24	24	Fr1	A	A	A	A	A	A	A	A	A	A
25	25	Fr1	P	S	S	S	S	S	S	S	S	S
26	26	CDR-H1	G	G	G	G	G	G	G	G	G	G

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VH (SEQ ID NO:4)	AEX29086-VH_huFwk (SEQ ID NO:161)	IMGT# IGHV3-21*01 (SEQ ID NO:162)	Hu5E20VHv1 (SEQ ID NO:163)	Hu5E20VHv2 (SEQ ID NO:164)	Hu5E20VHv3 (SEQ ID NO:165)	Hu5E20VHv4 (SEQ ID NO:166)	Hu5E20VHv5 (SEQ ID NO:167)	Hu5E20VHv6 (SEQ ID NO:168)	Hu5E20VHv7 (SEQ ID NO:169)
27	27	CDR-H1	F	F	F	F	F	F	F	F	F	F
28	28	CDR-H1	T	T	T	T	T	T	T	T	T	T
29	29	CDR-H1	F	F	F	F	F	F	F	F	F	F
30	30	CDR-H1	S	S	S	S	S	S	S	S	S	S
31	31	CDR-H1	T	S	S	T	T	T	T	T	T	T
32	32	CDR-H1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
33	33	CDR-H1	G	A	S	G	G	G	G	G	G	G
34	34	CDR-H1	M	M	M	M	M	M	M	M	M	M
35	35	CDR-H1	S	S	N	S	S	S	S	S	S	S
35A		CDR-H1	-	-	-	-	-	-	-	-	-	-
35B		CDR-H1	-	-	-	-	-	-	-	-	-	-
36	36	Fr2	W	W	W	W	W	W	W	W	W	W
37	37	Fr2	V	V	V	V	V	V	V	V	V	V
38	38	Fr2	R	R	R	R	R	R	R	R	R	R
39	39	Fr2	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
40	40	Fr2	T	A	A	A	A	A	A	A	T	T
41	41	Fr2	P	P	P	P	P	P	P	P	P	P
42	42	Fr2	D	G	G	G	G	G	D	D	G	D
43	43	Fr2	K	K	K	K	K	K	K	K	K	K
44	44	Fr2	R	G	G	G	G	R	R	R	R	R
45	45	Fr2	L	L	L	L	L	L	L	L	L	L
46	46	Fr2	E	E	E	E	E	E	E	E	E	E
47	47	Fr2	W	W	W	W	W	W	W	W	W	W
48	48	Fr2	V	V	V	V	V	V	V	V	V	V
49	49	Fr2	A	S	S	A	A	A	A	A	A	A
50	50	CDR-H2	I	A	S	I	I	I	I	I	I	I
51	51	CDR-H2	I	I	I	I	I	I	I	I	I	I
52	52	CDR-H2	S	S	S	S	S	S	S	S	S	S
52A	53	CDR-H2	S	G	S	S	S	S	S	S	S	S

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VH (SEQ ID NO:4)	AEX29086-VH_huFwk (SEQ ID NO:161)	IMGT# IGHV3-21*01 (SEQ ID NO:162)	Hu5E20VHv1 (SEQ ID NO:163)	Hu5E20VHv2 (SEQ ID NO:164)	Hu5E20VHv3 (SEQ ID NO:165)	Hu5E20VHv4 (SEQ ID NO:166)	Hu5E20VHv5 (SEQ ID NO:167)	Hu5E20VHv6 (SEQ ID NO:168)	Hu5E20VHv7 (SEQ ID NO:169)
52B		CDR-H2	-	-	-	-	-	-	-	-	-	-
52C		CDR-H2	-	-	-	-	-	-	-	-	-	-
53	54	CDR-H2	G	S	S	G	G	G	G	G	G	G
54	55	CDR-H2	G	G	S	G	G	G	G	G	G	G
55	56	CDR-H2	S	G	S	S	S	S	S	S	S	S
56	57	CDR-H2	Y	S	Y	Y	Y	Y	Y	Y	Y	Y
57	58	CDR-H2	T	T	I	T	T	T	T	T	T	T
58	59	CDR-H2	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
59	60	CDR-H2	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
60	61	CDR-H2	S	A	A	S	S	S	S	S	S	S
61	62	CDR-H2	D	D	D	D	D	D	D	D	D	D
62	63	CDR-H2	T	S	S	T	T	T	T	T	T	T
63	64	CDR-H2	V	V	V	V	V	V	V	V	V	V
64	65	CDR-H2	K	R	K	K	K	K	K	K	K	K
65	66	CDR-H2	G	G	G	G	G	G	G	G	G	G
66	67	Fr3	R	R	R	R	R	R	R	R	R	R
67	68	Fr3	F	F	F	F	F	F	F	F	F	F
68	69	Fr3	T	T	T	T	T	T	T	T	T	T
69	70	Fr3	I	I	I	I	I	I	I	I	I	I
70	71	Fr3	S	S	S	S	S	S	S	S	S	S
71	72	Fr3	R	R	R	R	R	R	R	R	R	R
72	73	Fr3	D	D	D	D	D	D	D	D	D	D
73	74	Fr3	N	N	N	N	N	N	N	N	N	N
74	75	Fr3	A	S	A	S	S	S	S	S	S	S
75	76	Fr3	K	K	K	K	K	K	K	K	K	K
76	77	Fr3	N	N	N	N	N	N	N	N	N	N
77	78	Fr3	T	T	S	T	S	S	S	S	S	S
78	79	Fr3	L	L	L	L	L	L	L	L	L	L
79	80	Fr3	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VH (SEQ ID NO:4)	AEX29086-VH_huFwk (SEQ ID NO:161)	IMGT# IGHV3-21*01 (SEQ ID NO:162)	Hu5E20VHv1 (SEQ ID NO:163)	Hu5E20VHv2 (SEQ ID NO:164)	Hu5E20VHv3 (SEQ ID NO:165)	Hu5E20VHv4 (SEQ ID NO:166)	Hu5E20VHv5 (SEQ ID NO:167)	Hu5E20VHv6 (SEQ ID NO:168)	Hu5E20VHv7 (SEQ ID NO:169)
80	81	Fr3	L	L	L	L	L	L	L	L	L	L
81	82	Fr3	Q	Q	Q	Q	Q	Q	Q	Q	Q	Q
82	83	Fr3	M	M	M	M	M	M	M	M	M	M
82A	84	Fr3	S	N	N	N	N	N	N	N	N	N
82B	85	Fr3	S	S	S	S	S	S	S	S	S	S
82C	86	Fr3	L	L	L	L	L	L	L	L	L	L
83	87	Fr3	K	R	R	R	R	R	R	K	R	R
84	88	Fr3	S	A	A	A	A	A	A	A	A	A
85	89	Fr3	E	E	E	E	E	E	E	E	E	E
86	90	Fr3	D	D	D	D	D	D	D	D	D	D
87	91	Fr3	T	T	T	T	T	T	T	T	T	T
88	92	Fr3	A	A	A	A	A	A	A	A	A	A
89	93	Fr3	M	V	V	V	V	V	V	V	V	V
90	94	Fr3	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
91	95	Fr3	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
92	96	Fr3	C	C	C	C	C	C	C	C	C	C
93	97	Fr3	S	A	A	S	S	S	S	S	S	S
94	98	Fr3	R	K	R	R	R	R	R	R	R	R
95	99	CDR-H3	S	S	D	S	S	S	S	S	S	S
96		CDR-H3	S	G	A	S	S	S	S	S	S	S
97		CDR-H3	S	T	F	S	S	S	S	S	S	S
98		CDR-H3	H	P	-	H	H	H	H	H	H	H
99		CDR-H3	W	W	-	W	W	W	W	W	W	W
100		CDR-H3	Y	Y	-	Y	Y	Y	Y	Y	Y	Y
100A		CDR-H3	F	F	-	F	F	F	F	F	F	F
100B		CDR-H3	-	-	-	-	-	-	-	-	-	-
100C		CDR-H3	-	-	-	-	-	-	-	-	-	-
100D		CDR-H3	-	-	-	-	-	-	-	-	-	-
100E		CDR-H3	-	-	-	-	-	-	-	-	-	-

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VH (SEQ ID NO:4)	AEX29086-VH_huFwk (SEQ ID NO:161)	IMGT# IGHV3-21*01 (SEQ ID NO:162)	Hu5E20VHv1 (SEQ ID NO:163)	Hu5E20VHv2 (SEQ ID NO:164)	Hu5E20VHv3 (SEQ ID NO:165)	Hu5E20VHv4 (SEQ ID NO:166)	Hu5E20VHv5 (SEQ ID NO:167)	Hu5E20VHv6 (SEQ ID NO:168)	Hu5E20VHv7 (SEQ ID NO:169)
100F		CDR-H3	-	-	-	-	-	-	-	-	-	-
100G		CDR-H3	-	-	-	-	-	-	-	-	-	-
100H		CDR-H3	-	-	-	-	-	-	-	-	-	-
100I		CDR-H3	-	-	-	-	-	-	-	-	-	-
100J		CDR-H3	-	-	-	-	-	-	-	-	-	-
100K		CDR-H3	-	-	-	-	-	-	-	-	-	-
101	100	CDR-H3	D	D	D	D	D	D	D	D	D	D
102	101	CDR-H3	V	Y	V	V	V	V	V	V	V	V
103	102	Fr4	W	W	W	W	W	W	W	W	W	W
104	103	Fr4	G	G	G	G	G	G	G	G	G	G
105	104	Fr4	T	Q	Q	Q	Q	Q	Q	Q	Q	Q
106	105	Fr4	G	G	G	G	G	G	G	G	G	G
107	106	Fr4	T	T	T	T	T	T	T	T	T	T
108	107	Fr4	T	L	M	L	L	L	L	L	L	L
109	108	Fr4	V	V	V	V	V	V	V	V	V	V
110	109	Fr4	T	T	T	T	T	T	T	T	T	T
111	110	Fr4	V	V	V	V	V	V	V	V	V	V
112	111	Fr4	S	S	S	S	S	S	S	S	S	S
113	112	Fr4	S	S	S	S	S	S	S	S	S	S

[0604] Table 15 5E20 VL sequences

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VL (SEQ ID NO:10)	BAH04687-VL_huFwk (SEQ ID NO:171)	IGKV1-12*01 (SEQ ID NO:172)	Hu5E20VLv1 (SEQ ID NO:173)	Hu5E20VLv2 (SEQ ID NO:174)	Hu5E20VLv3 (SEQ ID NO:175)	Hu5E20VLv4 (SEQ ID NO:176)
1	1	Fr1	D	D	D	D	D	D	D
2	2	Fr1	I	I	I	I	I	I	I
3	3	Fr1	L	Q	Q	Q	Q	Q	Q
4	4	Fr1	M	M	M	M	M	M	M
5	5	Fr1	T	T	T	T	T	T	T
6	6	Fr1	Q	Q	Q	Q	Q	Q	Q
7	7	Fr1	S	S	S	S	S	S	S
8	8	Fr1	P	P	P	P	P	P	P
9	9	Fr1	S	S	S	S	S	S	S
10	10	Fr1	S	S	S	S	S	S	S
11	11	Fr1	M	L	V	L	V	V	V
12	12	Fr1	S	S	S	S	S	S	S
13	13	Fr1	V	A	A	A	A	A	A
14	14	Fr1	S	S	S	S	S	S	S
15	15	Fr1	L	V	V	V	V	V	V
16	16	Fr1	G	G	G	G	G	G	G
17	17	Fr1	D	D	D	D	D	D	D
18	18	Fr1	T	R	R	R	R	R	R
19	19	Fr1	V	V	V	V	V	V	V
20	20	Fr1	S	T	T	T	T	T	T
21	21	Fr1	I	I	I	I	I	I	I
22	22	Fr1	T	T	T	T	T	T	T
23	23	Fr1	C	C	C	C	C	C	C
24	24	CDR-L1	H	R	R	H	H	H	H
25	25	CDR-L1	A	A	A	A	A	A	A
26	26	CDR-L1	S	S	S	S	S	S	S

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VL (SEQ ID NO:10)	BAH04687-VL_huFrwk (SEQ ID NO:171)	IGKV1-12*01 (SEQ ID NO:172)	Hu5E20VLv1 (SEQ ID NO:173)	Hu5E20VLv2 (SEQ ID NO:174)	Hu5E20VLv3 (SEQ ID NO:175)	Hu5E20VLv4 (SEQ ID NO:176)
27	27	CDR-L1	Q	Q	Q	Q	Q	Q	Q
27A	28	CDR-L1	-	-	-	-	-	-	-
27B	29	CDR-L1	-	-	-	-	-	-	-
27C	30	CDR-L1	-	-	-	-	-	-	-
27D	31	CDR-L1	-	-	-	-	-	-	-
27E	32	CDR-L1	-	-	-	-	-	-	-
27F		CDR-L1	-	-	-	-	-	-	-
28	33	CDR-L1	G	S	G	G	G	G	G
29	34	CDR-L1	I	I	I	I	I	I	I
30	35	CDR-L1	S	S	S	S	S	S	S
31	36	CDR-L1	S	S	S	S	S	S	S
32	37	CDR-L1	N	Y	W	N	N	N	N
33	38	CDR-L1	I	L	L	I	I	I	I
34	39	CDR-L1	G	N	A	G	G	G	G
35	40	Fr2	W	W	W	W	W	W	W
36	41	Fr2	L	Y	Y	L	L	L	L
37	42	Fr2	Q	Q	Q	Q	Q	Q	Q
38	43	Fr2	Q	Q	Q	Q	Q	Q	Q
39	44	Fr2	K	K	K	K	K	K	K
40	45	Fr2	P	P	P	P	P	P	P
41	46	Fr2	G	G	G	G	G	G	G
42	47	Fr2	K	K	K	K	K	K	K
43	48	Fr2	S	A	A	A	A	A	A
44	49	Fr2	F	P	P	F	F	F	F
45	50	Fr2	K	K	K	K	K	K	K
46	51	Fr2	G	L	L	G	G	G	G
47	52	Fr2	L	L	L	L	L	L	L
48	53	Fr2	I	I	I	I	I	I	I

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VL (SEQ ID NO:10)	BAH04687-VL_huFrwk (SEQ ID NO:171)	IGKV1-12*01 (SEQ ID NO:172)	Hu5E20VLv1 (SEQ ID NO:173)	Hu5E20VLv2 (SEQ ID NO:174)	Hu5E20VLv3 (SEQ ID NO:175)	Hu5E20VLv4 (SEQ ID NO:176)
49	54	Fr2	Y	Y	Y	Y	Y	Y	Y
50	55	CDR-L2	H	A	A	H	H	H	H
51	56	CDR-L2	G	A	A	G	G	G	G
52	57	CDR-L2	T	S	S	T	T	T	T
53	58	CDR-L2	N	S	S	N	N	N	N
54	59	CDR-L2	L	L	L	L	L	L	L
55	60	CDR-L2	K	Q	Q	K	K	K	K
56	61	CDR-L2	D	S	S	D	D	D	D
57	62	Fr3	G	G	G	G	G	G	G
58	63	Fr3	V	V	V	V	V	V	V
59	64	Fr3	P	P	P	P	P	P	P
60	65	Fr3	S	S	S	S	S	S	S
61	66	Fr3	R	R	R	R	R	R	R
62	67	Fr3	F	F	F	F	F	F	F
63	68	Fr3	S	S	S	S	S	S	S
64	69	Fr3	G	G	G	G	G	G	G
65	70	Fr3	S	S	S	S	S	S	S
66	71	Fr3	G	G	G	G	G	G	G
67	72	Fr3	S	S	S	S	S	S	S
68	73	Fr3	G	G	G	G	G	G	G
69	74	Fr3	A	T	T	A	A	A	A
70	75	Fr3	D	D	D	D	D	D	D
71	76	Fr3	F	F	F	F	F	F	F
72	77	Fr3	S	T	T	T	T	T	T
73	78	Fr3	L	L	L	L	L	L	L
74	79	Fr3	T	T	T	T	T	T	T
75	80	Fr3	I	I	I	I	I	I	I
76	81	Fr3	S	S	S	S	S	S	S

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VL (SEQ ID NO:10)	BAH04687-VL_huFrwk (SEQ ID NO:171)	IGKV1-12*01 (SEQ ID NO:172)	Hu5E20VLv1 (SEQ ID NO:173)	Hu5E20VLv2 (SEQ ID NO:174)	Hu5E20VLv3 (SEQ ID NO:175)	Hu5E20VLv4 (SEQ ID NO:176)
77	82	Fr3	S	S	S	S	S	S	S
78	83	Fr3	L	L	L	L	L	L	L
79	84	Fr3	E	Q	Q	Q	Q	Q	Q
80	85	Fr3	S	P	P	P	P	P	P
81	86	Fr3	E	E	E	E	E	E	E
82	87	Fr3	D	D	D	D	D	D	D
83	88	Fr3	F	F	F	F	F	F	F
84	89	Fr3	A	A	A	A	A	A	A
85	90	Fr3	D	T	T	T	T	T	D
86	91	Fr3	Y	Y	Y	Y	Y	Y	Y
87	92	Fr3	F	Y	Y	F	F	F	F
88	93	Fr3	C	C	C	C	C	C	C
89	94	CDR-L3	V	Q	Q	V	V	V	V
90	95	CDR-L3	Q	Q	Q	Q	Q	Q	Q
91	96	CDR-L3	Y	S	A	Y	Y	Y	Y
92	97	CDR-L3	A	Y	N	A	A	A	A
93	98	CDR-L3	Q	S	S	Q	Q	Q	Q
94	99	CDR-L3	F	T	F	F	F	F	F
95	100	CDR-L3	P	P	P	P	P	P	P
95A		CDR-L3	-	-	-	-	-	-	-
95B		CDR-L3	-	-	-	-	-	-	-
95C		CDR-L3	-	-	-	-	-	-	-
95D		CDR-L3	-	-	-	-	-	-	-
95E		CDR-L3	-	-	-	-	-	-	-
95F		CDR-L3	-	-	-	-	-	-	-
96	101	CDR-L3	Y	L	Y	Y	Y	Y	Y
97	102	CDR-L3	T	T	T	T	T	T	T
98	103	Fr4	F	F	F	F	F	F	F

Kabat residue #	Linear residue #	FR or CDR	Mouse 5E20 VL (SEQ ID NO:10)	BAH04687-VL_huFrwk (SEQ ID NO:171)	IGKV1-12*01 (SEQ ID NO:172)	Hu5E20VLv1 (SEQ ID NO:173)	Hu5E20VLv2 (SEQ ID NO:174)	Hu5E20VLv3 (SEQ ID NO:175)	Hu5E20VLv4 (SEQ ID NO:176)
99	104	Fr4	G	G	G	G	G	G	G
100	105	Fr4	G	G	Q	G	G	Q	Q
101	106	Fr4	G	G	G	G	G	G	G
102	107	Fr4	T	T	T	T	T	T	T
103	108	Fr4	K	K	K	K	K	K	K
104	109	Fr4	L	V	L	V	V	V	V
105	110	Fr4	E	E	E	E	E	E	E
106	111	Fr4	K	I	K	I	I	K	K
106A		Fr4	-	-	-	-	-	-	-
107	112	Fr4	R	R	K	R	R	R	R

[0605] Table 16: V_H, V_L Backmutations and Other Mutations for Humanized 5E20		
V_H or V_L Variant	V_H or V_L Exon Acceptor Sequence	Changes from Acceptor Framework (or CDR) Residues (based on Kabat/Chothia Composite CDRs)
Hu5E20VHv1 (SEQ ID NO:163)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H49, H93, H94
Hu5E20VHv2 (SEQ ID NO:164)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H5, H49, H77, H93, H94
Hu5E20VHv3 (SEQ ID NO:165)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H5, H44, H49, H77, H93, H94
Hu5E20VHv4 (SEQ ID NO:166)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H5, H42, H44, H49, H77, H93, H94
Hu5E20VHv5 (SEQ ID NO:167)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H5, H42, H44, H49, H77, H83, H93, H94
Hu5E20VHv6 (SEQ ID NO:168)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H5, H40, H44, H49, H77, H93, H94
Hu5E20VHv7 (SEQ ID NO:169)	GenBank Acc. # AEX29086-VH_huFrwk (SEQ ID NO:161) IMGT# IGHV3-21*01 (SEQ ID NO:162)	H5, H40, H42, H44, H49, H77, H93, H94
Hu5E20VLv1 (SEQ ID NO:173)	GenBank Acc. # BAH04687-VL_huFrwk (SEQ ID NO:171) IMGT# IGKV1-12*01 (SEQ ID NO:172)	L36, L44, L46, L69, L87
Hu5E20VLv2 (SEQ ID NO:174)	GenBank Acc. # BAH04687-VL_huFrwk (SEQ ID NO:171) IMGT# IGKV1-12*01 (SEQ ID NO:172)	L11, L36, L44, L46, L69, L87, L100
Hu5E20VLv3 (SEQ ID NO:175)	GenBank Acc. # BAH04687-VL_huFrwk (SEQ ID NO:171) IMGT# IGKV1-12*01 (SEQ ID NO:172)	L11, L36, L44, L46, L69, L87, L100, L106
Hu5E20VLv4 (SEQ ID NO:176)	GenBank Acc. # BAH04687-VL_huFrwk (SEQ ID NO:171) IMGT# IGKV1-12*01 (SEQ ID NO:172)	L11, L36, L44, L46, L69, L85, L87, L100, L106

[0606] Table 17: Kabat Numbering of Framework (or CDR) Residues (based on Kabat/Chothia Composite CDRs) for Backmutations and Other Mutations in Heavy Chains of Humanized 5E20 Antibodies

Residue	AEX29086-VH_huFrwk (SEQ ID NO:161)	IMGT# IGHV3-21*01 (SEQ ID NO:162)	Mouse 5E20 VH (SEQ ID NO:4)	Hu5E20VHv1 (SEQ ID NO:163)	Hu5E20VHv2 (SEQ ID NO:164)	Hu5E20VHv3 (SEQ ID NO:165)	Hu5E20VHv4 (SEQ ID NO:166)	Hu5E20VHv5 (SEQ ID NO:167)	Hu5E20VHv6 (SEQ ID NO:168)	Hu5E20VHv7 (SEQ ID NO:169)
H5	L	V	V	L	V	V	V	V	V	V
H40	A	A	T	A	A	A	A	A	T	T
H42	G	G	D	G	G	G	D	D	G	D
H44	G	G	R	G	G	R	R	R	R	R
H49	S	S	A	A	A	A	A	A	A	A
H77	T	S	T	T	S	S	S	S	S	S
H83	R	R	K	R	R	R	R	K	R	R
H93	A	A	S	S	S	S	S	S	S	S
H94	K	R	R	R	R	R	R	R	R	R

[0607] Table 18: Kabat Numbering of Framework Residues (based on Kabat/Chothia Composite CDRs) for Backmutations and Other Mutations in Light Chains of Humanized 5E20 Antibodies

Residue	BAH04687-VL_huFrwk (SEQ ID NO:171)	IGKV1-12*01 (SEQ ID NO:172)	Mouse 5E20 VL (SEQ ID NO:10)	Hu5E20VLv1 (SEQ ID NO:173)	Hu5E20VLv2 (SEQ ID NO:174)	Hu5E20VLv3 (SEQ ID NO:175)	Hu5E20VLv4 (SEQ ID NO:176)
L11	L	V	M	L	V	V	V
L36	Y	Y	L	L	L	L	L
L44	P	P	F	F	F	F	F
L46	L	L	G	G	G	G	G
L69	T	T	A	A	A	A	A
L85	T	T	D	T	T	T	D
L87	Y	Y	F	F	F	F	F
L100	G	Q	G	G	G	Q	Q
L106	I	K	K	I	I	K	K

[0608] Table 19
Percentage Humanness of Heavy and Light Chains of Humanized 5E20 Antibodies

V_H or V_L Variant	% Humanness
Hu5E20VHv1 (SEO ID NO:163)	87.8%
Hu5E20VHv2 (SEO ID NO:164)	89.8%
Hu5E20VHv3 (SEO ID NO:165)	86.7%
Hu5E20VHv4 (SEO ID NO:166)	85.7%
Hu5E20VHv5 (SEO ID NO:167)	84.7%
Hu5E20VHv6 (SEO ID NO:168)	85.7%
Hu5E20VHv7 (SEO ID NO:169)	84.7%
Hu5E20VLv1 (SEO ID NO:173)	78.9%
Hu5E20VLv2 (SEO ID NO:174)	80.0%
Hu5E20VLv3 (SEO ID NO:175)	80.0%
Hu5E20VLv4 (SEO ID NO:176)	78.9%

[0609] Positions at which canonical, vernier, or interface residues differ between mouse and human acceptor sequences are candidates for substitution. Examples of canonical/CDR interacting residues include Kabat residue H94 in Table 14. Examples of vernier residues include Kabat residues H49, H93, and H94 in Table 14 and L36, L46, and L69 in Table 15. Examples of interface/packing (VH+VL) residues include Kabat residues L36, L44, L46, and L87 in Table 15.

[0610] The rationales for selection of the positions indicated in Table 14 in the heavy chain variable region as candidates for substitution are as follows.

[0611] heavy chain variable regions

[0612] *hu5E20-VH_v1*

- consists of the CDR-H1, H2, and H3 loops of 5E20-VH grafted onto the framework of AEX29086, and reverts all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or localize to the VH/VL domain interface

[0613] *Hu5E20-VH_v2 through Hu5E20-VH_v7*

- revert all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or localize to the VH/VL domain interface or contribute to structural stability, also incorporate backmutations or substitution with most frequent residue at a given position.

[0614] L5V: is a frequency-based and germline-aligning mutation. Leu is rare at this position whereas, Val is most frequent. Germline gene IGHV3-21*01 (SEQ ID NO:162) has Val at this position.

[0615] A40T: is a backmutation. Thr at this position makes a bond with K43 heavy chain to stabilize the loop which in turn maintains the conformation of CDR-H2.

[0616] G42D: Asp stabilizes the loop by bonding with K43 and T40 both in the heavy chain. This tetraloop bridging maintains conformation of the loop. Gly at this position distorts the conformation which does not allow bonding between R44 heavy chain and F98 light chain. This back mutation is made to conserve conformation.

[0617] G44R: Arg 44 makes an interchain bond with light chain Phe 98. Gly at this position would not make an interchain bond with light chain Phe98, and may make antibody unstable. Stabilization with Arg at position 44 may preserve CDR conformation. Back-mutation is made to preserve conformation and stability.

[0618] S49A: backmutation of a vernier zone residue.

[0619] T77S: is a germline-aligning mutation. Germline gene IGHV3-21*01 (SEQ ID NO:162) has Ser at this position.

[0620] R83K: As Arg and Lys are very similar residues, mutation to Lys is made to enhance stability.

[0621] A93S: backmutation of a vernier zone and VH/VL domain interface residue

[0622] K94R: backmutation of a canonical and vernier zone residue

[0623] The rationales for selection of the positions indicated in Table 15 in the light chain variable region as candidates for substitution are as follows.

[0624] *kappa light chain variable regions*

hu5E20-VL_v1

- consists of the CDR-L1, L2, and L3 loops of 5E20-VL grafted onto the framework of BAH04687 VL along with reverting all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or locate to the VH/VL domain interface.

Hu5E20-VL_v2, hu5E20-VL_v3 and hu5E20-VL_v4

- consists of the CDR-L1, L2, and L3 loops of 5E20-VL grafted onto the framework of BAH04687 VL along with reverting all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or locate to the VH/VL domain interface.
- also include substitutions that contribute to structural stability or increases the humanness of the antibody.

[0625] L11V: is a germline-aligning mutation. Germline gene sequence IGKV1-12*01 (SEQ ID NO:172) has Val at this position.

[0626] Y36L: backmutation of a vernier zone and VH/VL domain interface residue

[0627] P44F: backmutation of a VH/VL domain interface residue

[0628] L46G: backmutation of a vernier zone and VH/VL domain interface residue

[0629] T69A: backmutation of a vernier zone residue

[0630] T85D: is a mutation to enhance interchain bonding,

[0631] Y87F: backmutation of a VH/VL domain interface residue

[0632] G100Q: is a germline-aligning and frequency-based mutation. Germline gene sequence IGKV1-12*01 (SEQ ID NO:172) has Gln at this position. Gln is most frequent at this position.

[0633] I106K: is a germline-aligning and frequency-based mutation. Germline gene sequence IGKV1-12*01 (SEQ ID NO:172) has Lys at this position. Lys at this position is more frequent.

[0634] Sequences of heavy chain variable regions

[0635] SEQ ID NO:163 >h5E20VH version1 (87.8% human)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQAPGKGLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNTLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0636] SEQ ID NO:164 >h5E20VH version2 (89.8% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQAPGKGLEWVAIISSGGSYTYSDTVKGRFTISR
DNSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0637] SEQ ID NO:165 >h5E20VH version3 (86.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQAPGKRLEWVAIISSGGSYTYSDTVKGRFTISR
DNSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0638] SEQ ID NO:166 >h5E20VH version4 (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQAPDKRLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0639] SEQ ID NO:167 >h5E20VH version5 (84.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQAPDKRLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNSLYLQMNSLKAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0640] SEQ ID NO:168 >h5E20VH version6 (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQTPGKRLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0641] SEQ ID NO:169 >h5E20VH version7 (84.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMWVRQTPDKRLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLVTVSS

[0642] Sequences of kappa light chain variable regions

[0643] SEQ ID NO:173 >h5E20VL version1 (78.9% human)
DIQMTQSPSSLSASVGDRVTITCHASQGISSNIGWLQKKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFTL
TISSLQPEDFATYFCVQYAQFPYTFGGGTKVEIR

[0644] SEQ ID NO:174 >h5E20VL version2 (80.0% human)
DIQMTQSPSSVSASVGDRVTITCHASQGISSNIGWLQKKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFT
LTISLQPEDFATYFCVQYAQFPYTFGQGTKVEIR

[0645] SEQ ID NO:175 >h5E20VL version3 (80.0% human)
 DIQMTQSPSSVSASVGDRTITCHASQGISSNIGWLQKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFT
 LTISSLQPEDFATYFCVQYAQFPYTFGQGGTKVEKR

[0646] SEQ ID NO:176 >h5E20VL version4 (78.9% human)
 DIQMTQSPSSVSASVGDRTITCHASQGISSNIGWLQKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFT
 LTISSLQPEDFADYFCVQYAQFPYTFGQGGTKVEKR

[0647] Example 15. Design of Humanized 8H24 Antibodies

[0648] The starting point or donor antibody for humanization was the mouse antibody 8H24. The heavy chain variable amino acid sequence of mature m8H24 is provided as SEQ ID NO:28. The light chain variable amino acid sequence of mature m8H24 is provided as SEQ ID NO:34. The heavy chain Kabat/Chothia Composite CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:29-31, respectively. The light chain Kabat CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:35-37, respectively. Kabat numbering is used throughout.

[0649] The variable kappa (Vk) of 8H24 belongs to mouse Vk subgroup 1b which corresponds to human Vk subgroup 1 and the variable heavy (Vh) to mouse Vh subgroup 3d which corresponds to human Vh subgroup 1 [Kabat E.A., et al., (1991), Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242]. 16 residue Chothia CDR-L1 is similar to Chothia canonical class 4, 7 residue Chothia CDR-L2 is of Chothia canonical class 1, 9 residue Chothia CDR-L3 is similar to Chothia canonical class 1 [Martin ACR. (2010) Protein sequence and structure analysis of antibody variable domains. In: Kontermann R and Dübel S (eds). *Antibody Engineering*. Heidelberg, Germany: Springer International Publishing AG.. [Martin, 2010]. 10 residue Chothia CDR-H1 is similar to Chothia canonical class 1, 17 residue Chothia CDR-H2 is similar to Chothia canonical class 2 [Martin, 2010]. 3 residue CDR-H3 has no canonical classes. A search was made over the protein sequences in the PDB database [Deshpande N, et al., (2005) Nucleic Acids Res. 33: D233-7] to find structures which would provide a rough structural model of 8H24. The crystal structure of an antibody fab PDB code 1MRC [Pokkuluri, P.R., et al. (1994) J Mol Biol 243: 283-297] for both Vh and Vk structure since it had good resolution (2.4 Å) and overall sequence similarity to 8H24 Vh and Vk, retaining the same canonical structures for the loops.

[0650] The frameworks of 8H24 VH share a high degree of sequence similarity with the corresponding regions of human antibody AAC51714 VH, cloned by Johnson, T.A., et al. (J. Immunol. 158 (1), 235-246, 1997). The variable heavy domains of 8H24 and AAC51714 also share identical lengths for the CDR-H1, H2 loops. Similarly, the frameworks of 8H24 VL share a high degree of sequence similarity with the corresponding regions of human antibody ABC66914 VL cloned by Shriner A.K., et al (Vaccine 24 (49-50), 7159-7166 (2006)). The variable light domain of 8H24 and ABC66914 antibody also share identical lengths for the CDR-L1, L2 and L3 loops. Accordingly, the framework regions of AAC51714 VH and ABC66914 VL were chosen as the acceptor sequences for the CDRs of 8H24. A model of the 8H24 CDRs grafted onto the respective human frameworks for VH and VL was built and used as a guidance for further backmutations.

[0651] Heavy and light chain variant sequences resulting from antibody humanization process were further aligned to human germ line sequences using IMGT Domain GapAlign tool to assess the humanness of the heavy and light chain as outlined by WHO INN committee guidelines. (WHO-INN: International nonproprietary names (INN) for biological and biotechnological substances (a review) (Internet) 2014. Available from: <http://www.who.int/medicines/services/inn/BioRev2014.pdf>) Residues were changed to align with corresponding human germ line sequence, where possible, to enhance humanness and to reduce potential immunogenicity.

[0652] The amino acid sequences consisting of AAC51714VH human frameworks and 8H24 CDRs are designated hu8H24VHv1 and the amino acid sequences consisting of ABC66914 VL human framework and 8H24 CDRs are designated hu8H24VLv1. Additional versions of hu8H24-VH and hu8H24-VL were designed to enable assessment of various framework residues for their contributions to antigen binding, thermostability, developability (deamination, oxidation, N-glycosylation, proteolysis and aggregation) and immunogenicity. The positions considered for mutation include those that:

- define the canonical CDR conformations (summarized in Martin),
- are within the Vernier zone (Foote and Winter),
- localize to the VH/VL domain interface (summarized in Léger and Saldanha),
- are susceptible to post-translational modifications, such as glycosylation or pyroglutamination,

- are occupied by residues that are predicted to clash with CDRs, according to the model of 8H24 CDRs grafted onto VH and VL frameworks, or
- are occupied by residues that are rare among sequenced human antibodies, where either the parental mouse 8H24 residue or some other residue is much more prevalent within human antibody repertoire.

[0653] Alignments of the murine 8H24 and various humanized antibodies are shown for the light chain variable regions (Table 21 and Figure 4), and heavy chain variable regions (Table 20 and Figure 3).

[0654] 2 humanized heavy chain variable region variants and 2 humanized light chain variable region variants were constructed containing different permutations of substitutions:

hu8H24VHv1 or hu8H24VHv2, (SEQ ID NOS:180-181, respectively); and hu8H24VLv1 or hu8H24VLv2 (SEQ ID NOS:185-186, respectively) (Tables 20 and 21). The exemplary humanized Vk and Vh designs, with backmutations and other mutations based on selected human frameworks, are shown in Tables 21 and 20, respectively. The bolded areas in Tables 20 and 21 indicate the CDRs as defined by Kabat/Chothia Composite. A “-” in the columns in Tables 20 and 21 indicates no residue at the indicated position. SEQ ID NOS:180-181, and SEQ ID NOS:185-186 contain backmutations and other mutations as shown in Table 22. The amino acids at positions in hu8H24VHv1 and hu8H24VHv2 are listed in Table 23. The amino acids at positions in hu8H24VLv1 and hu8H24VLv2 are listed in Table 24.

[0655] The percentage humanness for humanized VH chains hu8H24VHv1 and hu8H24VHv2 (SEQ ID NOS:180-181, respectively) with respect to the most similar human germline gene IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179), and for humanized VL chains hu8H24VLv1 and hu8H24VLv2 (SEQ ID NOS:185-186 respectively) with respect to the most similar human germline gene IMGT# IGKV2-40*01 (SEQ ID NO:184) is shown in Table 25.

[0656] Table 20: 8H24 VH sequences

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VH (SEQ ID NO:28)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178)	IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	Hu8H24VHv1 (SEQ ID NO:180)	Hu8H24VHv2 (SEQ ID NO:181)
1	1	Fr1	Q	Q	Q	Q	Q
2	2	Fr1	A	V	V	A	A
3	3	Fr1	Y	Q	Q	Q	Q
4	4	Fr1	L	L	L	L	L
5	5	Fr1	Q	V	V	V	V
6	6	Fr1	Q	Q	Q	Q	Q
7	7	Fr1	S	S	S	S	S
8	8	Fr1	G	G	G	G	G
9	9	Fr1	T	A	A	A	A
10	10	Fr1	E	E	E	E	E
11	11	Fr1	L	V	V	V	V
12	12	Fr1	V	K	K	K	V
13	13	Fr1	R	K	K	K	K
14	14	Fr1	P	P	P	P	P
15	15	Fr1	G	G	G	G	G
16	16	Fr1	A	S	S	S	S
17	17	Fr1	S	S	S	S	S
18	18	Fr1	V	V	V	V	V
19	19	Fr1	K	K	K	K	K
20	20	Fr1	M	V	V	V	V
21	21	Fr1	S	S	S	S	S
22	22	Fr1	C	C	C	C	C
23	23	Fr1	K	K	K	K	K
24	24	Fr1	A	A	A	A	A
25	25	Fr1	S	S	S	S	S
26	26	CDR-H1	G	G	G	G	G

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VH (SEQ ID NO:28)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178)	IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	Hu8H24VHv1 (SEQ ID NO:180)	Hu8H24VHv2 (SEQ ID NO:181)
27	27	CDR-H1	Y	G	G	Y	Y
28	28	CDR-H1	T	T	T	T	T
29	29	CDR-H1	F	F	F	F	F
30	30	CDR-H1	T	S	S	T	T
31	31	CDR-H1	S	S	S	S	S
32	32	CDR-H1	Y	Y	Y	Y	Y
33	33	CDR-H1	S	A	T	S	S
34	34	CDR-H1	M	I	I	M	M
35	35	CDR-H1	H	S	S	H	H
35A		CDR-H1	-	-	-	-	-
35B		CDR-H1	-	-	-	-	-
36	36	Fr2	W	W	W	W	W
37	37	Fr2	V	V	V	V	V
38	38	Fr2	K	R	R	R	R
39	39	Fr2	Q	Q	Q	Q	Q
40	40	Fr2	T	A	A	A	A
41	41	Fr2	P	P	P	P	P
42	42	Fr2	R	G	G	G	G
43	43	Fr2	Q	Q	Q	Q	Q
44	44	Fr2	G	G	G	G	G
45	45	Fr2	L	L	L	L	L
46	46	Fr2	E	E	E	E	E
47	47	Fr2	W	W	W	W	W
48	48	Fr2	I	M	M	I	I
49	49	Fr2	G	G	G	G	G
50	50	CDR-H2	A	G	R	A	A
51	51	CDR-H2	I	I	I	I	I
52	52	CDR-H2	Y	I	I	Y	Y

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VH (SEQ ID NO:28)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178)	IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	Hu8H24VHv1 (SEQ ID NO:180)	Hu8H24VHv2 (SEQ ID NO:181)
52A	53	CDR-H2	P	P	P	P	P
52B		CDR-H2	-	-	-	-	-
52C		CDR-H2	-	-	-	-	-
53	54	CDR-H2	G	I	I	G	G
54	55	CDR-H2	N	F	L	N	N
55	56	CDR-H2	D	G	G	D	D
56	57	CDR-H2	A	T	T	A	A
57	58	CDR-H2	T	A	A	T	T
58	59	CDR-H2	S	N	N	S	S
59	60	CDR-H2	Y	Y	Y	Y	Y
60	61	CDR-H2	N	A	A	N	N
61	62	CDR-H2	Q	Q	Q	Q	Q
62	63	CDR-H2	K	K	K	K	K
63	64	CDR-H2	F	F	F	F	F
64	65	CDR-H2	K	Q	Q	K	K
65	66	CDR-H2	G	G	G	G	G
66	67	Fr3	K	R	R	R	R
67	68	Fr3	A	V	V	A	A
68	69	Fr3	T	T	T	T	T
69	70	Fr3	L	I	I	L	L
70	71	Fr3	T	T	T	T	T
71	72	Fr3	V	A	A	V	V
72	73	Fr3	D	D	D	D	D
73	74	Fr3	K	K	K	K	K
74	75	Fr3	S	S	S	S	S
75	76	Fr3	S	T	T	T	T
76	77	Fr3	S	S	S	S	S
77	78	Fr3	T	T	T	T	T

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VH (SEQ ID NO:28)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178)	IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	Hu8H24VHv1 (SEQ ID NO:180)	Hu8H24VHv2 (SEQ ID NO:181)
78	79	Fr3	A	A	A	A	A
79	80	Fr3	Y	Y	Y	Y	Y
80	81	Fr3	M	M	M	M	M
81	82	Fr3	Q	E	E	E	E
82	83	Fr3	L	L	L	L	L
82A	84	Fr3	S	S	S	S	S
82B	85	Fr3	S	S	S	S	S
82C	86	Fr3	L	L	L	L	L
83	87	Fr3	T	R	R	R	R
84	88	Fr3	S	S	S	S	S
85	89	Fr3	E	E	E	E	E
86	90	Fr3	D	D	D	D	D
87	91	Fr3	S	T	T	T	T
88	92	Fr3	A	A	A	A	A
89	93	Fr3	V	V	V	V	V
90	94	Fr3	Y	Y	Y	Y	Y
91	95	Fr3	F	Y	Y	F	F
92	96	Fr3	C	C	C	C	C
93	97	Fr3	A	A	A	A	A
94	98	Fr3	R	R	R	R	R
95	99	CDR-H3	E	A	A	E	E
96		CDR-H3	G	Y	E	G	G
97		CDR-H3	Y	C	Y	Y	Y
98		CDR-H3	Y	S	F	Y	Y
99		CDR-H3	G	S	-	G	G
100		CDR-H3	S	T	-	S	S
100A		CDR-H3	S	S	-	S	S
100B		CDR-H3	F	C	-	F	F

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VH (SEQ ID NO:28)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178)	IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	Hu8H24VHv1 (SEQ ID NO:180)	Hu8H24VHv2 (SEQ ID NO:181)
100C		CDR-H3	E	Y	-	E	E
100D		CDR-H3	A	K	-	A	A
100E		CDR-H3	W	T	-	W	W
100F		CDR-H3	F	G	-	F	F
100G		CDR-H3	-	-	-	-	-
100H		CDR-H3	-	-	-	-	-
100I		CDR-H3	-	-	-	-	-
100J		CDR-H3	-	-	-	-	-
100K		CDR-H3	-	-	-	-	-
101	100	CDR-H3	A	F	Q	A	A
102	101	CDR-H3	S	V	H	S	S
103	102	Fr4	W	W	W	W	W
104	103	Fr4	G	G	G	G	G
105	104	Fr4	Q	Q	Q	Q	Q
106	105	Fr4	G	G	G	G	G
107	106	Fr4	T	T	T	T	T
108	107	Fr4	L	L	L	T	T
109	108	Fr4	V	V	V	V	V
110	109	Fr4	T	T	T	T	T
111	110	Fr4	V	V	V	V	V
112	111	Fr4	S	S	S	S	S
113	112	Fr4	A	S	S	S	S

[0657] Table 21: 8H24 VL sequences

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VL (SEQ ID NO:34)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183)	IMGT# IGKV2-40*01 (SEQ ID NO:184)	Hu8H24VLv1 (SEQ ID NO:185)	Hu8H24VLv2 (SEQ ID NO:186)
1	1	Fr1	D	D	D	D	D
2	2	Fr1	V	I	I	V	V
3	3	Fr1	L	V	V	V	V
4	4	Fr1	M	M	M	M	M
5	5	Fr1	T	T	T	T	T
6	6	Fr1	Q	Q	Q	Q	Q
7	7	Fr1	T	T	T	T	T
8	8	Fr1	P	P	P	P	P
9	9	Fr1	L	L	L	L	S
10	10	Fr1	S	S	S	S	S
11	11	Fr1	L	L	L	L	L
12	12	Fr1	P	P	P	P	P
13	13	Fr1	V	V	V	V	V
14	14	Fr1	S	T	T	T	T
15	15	Fr1	L	P	P	P	P
16	16	Fr1	G	G	G	G	G
17	17	Fr1	D	E	E	E	E
18	18	Fr1	Q	P	P	P	P
19	19	Fr1	A	A	A	A	A
20	20	Fr1	S	S	S	S	S
21	21	Fr1	I	I	I	I	I
22	22	Fr1	S	S	S	S	S
23	23	Fr1	C	C	C	C	C

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VL (SEQ ID NO:34)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183)	IMGT# IGKV2-40*01 (SEQ ID NO:184)	Hu8H24VLv1 (SEQ ID NO:185)	Hu8H24VLv2 (SEQ ID NO:186)
24	24	CDR-L1	R	R	R	R	R
25	25	CDR-L1	S	S	S	S	S
26	26	CDR-L1	S	S	S	S	S
27	27	CDR-L1	Q	Q	Q	Q	Q
27A	28	CDR-L1	S	S	S	S	S
27B	29	CDR-L1	I	L	L	I	I
27C	30	CDR-L1	V	L	L	V	V
27D	31	CDR-L1	H	H	D	H	H
27E	32	CDR-L1	S	S	S	S	S
27F		CDR-L1	-	-	D	-	-
28	33	CDR-L1	N	N	D	N	N
29	34	CDR-L1	G	G	G	G	G
30	35	CDR-L1	N	Y	N	N	N
31	36	CDR-L1	T	N	T	T	T
32	37	CDR-L1	Y	Y	Y	Y	Y
33	38	CDR-L1	L	L	L	L	L
34	39	CDR-L1	E	D	D	E	E
35	40	Fr2	W	W	W	W	W
36	41	Fr2	Y	Y	Y	Y	Y
37	42	Fr2	L	L	L	L	L
38	43	Fr2	Q	Q	Q	Q	Q
39	44	Fr2	K	K	K	K	K
40	45	Fr2	P	P	P	P	P
41	46	Fr2	G	G	G	G	G
42	47	Fr2	Q	Q	Q	Q	Q
43	48	Fr2	S	S	S	S	S

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VL (SEQ ID NO:34)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183)	IMGT# IGKV2-40*01 (SEQ ID NO:184)	Hu8H24VLv1 (SEQ ID NO:185)	Hu8H24VLv2 (SEQ ID NO:186)
44	49	Fr2	P	P	P	P	P
45	50	Fr2	K	Q	Q	Q	Q
46	51	Fr2	L	L	L	L	L
47	52	Fr2	L	L	L	L	L
48	53	Fr2	I	I	I	I	I
49	54	Fr2	Y	Y	Y	Y	Y
50	55	CDR-L2	K	L	T	K	K
51	56	CDR-L2	V	G	L	V	V
52	57	CDR-L2	S	S	S	S	S
53	58	CDR-L2	N	N	Y	N	N
54	59	CDR-L2	R	R	R	R	R
55	60	CDR-L2	F	A	A	F	F
56	61	CDR-L2	S	S	S	S	S
57	62	Fr3	G	G	G	G	G
58	63	Fr3	V	V	V	V	V
59	64	Fr3	P	P	P	P	P
60	65	Fr3	D	D	D	D	D
61	66	Fr3	R	R	R	R	R
62	67	Fr3	F	F	F	F	F
63	68	Fr3	S	S	S	S	S
64	69	Fr3	G	G	G	G	G
65	70	Fr3	S	S	S	S	S
66	71	Fr3	G	G	G	G	G
67	72	Fr3	S	S	S	S	S
68	73	Fr3	G	G	G	G	G
69	74	Fr3	T	T	T	T	T

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VL (SEQ ID NO:34)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183)	IMGT# IGKV2-40*01 (SEQ ID NO:184)	Hu8H24VLv1 (SEQ ID NO:185)	Hu8H24VLv2 (SEQ ID NO:186)
70	75	Fr3	D	D	D	D	D
71	76	Fr3	F	F	F	F	F
72	77	Fr3	T	T	T	T	T
73	78	Fr3	L	L	L	L	L
74	79	Fr3	K	K	K	K	T
75	80	Fr3	I	I	I	I	I
76	81	Fr3	S	S	S	S	S
77	82	Fr3	R	R	R	R	R
78	83	Fr3	V	V	V	V	V
79	84	Fr3	E	E	E	E	E
80	85	Fr3	A	A	A	A	A
81	86	Fr3	E	E	E	E	E
82	87	Fr3	D	D	D	D	D
83	88	Fr3	L	V	V	V	V
84	89	Fr3	G	G	G	G	G
85	90	Fr3	V	V	V	V	V
86	91	Fr3	Y	Y	Y	Y	Y
87	92	Fr3	Y	Y	Y	Y	Y
88	93	Fr3	C	C	C	C	C
89	94	CDR-L3	F	M	M	F	F
90	95	CDR-L3	Q	Q	Q	Q	Q
91	96	CDR-L3	G	A	R	G	G
92	97	CDR-L3	S	L	I	S	S
93	98	CDR-L3	H	Q	E	H	H
94	99	CDR-L3	V	T	F	V	V
95	100	CDR-L3	L	P	P	L	L

Kabat residue #	Linear residue #	FR or CDR	Mouse 8H24 VL (SEQ ID NO:34)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183)	IMGT# IGKV2-40*01 (SEQ ID NO:184)	Hu8H24VLv1 (SEQ ID NO:185)	Hu8H24VLv2 (SEQ ID NO:186)
95A		CDR-L3	-	-	-	-	-
95B		CDR-L3	-	-	-	-	-
95C		CDR-L3	-	-	-	-	-
95D		CDR-L3	-	-	-	-	-
95E		CDR-L3	-	-	-	-	-
95F		CDR-L3	-	-	-	-	-
96	101	CDR-L3	P	L	L	P	P
97	102	CDR-L3	T	T	T	T	T
98	103	Fr4	F	F	F	F	F
99	104	Fr4	G	G	G	G	G
100	105	Fr4	G	G	G	G	G
101	106	Fr4	G	G	G	G	G
102	107	Fr4	T	T	T	T	T
103	108	Fr4	K	K	K	K	K
104	109	Fr4	L	V	V	V	V
105	110	Fr4	E	E	E	E	E
106	111	Fr4	I	I	I	I	I
106A		Fr4	-	-	-	-	-
107	112	Fr4	R	K	K	K	K

[0658] Table 22: V_H, V_L Backmutations and Other Mutations for Humanized 8H24		
V_H or V_L Variant	V_H or V_L Exon Acceptor Sequence	Changes from Acceptor Framework (or CDR) Residues (based on Kabat/Chothia Composite CDRs)
Hu8H24VHv1 (SEQ ID NO:180)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178) IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	H2, H48, H67, H71, H91, H108
Hu8H24VHv2 (SEQ ID NO:181)	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178) IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	H2, H12, H48, H67, H71, H91, H108
Hu8H24VLv1 (SEQ ID NO:185)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183) IMGT# IGKV2-40*01 (SEQ ID NO:184)	L2
Hu8H24VLv2 (SEQ ID NO:186)	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183) IMGT# IGKV2-40*01 (SEQ ID NO:184)	L2, L9, L74

[0659] Table 23: Kabat Numbering of Framework (or CDR) Residues (based on Kabat/Chothia Composite CDRs) for Backmutations and Other Mutations in Heavy Chains of Humanized 8H24 Antibodies

Residue	GenBank Acc. # AAC51714-VH_huFrwk (SEQ ID NO:178)	IMGT# IGHV1-69*08_IGHJ1*01 (SEQ ID NO:179)	Mouse 8H24 VH (SEQ ID NO:28)	Hu8H24VHv1 (SEQ ID NO:180)	Hu8H24VHv2 (SEQ ID NO:181)
H2	V	V	A	A	A
H12	K	K	V	K	V
H48	M	M	I	I	I
H67	V	V	A	A	A
H71	A	A	V	V	V
H91	Y	Y	F	F	F
H108	L	L	L	T	T

[0660] Table 24: Kabat Numbering of Framework Residues (based on Kabat/Chothia Composite CDRs) for Backmutations and Other Mutations in Light Chains of Humanized 8H24 Antibodies

Residue	GenBank Acc. # ABC66914-VL_huFrwk (SEQ ID NO:183)	IMGT# IGKV2-40*01 (SEQ ID NO:184)	Mouse 8H24 VL (SEQ ID NO:34)	Hu8H24VLv1 (SEQ ID NO:185)	Hu8H24VLv2 (SEQ ID NO:186)
L2	I	I	V	V	V
L9	L	L	L	L	S
L74	K	K	K	K	T

[0661] Table 25
Percentage Humanness of Heavy and Light Chains of Humanized 8H24 Antibodies

V_H or V_L Variant	% Humanness
Hu8H24VHv1 (SEQ ID NO:180)	81.6%
Hu8H24VHv2 (SEQ ID NO:181)	80.6%
Hu8H24VLv1 (SEQ ID NO:185)	88.4%
Hu8H24VLv2 (SEQ ID NO:186)	86.3%

[0662] Positions at which canonical, vernier, or interface residues differ between mouse and human acceptor sequences are candidates for substitution. Examples of canonical/CDR interacting residues include Kabat residues H71 in Table 20 and L2 in Table 21. Examples of vernier residues include Kabat residues H2, H48, H67, and H71 in Table 20 and L2 in Table 21. Examples of interface/packing (VH+VL) residues include Kabat residue H91 in Table 20.

[0663] The rationales for selection of the positions indicated in Table 20 in the heavy chain variable region as candidates for substitution are as follows.

[0664] heavy chain variable regions

hu8H24-VH_v1

- consists of the CDR-H1, H2, and H3 loops of 8H24-VH grafted onto the framework of AAC51714 VH, and reverts all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or localize to the VH/VL domain interface

Hu8H24-VH_v2

- reverts all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or localize to the VH/VL domain interface or contribute to structural stability. H824-VH_v2 incorporates backmutations or substitution with most frequent residue at a given position.

[0665] V2A: Backmutation of a vernier zone residue

[0666] K12V: Side chain of Lys clashes with Val18, murine sequence at this position is Val, Val is most frequent residue at this position. Backmutation also increases humanness of the sequence.

[0667] M48I: Backmutation of a vernier zone residue

[0668] V67A: Backmutation of a vernier zone residue

[0669] A71V: Backmutation of a canonical and a vernier zone residue

[0670] Y91F: Backmutation of a VH/VL domain interface residue

[0671] L108T: This back mutation is made to reduce immunogenicity

[0672] The rationales for selection of the positions indicated in Table 21 in the light chain variable region as candidates for substitution are as follows.

[0673] *kappa light chain variable regions*

hu8H24-VL_v1

- consists of the CDR-L1, L2, and L3 loops of 8H24-VL grafted onto the framework of ABC66914 VL along with reverting all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or locate to the VH/VL domain interface.

Hu8H24-VL_v2

- Hu8H24-VL_v2 includes substitutions that contribute to structural stability or increase the humanness of the antibody.

[0674] I2V: Backmutation of a canonical and a vernier zone residue

[0675] L9S: Corresponding germline sequence has Leu at this position, but Ser is most frequent at this position. Given that this is a surface exposed residue and Leu is hydrophobic whereas Ser is hydrophilic, having a hydrophilic residue at this position may increase developability (optimization of deamination, oxidation, N-glycosylation, proteolysis and aggregation).

[0676] K74T: murine and germline have Lys at this position but Thr is most frequent residue at this position. Being surface exposed Lys could lead to increase in surface charge patches. Thr at this position may increase developability (optimization of deamination, oxidation, N-glycosylation, proteolysis and aggregation).

[0677] *Sequences of heavy chain variable regions*

[0678] >h8H24VH version1 (81.6% human)

QAQLVQSGAEVKKPGSSVKVSKASGYTFTSYSMHWVRQAPGQGLEWIGAIYPGNDATSYNQKFKGRATL
TVDKSTSTAYMELSSLRSEDVAVFCAREGGYGSFEAWFASWGQGTTVTVSS

[0679] >h8H24VH version2 (80.6% human)

QAQLVQSGAEVVKPGSSVKVSKASGYTFTSYSMHWVRQAPGQGLEWIGAIYPGNDATSYNQKFKGRATL
TVDKSTSTAYMELSSLRSEDVAVFCAREGGYGSFEAWFASWGQGTTVTVSS

[0680] *Sequences of kappa light chain variable regions*

[0681] >h8H24VL version1 (88.4% human)

DVVMVTQTPLSLPVTGPGEPAISCRSSQIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSG
TDFTLKISRVEAEDVGVYYCFQGSHVLPFTGGGTKVEIK

[0682] >h8H24VL version2 (86.3% human)

DVVMVTQTPSSLPVTGPGEPAISCRSSQIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSG
TDFTLTISRVEAEDVGVYYCFQGSHVLPFTGGGTKVEIK

[0683] **Example 16.** Design of Humanized 11M14 Antibodies

[0684] The starting point or donor antibody for humanization was the mouse antibody 11M14. The heavy chain variable amino acid sequence of mature m11M14 is provided as SEQ ID NO:52. The light chain variable amino acid sequence of mature m11M14 is provided as SEQ ID NO:58. The heavy chain Kabat/Chothia Composite CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:53-55, respectively. The light chain Kabat CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:59-61, respectively. Kabat numbering is used throughout.

[0685] The variable kappa (Vk) of 11M14 belongs to mouse Vk subgroup 5 which corresponds to human Vk subgroup 1 and the variable heavy (Vh) to mouse Vh subgroup 3d which corresponds to human Vh subgroup 3 [Kabat E.A., et al., (1991), Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242]. 11 residue Chothia CDR-L1 is similar to Chothia canonical class 2, 7 residue Chothia CDR-L2 is of Chothia canonical class 1, 10 residue Chothia CDR-L3 is not similar to any Chothia canonical class [Martin ACR. (2010) Protein sequence and structure analysis of antibody variable domains. In: Kontermann R and Dübel S (eds). *Antibody Engineering*. Heidelberg, Germany: Springer International Publishing AG.. [Martin, 2010]. 10 residue Chothia CDR-H1 is similar to Chothia canonical

class 1, 17 residue Chothia CDR-H2 is similar to Chothia canonical class 3 [Martin, 2010]. 8 residue CDR-H3 has no canonical classes. A search was made over the protein sequences in the PDB database [Deshpande N, et al., (2005) *Nucleic Acids Res.* 33: D233-7] to find structures, which would provide a rough structural model of 11M14. The crystal structure of an antibody fab PDB code 1MQK [Essen, L.O. et al., 2003, *Acta Crystallogr., Sect. D* 59: 677-687] for both Vh and Vk structure since it had good resolution (1.28 Å) and overall sequence similarity to 11M14 Vh and Vk, retaining the same canonical structures for the loops.

[0686] A search of the non-redundant protein sequence database from NCBI allowed selection of suitable human frameworks into which to graft the murine 11M14 CDRs. For Vk, a human kappa light chain with NCBI accession code CBZ39892 (EMBL accession:FR820882) [Colombo, M. et al, Direct Submission 2011] was chosen. This has the same canonical classes for CDR-L1 & L2 and belongs to human germline IGKV1D-39*01 according to IMGT convention. It is a member of Kabat human kappa subgroup 1. For Vh, human Ig heavy chain ACS96198 (DBSOURCE:FJ489037) [Jimenez-Gomez, G. et al, 2010, *J. Leukoc. Biol.* 87 (3), 523-530] was chosen, again with the same canonical classes and belonging to human germline IGHV3-48*03. It is a member of Kabat human heavy subgroup 3. Accordingly, the framework regions of ACS96198 VH and CBZ39892 VL were chosen as the acceptor sequences for the CDRs of 11M14. A model of the 11M14 CDRs grafted onto the respective human frameworks for VH and VL was built and used as a guidance for further backmutations.

[0687] Heavy and light chain variant sequences resulting from antibody humanization process were further aligned to human germ line sequences using IMGT Domain GapAlign tool to assess the humanness of the heavy and light chain as outlined by WHO INN committee guidelines. (WHO-INN: International nonproprietary names (INN) for biological and biotechnological substances (a review) (Internet) 2014. Available from: <http://www.who.int/medicines/services/inn/BioRev2014.pdf>) Residues were changed to align with corresponding human germ line sequence, where possible, to enhance humanness and to reduce potential immunogenicity. For humanized VLv2b, VL3b, and VL4b variants, mutations were introduced to render the sequences more similar to human germ line IGKV1-39*01 (SEQ ID NO:195)

[0688] Additional versions of hu11M14-VH and hu11M14-VL were designed to enable assessment of various framework residues for their contributions to antigen binding, thermostability, developability (deamination, oxidation, N-glycosylation, proteolysis and aggregation) and immunogenicity. The positions considered for mutation include those that...

- define the canonical CDR conformations (summarized in Martin ACR. (2010) Protein sequence and structure analysis of antibody variable domains. In: Kontermann R and Dübel S (eds). *Antibody Engineering*. Heidelberg, Germany: Springer International Publishing AG).
- are within the Vernier zone (Foote J and Winter G. (1992) Antibody framework residues affecting the conformation of the hypervariable loops. *J Mol Biol.* 224(2):487-99).
- localize to the VH/VL domain interface (summarized in Léger OJP and Saldanha J. (2000) Preparation of recombinant antibodies from immune rodent spleens and the design of their humanisation by CDR grafting. In: Shepherd P and Dean C (eds). *Monoclonal Antibodies: A Practical Approach*. Oxford, UK: Oxford University Press).
- are susceptible to post-translational modifications, such as glycosylation or pyroglutamination,
- are occupied by residues that are predicted to clash with CDRs, according to the model of 11M14 CDRs grafted onto VH and VL frameworks, or
- are occupied by residues that are rare among sequenced human antibodies, where either the parental mouse 11M14 residue or some other residue is much more prevalent within human antibody repertoire.

[0689] Alignments of the murine 11M14 and various humanized antibodies are shown for the light chain variable regions (Table 27 and Figure 6), and heavy chain variable regions (Table 26 and Figure 5).

[0690] 3 humanized heavy chain variable region variants and 4 humanized light chain variable region variants were constructed containing different permutations of substitutions: hu11M14VHv1b, hu11M14VHv2b, or hu11M14VHv3b, (SEQ ID NOS:190-192, respectively); and hu11M14VLv1b, hu11M14VLv2b, hu11M14VLv3b, or hu11M14VLv4b (SEQ ID NOS:196-199 respectively) (Tables 26 and 27). The exemplary humanized Vk and Vh designs, with backmutations and other mutations based on selected human frameworks, are shown in Tables 27 and 26, respectively. The bolded areas in Tables 26 and 27 indicate the CDRs as defined by Kabat/Chothia Composite. A “-“ in the columns in Tables 26 and 27 indicates no residue at the indicated position. SEQ ID NOS:190-192, and SEQ ID NOS:196-199 contain

backmutations and other mutations as shown in Table 28. The amino acids at positions in hu11M14VHv1b, hu11M14VHv2b, and hu11M14VHv3b are listed in Table 29. The amino acids at positions in hu11M14VLv1b, hu11M14VLv2b, hu11M14VLv3b, and hu11M14VLv4b are listed in Table 30.

[0691] The percentage humanness for humanized VH chains hu11M14VHv1b, hu11M14VHv2b, and hu11M14VHv3b (SEQ ID NOS:190-192, respectively) with respect to the most similar human germline gene IGHV3-48*03 (SEQ ID NO:189), and for humanized VL chains in hu11M14VLv1b, hu11M14VLv2b, hu11M14VLv3b, and hu11M14VLv4b (SEQ ID NOS:196-199 respectively) with respect to the most similar human germline gene IGKV1-39*01 (SEQ ID NO:195) is shown in Table 31. NOS:196-199 respectively) with respect to the most similar human germline gene IGKV1-39*01 (SEQ ID NO:195) is shown in Table 31.

[0692] Table 26 11M14 VH sequences

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VH (SEQ ID NO:)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188)	IMGT# IGHV3-48*03 (SEQ ID NO:189)	Hu11M14VHv1b (SEQ ID NO:190)	Hu11M14VHv2b (SEQ ID NO:191)	Hu11M14VHv3b (SEQ ID NO:192)
1	1	Fr1	E	E	E	E	E	E
2	2	Fr1	V	V	V	V	V	V
3	3	Fr1	Q	Q	Q	Q	Q	Q
4	4	Fr1	L	L	L	L	L	L
5	5	Fr1	V	V	V	V	V	V
6	6	Fr1	E	E	E	E	E	E
7	7	Fr1	S	S	S	S	S	S
8	8	Fr1	G	G	G	G	G	G
9	9	Fr1	G	G	G	G	G	G
10	10	Fr1	D	G	G	G	G	G
11	11	Fr1	L	L	L	L	L	L
12	12	Fr1	V	V	V	V	V	V
13	13	Fr1	K	Q	Q	Q	Q	Q
14	14	Fr1	P	P	P	P	P	P
15	15	Fr1	G	G	G	G	G	G
16	16	Fr1	G	G	G	G	G	G
17	17	Fr1	S	S	S	S	S	S
18	18	Fr1	L	L	L	L	L	L
19	19	Fr1	K	R	R	R	R	R
20	20	Fr1	L	L	L	L	L	L
21	21	Fr1	S	S	S	S	S	S
22	22	Fr1	C	C	C	C	C	C
23	23	Fr1	A	A	A	A	A	A
24	24	Fr1	A	A	A	A	A	A

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VH (SEQ ID NO:)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188)	IMGT# IGHV3-48*03 (SEQ ID NO:189)	Hu11M14VHv1b (SEQ ID NO:190)	Hu11M14VHv2b (SEQ ID NO:191)	Hu11M14VHv3b (SEQ ID NO:192)
25	25	Fr1	S	S	S	S	S	S
26	26	CDR-H1	G	G	G	G	G	G
27	27	CDR-H1	F	F	F	F	F	F
28	28	CDR-H1	T	T	T	T	T	T
29	29	CDR-H1	F	F	F	F	F	F
30	30	CDR-H1	N	S	S	N	N	N
31	31	CDR-H1	I	S	S	I	I	I
32	32	CDR-H1	Y	Y	Y	Y	Y	Y
33	33	CDR-H1	G	E	E	G	G	G
34	34	CDR-H1	M	M	M	M	M	M
35	35	CDR-H1	S	N	N	S	S	S
35A		CDR-H1	-	-	-	-	-	-
35B		CDR-H1	-	-	-	-	-	-
36	36	Fr2	W	W	W	W	W	W
37	37	Fr2	V	V	V	V	V	V
38	38	Fr2	R	R	R	R	R	R
39	39	Fr2	Q	Q	Q	Q	Q	Q
40	40	Fr2	T	A	A	A	A	A
41	41	Fr2	P	P	P	P	P	P
42	42	Fr2	D	G	G	G	G	G
43	43	Fr2	K	K	K	K	K	K
44	44	Fr2	R	G	G	G	G	G
45	45	Fr2	L	L	L	L	L	L
46	46	Fr2	E	E	E	E	E	E
47	47	Fr2	W	W	W	W	W	W
48	48	Fr2	V	V	V	V	V	V
49	49	Fr2	A	S	S	A	A	A
50	50	CDR-H2	T	Y	Y	T	T	T

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VH (SEQ ID NO:)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188)	IMGT# IGHV3-48*03 (SEQ ID NO:189)	Hu11M14VHv1b (SEQ ID NO:190)	Hu11M14VHv2b (SEQ ID NO:191)	Hu11M14VHv3b (SEQ ID NO:192)
51	51	CDR-H2	I	I	I	I	I	I
52	52	CDR-H2	S	S	S	S	S	S
52A	53	CDR-H2	S	S	S	S	S	S
52B		CDR-H2	-	-	-	-	-	-
52C		CDR-H2	-	-	-	-	-	-
53	54	CDR-H2	G	S	S	G	G	G
54	55	CDR-H2	G	G	G	G	G	G
55	56	CDR-H2	I	S	S	I	I	I
56	57	CDR-H2	Y	T	T	Y	Y	Y
57	58	CDR-H2	T	I	I	T	T	T
58	59	CDR-H2	Y	Y	Y	Y	Y	Y
59	60	CDR-H2	Y	Y	Y	Y	Y	Y
60	61	CDR-H2	P	A	A	P	P	P
61	62	CDR-H2	D	D	D	D	D	D
62	63	CDR-H2	I	S	S	I	I	I
63	64	CDR-H2	L	V	V	L	L	L
64	65	CDR-H2	K	K	K	K	K	K
65	66	CDR-H2	G	G	G	G	G	G
66	67	Fr3	R	R	R	R	R	R
67	68	Fr3	F	F	F	F	F	F
68	69	Fr3	T	T	T	T	T	T
69	70	Fr3	I	I	I	I	I	I
70	71	Fr3	S	S	S	S	S	S
71	72	Fr3	R	R	R	R	R	R
72	73	Fr3	D	D	D	D	D	D
73	74	Fr3	N	N	N	N	N	N
74	75	Fr3	A	A	A	A	A	A
75	76	Fr3	K	K	K	K	K	K

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VH (SEQ ID NO:)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188)	IMGT# IGHV3-48*03 (SEQ ID NO:189)	Hu11M14VHv1b (SEQ ID NO:190)	Hu11M14VHv2b (SEQ ID NO:191)	Hu11M14VHv3b (SEQ ID NO:192)
76	77	Fr3	N	N	N	N	N	N
77	78	Fr3	T	S	S	S	S	S
78	79	Fr3	L	L	L	L	L	L
79	80	Fr3	Y	Y	Y	Y	Y	Y
80	81	Fr3	L	L	L	L	L	G
81	82	Fr3	Q	Q	Q	Q	Q	Q
82	83	Fr3	M	M	M	M	M	M
82A	84	Fr3	S	N	N	N	N	N
82B	85	Fr3	S	S	S	S	S	S
82C	86	Fr3	L	L	L	L	G	L
83	87	Fr3	K	R	R	R	R	R
84	88	Fr3	S	A	A	A	A	A
85	89	Fr3	E	E	E	E	E	E
86	90	Fr3	D	D	D	D	D	D
87	91	Fr3	T	T	T	T	T	T
88	92	Fr3	A	A	A	A	A	A
89	93	Fr3	M	V	V	V	V	V
90	94	Fr3	Y	Y	Y	Y	Y	Y
91	95	Fr3	Y	Y	Y	Y	Y	Y
92	96	Fr3	C	C	C	C	C	C
93	97	Fr3	A	A	A	A	A	A
94	98	Fr3	R	R	R	R	R	R
95	99	CDR-H3	H	E	Y	H	H	H
96		CDR-H3	P	N	F	P	P	P
97		CDR-H3	G	I	-	G	G	G
98		CDR-H3	G	A	-	G	G	G
99		CDR-H3	A	A	-	A	A	A
100		CDR-H3	M	F	-	M	M	M

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VH (SEQ ID NO:)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188)	IMGT# IGHV3-48*03 (SEQ ID NO:189)	Hu11M14VHv1b (SEQ ID NO:190)	Hu11M14VHv2b (SEQ ID NO:191)	Hu11M14VHv3b (SEQ ID NO:192)
100A		CDR-H3		-	-	-	-	-
100B		CDR-H3		-	-	-	-	-
100C		CDR-H3		-	-	-	-	-
100D		CDR-H3		-	-	-	-	-
100E		CDR-H3		-	-	-	-	-
100F		CDR-H3		-	-	-	-	-
100G		CDR-H3	-	-	-	-	-	-
100H		CDR-H3	-	-	-	-	-	-
100I		CDR-H3	-	-	-	-	-	-
100J		CDR-H3	-	-	-	-	-	-
100K		CDR-H3	-	-	-	-	-	-
101	100	CDR-H3	D	D	D	D	D	D
102	101	CDR-H3	Y	Y	Y	Y	Y	Y
103	102	Fr4	W	W	W	W	W	W
104	103	Fr4	G	G	G	G	G	G
105	104	Fr4	Q	Q	Q	Q	Q	Q
106	105	Fr4	G	G	G	G	G	G
107	106	Fr4	T	T	T	T	T	T
108	107	Fr4	S	L	L	L	L	L
109	108	Fr4	V	V	V	V	V	V
110	109	Fr4	T	T	T	T	T	T
111	110	Fr4	V	V	V	V	V	V
112	111	Fr4	S	S	S	S	S	S
113	112	Fr4	S	S	S	S	S	S

[0693] Table 27: 11M14 VL sequences

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VL (SEQ ID NO:58)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194)	IMGT# IGKV1-39*01 (SEQ ID NO:195)	Mouse 11M14 VH (SEQ ID NO:52)	Hu11M14VLv1b (SEQ ID NO:196)	Hu11M14VLv2b (SEQ ID NO:197)	Hu11M14VLv3b (SEQ ID NO:198)
1	1	Fr1	D	D	D	D	D	D	D
2	2	Fr1	I	I	I	I	I	I	I
3	3	Fr1	Q	Q	Q	Q	Q	Q	Q
4	4	Fr1	M	M	M	M	M	M	M
5	5	Fr1	T	T	T	T	T	T	T
6	6	Fr1	Q	Q	Q	Q	Q	Q	Q
7	7	Fr1	S	S	S	S	S	S	S
8	8	Fr1	P	P	P	P	P	P	P
9	9	Fr1	A	S	S	S	S	S	S
10	10	Fr1	S	S	S	S	S	S	S
11	11	Fr1	L	L	L	L	L	L	L
12	12	Fr1	S	S	S	S	S	S	S
13	13	Fr1	V	A	A	A	A	A	A
14	14	Fr1	S	S	S	S	S	S	S
15	15	Fr1	V	V	V	V	V	V	V
16	16	Fr1	G	G	G	G	G	G	G
17	17	Fr1	E	D	D	D	D	D	D
18	18	Fr1	T	R	R	R	R	R	R
19	19	Fr1	V	V	V	V	V	V	V
20	20	Fr1	T	T	T	T	T	T	T
21	21	Fr1	I	I	I	I	I	I	I
22	22	Fr1	T	T	T	T	T	T	T
23	23	Fr1	C	C	C	C	C	C	C
24	24	CDR-L1	R	R	R	R	R	R	R
25	25	CDR-L1	V	A	A	V	V	V	V
26	26	CDR-L1	S	S	S	S	S	S	S

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VL (SEQ ID NO:58)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194)	IMGT# IGKV1-39*01 (SEQ ID NO:195)	Mouse 11M14 VH (SEQ ID NO:52)	Hu11M14VLv1b (SEQ ID NO:196)	Hu11M14VLv2b (SEQ ID NO:197)	Hu11M14VLv3b (SEQ ID NO:198)
27	27	CDR-L1	E	Q	Q	E	E	E	E
27A	28	CDR-L1	-	S	S	-	-	-	
27B	29	CDR-L1	-	I	I	-	-	-	
27C	30	CDR-L1	-	S	S	-	-	-	
27D	31	CDR-L1	-	S	S	-	-	-	-
27E	32	CDR-L1	-	Y	Y	-	-	-	-
27F		CDR-L1	-	-	-	-	-	-	-
28	33	CDR-L1	N	-	-	N	N	N	N
29	34	CDR-L1	I	-	-	I	I	I	I
30	35	CDR-L1	Y	-	-	Y	Y	Y	Y
31	36	CDR-L1	S	-	-	S	S	S	S
32	37	CDR-L1	N	-	-	N	N	N	N
33	38	CDR-L1	L	L	L	L	L	L	L
34	39	CDR-L1	A	N	N	A	A	A	A
35	40	Fr2	W	W	W	W	W	W	W
36	41	Fr2	Y	Y	Y	Y	Y	Y	Y
37	42	Fr2	Q	Q	Q	Q	Q	Q	Q
38	43	Fr2	Q	Q	Q	Q	Q	Q	Q
39	44	Fr2	K	K	K	K	K	K	K
40	45	Fr2	Q	P	P	P	P	P	P
41	46	Fr2	G	G	G	G	G	G	G
42	47	Fr2	K	K	K	K	K	K	K
43	48	Fr2	S	A	A	A	S	S	S
44	49	Fr2	P	P	P	P	P	P	P
45	50	Fr2	H	K	K	K	K	K	K
46	51	Fr2	L	L	L	L	L	L	L
47	52	Fr2	L	L	L	L	L	L	L
48	53	Fr2	V	I	I	V	V	V	V

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VL (SEQ ID NO:58)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194)	IMGT# IGKV1-39*01 (SEQ ID NO:195)	Mouse 11M14 VH (SEQ ID NO:52)	Hu11M14VLv1b (SEQ ID NO:196)	Hu11M14VLv2b (SEQ ID NO:197)	Hu11M14VLv3b (SEQ ID NO:198)
49	54	Fr2	Y	Y	Y	Y	Y	Y	Y
50	55	CDR-L2	A	A	A	A	A	A	A
51	56	CDR-L2	A	A	A	A	A	A	A
52	57	CDR-L2	T	S	S	T	T	T	T
53	58	CDR-L2	N	S	S	N	N	N	N
54	59	CDR-L2	L	L	L	L	L	G	I
55	60	CDR-L2	A	Q	Q	A	A	A	A
56	61	CDR-L2	D	S	S	D	D	D	D
57	62	Fr3	G	G	G	G	G	G	G
58	63	Fr3	V	V	V	V	V	V	V
59	64	Fr3	P	P	P	P	P	P	P
60	65	Fr3	S	S	S	S	S	S	S
61	66	Fr3	R	R	R	R	R	R	R
62	67	Fr3	F	F	F	F	F	F	F
63	68	Fr3	S	S	S	S	S	S	S
64	69	Fr3	G	G	G	G	G	G	G
65	70	Fr3	S	S	S	S	S	S	S
66	71	Fr3	G	G	G	G	G	G	G
67	72	Fr3	S	S	S	S	S	S	S
68	73	Fr3	G	G	G	G	G	G	G
69	74	Fr3	T	T	T	T	T	T	T
70	75	Fr3	Q	D	D	D	D	D	D
71	76	Fr3	Y	F	F	Y	Y	Y	Y
72	77	Fr3	S	T	T	T	T	T	T
73	78	Fr3	L	L	L	L	L	L	L
74	79	Fr3	K	T	T	T	T	T	T
75	80	Fr3	I	I	I	I	I	I	I
76	81	Fr3	N	N	S	N	S	S	S

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VL (SEQ ID NO:58)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194)	IMGT# IGKV1-39*01 (SEQ ID NO:195)	Mouse 11M14 VH (SEQ ID NO:52)	Hu11M14VLv1b (SEQ ID NO:196)	Hu11M14VLv2b (SEQ ID NO:197)	Hu11M14VLv3b (SEQ ID NO:198)
77	82	Fr3	S	S	S	S	S	S	S
78	83	Fr3	L	L	L	L	L	L	L
79	84	Fr3	Q	Q	Q	Q	Q	Q	Q
80	85	Fr3	S	P	P	P	P	P	P
81	86	Fr3	E	E	E	E	E	E	E
82	87	Fr3	D	D	D	D	D	D	D
83	88	Fr3	F	F	F	F	F	F	F
84	89	Fr3	G	A	A	A	A	A	A
85	90	Fr3	S	T	T	T	T	T	T
86	91	Fr3	Y	Y	Y	Y	Y	Y	Y
87	92	Fr3	Y	Y	Y	Y	Y	Y	Y
88	93	Fr3	C	C	C	C	C	C	C
89	94	CDR-L3	Q	Q	Q	Q	Q	Q	Q
90	95	CDR-L3	H	Q	Q	H	H	H	H
91	96	CDR-L3	F	S	S	F	F	F	F
92	97	CDR-L3	W	Y	Y	W	W	W	W
93	98	CDR-L3	G	S	S	G	G	G	G
94	99	CDR-L3	T	T	T	T	T	T	T
95	100	CDR-L3	P	P	P	P	P	P	P
95A		CDR-L3	P	-	-	P	P	P	P
95B		CDR-L3	-	-	-	-	-	-	-
95C		CDR-L3	-	-	-	-	-	-	-
95D		CDR-L3	-	-	-	-	-	-	-
95E		CDR-L3	-	-	-	-	-	-	-
95F		CDR-L3	-	-	-	-	-	-	-
96	101	CDR-L3	W	Y	Y	W	W	W	W
97	102	CDR-L3	T	T	T	T	T	T	T
98	103	Fr4	F	F	F	F	F	F	F

Kabat residue #	Linear residue #	FR or CDR	Mouse 11M14 VL (SEQ ID NO:58)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194)	IMGT# IGKV1-39*01 (SEQ ID NO:195)	Mouse 11M14 VH (SEQ ID NO:52)	Hu11M14VLv1b (SEQ ID NO:196)	Hu11M14VLv2b (SEQ ID NO:197)	Hu11M14VLv3b (SEQ ID NO:198)
99	104	Fr4	G	G	G	G	G	G	G
100	105	Fr4	G	Q	Q	Q	Q	Q	Q
101	106	Fr4	G	G	G	G	G	G	G
102	107	Fr4	T	T	T	T	T	T	T
103	108	Fr4	K	K	K	K	K	K	K
104	109	Fr4	L	L	L	L	L	L	L
105	110	Fr4	E	E	E	E	E	E	E
106	111	Fr4	I	I	I	I	I	I	I
106A		Fr4	-	-	-	-	-	-	-
107	112	Fr4	R	K	K	K	K	K	K

[0694] Table 28: V_H, V_L Backmutations and Other Mutations for Humanized 11M14		
V_H or V_L Variant	V_H or V_L Exon Acceptor Sequence	Changes from Acceptor Framework (or CDR) Residues (based on Kabat/Chothia Composite CDRs)
Hu11M14VHv1b (SEQ ID NO:190)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188) IMGT# IGHV3-48*03 (SEQ ID NO:189)	H49
Hu11M14VHv2b (SEQ ID NO:191)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188) IMGT# IGHV3-48*03 (SEQ ID NO:189)	H49, H82c
Hu11M14VHv3b (SEQ ID NO:192)	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188) IMGT# IGHV3-48*03 (SEQ ID NO:189)	H49, H80
Hu11M14VLv1b (SEQ ID NO:196)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194) IMGT# IGKV1-39*01 (SEQ ID NO:195)	L48, L71
Hu11M14VLv2b (SEQ ID NO:197)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194) IMGT# IGKV1-39*01 (SEQ ID NO:195)	L43, L48, L71, L76
Hu11M14VLv3b (SEQ ID NO:198)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194) IMGT# IGKV1-39*01 (SEQ ID NO:195)	L43, L48, L54, L71, L76
Hu11M14VLv4b (SEQ ID NO:199)	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194) IMGT# IGKV1-39*01 (SEQ ID NO:195)	L43, L48, L54, L71, L76

[0695] Table 29: Kabat Numbering of Framework (or CDR) Residues (based on Kabat/Chothia Composite CDRs) for Backmutations and Other Mutations in Heavy Chains of Humanized 11M14 Antibodies

Residue	GenBank Acc. # ACS96198-VH_huFrwk (SEQ ID NO:188)	IMGT# IGHV3-48*03 (SEQ ID NO:189)	Mouse 11M14 VH (SEQ ID NO:52)	Hu11M14VHv1b (SEQ ID NO:190)	Hu11M14VHv2b (SEQ ID NO:191)	Hu11M14VHv3b (SEQ ID NO:192)
H49	S	S	A	A	A	A
H80	L	L	L	L	L	G
H82c	L	L	L	L	G	L

[0696] Table 30: Kabat Numbering of Framework Residues (based on Kabat/Chothia Composite CDRs) for Backmutations and Other Mutations in Light Chains of Humanized 11M14 Antibodies

Residue	GenBank Acc. # CBZ39892-VL_huFrwk (SEQ ID NO:194)	IMGT# IGKV1-39*01 (SEQ ID NO:195)	Mouse 11M14 VL (SEQ ID NO:58)	Hu11M14VLv1b (SEQ ID NO:196)	Hu11M14VLv2b (SEQ ID NO:197)	Hu11M14VLv3b (SEQ ID NO:198)	Hu11M14VLv4b (SEQ ID NO:199)
L43	A	A	S	A	S	S	S
L48	I	I	V	V	V	V	V
L54	L	L	L	L	L	G	I
L71	F	F	Y	Y	Y	Y	Y
L76	N	S	N	N	S	S	S

[0697] Table 31
Percentage Humanness of Heavy and Light Chains of Humanized 11M14 Antibodies

V_H or V_L Variant	% Humanness
Hu11M14VHv1b (SEQ ID NO:190)	86.7%
Hu11M14VHv2b (SEQ ID NO:191)	85.7%
Hu11M14VHv3b (SEQ ID NO:192)	85.7%
Hu11M14VLv1b (SEQ ID NO:196)	83.2%
Hu11M14VLv2b (SEQ ID NO:197)	83.2%
Hu11M14VLv3b (SEQ ID NO:198)	82.1%
Hu11M14VLv4b (SEQ ID NO:199)	82.1%

[0698] Positions at which canonical, vernier, or interface residues differ between mouse and human acceptor sequences are candidates for substitution. Examples of canonical/CDR interacting residues include Kabat residue L71 in Table 27. Examples of vernier residues include Kabat residues H49 in Table 26 and L48 and L71 in Table 27.

[0699] The rationales for selection of the positions indicated in Table 26 in the heavy chain variable region as candidates for substitution are as follows.

[0700] heavy chain variable regions

[0701] *hu11M14-VH_v1b*

- consists of the CDR-H1, H2, and H3 loops of 11M14-VH grafted onto the framework of ACS96198 VH. And reverts all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or localize to the VH/VL domain interface or contribute to structural stability

[0702] *hu11M14-VH_v2b and hu11M14-VH_v3b*

- incorporates backmutations or substitution with most frequent residue at a given position. along with reverting all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or locate to the VH/VL domain interface, or reduce immunogenicity.

[0703] S49A: Backmutation of a vernier zone residue.

[0704] L80G: Leu at this position is immunogenic as indicated by IEDB analysis. A deimmunization analysis predicts immunogenicity reduction by Gly substitution. Gly substitution at positions 80 and 82c to mitigate immunogenicity are mutually exclusive and are tried in different VH versions.

[0705] L82cG: Leu at this position is immunogenic as indicated by IEDB analysis. A deimmunization analysis predicts immunogenicity reduction by Gly substitution.

[0706] The rationales for selection of the positions indicated in Table 27 in the light chain variable region as candidates for substitution are as follows.

[0707] *kappa light chain variable regions*

[0708] *hu11M14-VL_v1b*

- consists of the CDR-L1, L2, and L3 loops of 11M14-VL grafted onto the framework of CBZ39892 VL along with reverting all framework substitutions at positions that are key for defining the Chothia canonical classes, are part of the Vernier zone, or locate to the VH/VL domain interface.

[0709] *hu11M14-VL_v2b, hu11M14-VL_v3b, hu11M14-VL_v4b*

- include substitutions that contribute to structural stability or increases the humanness of the antibody or reduce immunogenicity.

[0710] A43S: Stability-enhancing mutation. Ser stabilizes the structure by making inter chain bonds with Tyr 91 and Gly 104 both in the heavy chain. This back mutation is made to maintain conformation and to keep the antibody structure stable.

[0711] I48V: Backmutation of a vernier zone residue.

[0712] L54G: Leu at this position is immunogenic as indicated by IEDB analysis; a deimmunization analysis predicts immunogenicity reduction by Gly substitution. Gly substitution at position 54 is predicted to mitigate immunogenicity.

[0713] L54I: Leu at this position is immunogenic as indicated by IEDB analysis; a deimmunization analysis predicts immunogenicity reduction by Ile substitution. Ile substitution at position 54 is predicted to mitigate immunogenicity.

[0714] F71Y: Backmutation of a canonical and vernier zone residue.

[0715] N76S: Germline-aligning mutation. Germline gene IGKV1-39*01 (SEQ ID NO:195) has Ser at this position.

[0716] The designs based on these human frameworks were:

[0717] Sequences of heavy chain variable regions

[0718] SEQ ID NO:190>h11M14VH version1b (86.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNIGMSWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARHPGGAMDYWGQGTLVTVSS

[0719] SEQ ID NO:191>h11M14VH version2b (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNIGMSWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRD
NAKNSLYLQMNSGRAEDTAVYYCARHPGGAMDYWGQGTLVTVSS

[0720] SEQ ID NO:192>h11M14VH version3b (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNIGMSWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRD
NAKNSLYGQMNSLRAEDTAVYYCARHPGGAMDYWGQGTLVTVSS

[0721] Sequences of kappa light chain variable regions

[0722] SEQ ID NO:196>h11M14VL version1b (83.2% human)
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLVYAATNLADGVPSRFSGSGSGTDYTL
TINSIQPEDFATYYCQHFWGTPPWTFGQGTKLEIK

[0723] SEQ ID NO:197>h11M14VL version2b (83.2% human)
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKSPKLLVYAATNLADGVPSRFSGSGSGTDYTL
TISSLPEDFATYYCQHFWGTPPWTFGQGTKLEIK

[0724] SEQ ID NO:198>h11M14VL version3b (82.1% human)
 DIQMTQSPSSLSASVGDRVITICRVSENIYSNLAWYQQKPGKSPKLLVYAATNGADGVPSRFSGSGSGTDYTL
 TISSLQPEDFATYYCQHFWGTPPWTFGQGTKLEIK

[0725] SEQ ID NO:199>h11M14VL version4b (82.1% human)
 DIQMTQSPSSLSASVGDRVITICRVSENIYSNLAWYQQKPGKSPKLLVYAATNIADGVPSRFSGSGSGTDYTL
 TISSLQPEDFATYYCQHFWGTPPWTFGQGTKLEIK

[0726] Example 17. Antibody epitope mapping by peptide microarray analysis

[0727] PEPperMAP Conformational epitope mapping was carried out by PEPperPrint GmbH. Epitope analysis of 11M14 and 8H24 was performed by peptide microarray analysis. The sequence of the extracellular domain of human sortilin (756 amino acids; SEQ ID NO:1) was elongated with neutral GSGSGSG (SEQ ID NO:207) linkers at the C- and N-terminus to avoid truncated peptides. The linked and elongated antigen sequence was translated into 7, 10 and 13 amino acid peptides with a peptide-peptide overlap of 6, 9 and 12 amino acids. After peptide synthesis, all peptides were cyclized via a thioether linkage between a C-terminal cysteine and an appropriately modified N-terminus. The resulting conformational peptide microarrays contained 2,283 different peptides printed in duplicate (4,566 peptide spots), and were framed by additional HA (YPYDVDPDYAG, SEQ ID NO:200; 70 spots) and c-Myc (EQKLISEEDL, SEQ ID NO:201; 70 spots) control peptides linear 15 amino acid peptides with a peptide-peptide overlap of 14 amino acids. The resulting human sortilin peptide microarrays contained 756 different peptides printed in duplicate (1,512 spots) and were framed by additional HA (YPYDVDPDYAG, SEQ ID NO:200; 46 spots) and c-Myc (EQKLISEEDL, SEQ ID NO:201; 46 spots) control peptides.

[0728] After synthesis, the microarray was blocked to prevent nonspecific binding (Rockland catalog #MB-070). Murine 11M14 or 8H24 was then applied to the microarray at concentrations ranging from 10 µg/mL to 100 µg/mL along with positive control mouse monoclonal anti-HA (12CA5) DyLight800 (0.5 µg/ml) for 16h at 4°C with shaking at 140 rpm. The microarray was washed, and secondary antibody (Goat anti-mouse IgG (H+L) DyLight680 (0.2 µg/ml) was applied for 45 minutes at room temperature. After further washing, the microarray was imaged using a Licor Odyssey Imaging System.

[0729] Quantification of spot intensities and peptide annotation were based on the 16-bit gray scale tiff files that exhibit a higher dynamic range than the 24-bit colorized tiff files. Microarray image analysis was done with PepSlide™. A software algorithm breaks down fluorescence intensities of each spot into raw, foreground and background signal, and calculates averaged median foreground intensities and spot-to-spot deviations of spot duplicates. Based on averaged median foreground intensities, intensity maps were generated and interactions in the peptide maps highlighted by an intensity color code with red for high and white for low spot intensities. We tolerated a maximum spot-to-spot deviation of 40%, otherwise the corresponding intensity value was zeroed.

[0730] Results

[0731] For 8H24, An antibody response against an epitope-like spot pattern formed by peptides with the consensus motif RTEFGMAIGP. (SEQ ID NO:213).

[0732] For 11M14, a very weak antibody response was observed against an epitope-like spot pattern formed by adjacent peptides with the consensus motif WGFTEFLTS (SEQ IDNO:214).

[0733] Example 18. Antibody epitope mapping via structural guided mutagenesis

[0734] Rationale:

[0735] Rough epitope of the 5E20 antibody was previously determined to be within amino acids 557-560 of SEQ ID NO:215 (ESFL; SEQ ID NO:203) using peptide arrays at PEPperPrint GmbH; see Example 10. Similarly, SORT1-Progranulin interaction experimental data indicated that Progranulin binding to Sortilin is blocked by 5E20 mAb (See Example 7). SORT1-PRGN interaction data for 8H24 and 11M24 antibodies showed that they also block binding of PGRN to sortilin (See Example 7). Therefore, it was reasoned that 8H24 and 11M14 must also bind to the SORT1 loops/domains projecting in the similar plane on 3D structure.

[0736] During the first round of mutagenesis multiple amino acid mutations in hSORT1 were made within the loops/domains that were structurally adjacent to the 5E20 epitope. Fourteen hSORT1 mutants with multiple amino acid mutations in targeted loop regions were generated during de novo DNA synthesis at Atum Bio (CRO) during the first round of mutagenesis (Table 32). Numbering of Loop Area and Mutations is based on sortilin ECD without the 33-amino acid signal peptide (SEQ ID NO:215). These mutants were subcloned into expression vector and

a Hisx8 tag was attached to the C-terminus of all mutants. Mutant proteins were generated by transient transfection of plasmids into HEK293 cells. Conditioned media was directly used for ELISA assay to measure antibody binding, wild type SORT1 protein was used a control.

[0737] Table 32: Sortilin antibody epitope mutagenesis round 1 mutants:

Mutant	SEQ ID NO:	Loop area - aa	Mutations
hSORT1_ECD_Emut1	216	70-76	H70A, D74A, L75A, R76A
hSORT1_ECD_Emut2a	217	97-104	F97A, H98A, V102A, M104A
hSORT1_ECD_Emut2b	218	106-112	F106A, Q108A, K110A, L112
hSORT1_ECD_Emut3	219	123-131	D123A, D126A, N130A, T131A
hSORT1_ECD_Emut4	220	155-163	V155A, S156A, S159A, R160A, R163
hSORT1_ECD_Emut5	221	176-182	Q176A, D178A, H182A
hSORT1_ECD_Emut6N	222	203-207 & 218-221	E203A, W207A, E218A, H220A, K221A
hSORT1_ECD_Emut8N	223	238-247, 247-250 & 285-289	Y238A, K244A, L247A, Q247A, E248A, F250A, M285A, D288A, D289A
hSORT1_ECD_Emut11N	224	333-338, 362-365 & 386-392	D333A, D337A, T338A, Y362A, T364A, T365A, L386A, E388A, N390A, Q392A
hSORT1_ECD_Emut14N	225	437-440 & 463-471	Q437A, L439A, N440A, D463A, T465A, V469A, D471A
hSORT1_ECD_Emut16	226	484-490	K484A, K483A, H490A
hSORT1_ECD_Emut17	227	505-512	E505A, S507A, R509A, N512A
hSORT1_ECD_Emut18	228	528-531	T528A, R531A
hSORT1_ECD_Emut19	229	557-560	E557A, S558A, F559A, L560A

[0738] Amino acid sequence of the human Sortilin extra-cellular domain mutants to determine anti-SORT1 antibody epitope – mutagenesis round 2:

[0739] hSORT1 ECD Loop domain mutants that showed reduced binding for any of the antibodies (5E20, 8H24 or 11M14) were considered for second round of mutagenesis to further refine the antibody epitope. Thirteen single amino acid mutants and one double mutant were generated during the epitope refinement round 2 (Table 33) Numbering of mutations is based on sortilin ECD without the 33-amino acid signal peptide (SEQ ID NO:215). Mutant binding to the antibodies was measured using ELISA assay and Biacore. Mutants that showed reduced or

abolished binding were considered to be constituting respective antibody. These mutants were mapped on hSORT1 3D model.

[0740] Table 33 SORT1 Epitope mutagenesis Round 2

Mutant	SEQ ID NO:	Mutations
hSORT1_ECD_Emut20	230	D74A
hSORT1_ECD_Emut21	231	R76A
hSORT1_ECD_Emut22	232	F97A
hSORT1_ECD_Emut23	233	K110
hSORT1_ECD_Emut24	234	L560A
hSORT1_ECD_Emut25	235	T561A
hSORT1_ECD_Emut26	236	Q563
hSORT1_ECD_Emut27	237	P510A
hSORT1_ECD_Emut28	238	P533A
hSORT1_ECD_Emut29	239	Y535A
hSORT1_ECD_Emut30	240	E557A
hSORT1_ECD_Emut31	241	F559A
hSORT1_ECD_Emut32	243	E558
hSORT1_ECD_Emut33	243	F137 & 141

[0741] Results:

[0742] After two rounds of mutagenesis and structural analysis, epitopes for 8H24 and 11M14 were mapped to the amino acids listed below. Additionally, mutagenesis allowed further refinement of the epitope for 5E20, also listed below. Numbering of residues in epitopes below are based on sortilin ECD without the 33-amino acid signal peptide (SEQ ID NO:215).

[0743] 8H24 epitope: D74, R76, F97, K110, Y535, L560, and E557

11M14 epitope: K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560

5E20 epitope (refined): E557, S558, F559, L560, P510, and Y535

[0744] Example 19. Fc LALA and YTE mutant generation

[0745] The Fc region of human IgG antibodies (hIgGs) interacts with multiple Fcγ receptor (FcγR) and complement proteins and mediates immune effector functions, which are important

for many therapeutic applications, e.g. elimination of targeted cells via antibody-dependent cellular-cytotoxicity (ADCC), -phagocytosis (ADCP) or complement-dependent cytotoxicity (CDC).

[0746] To develop antibodies with reduced immune effector functions, different Fc mutations were employed. The so-called 'LALA' double mutation (Leu234Ala together with Leu235Ala) was first described as a molecule with diminished effector functions (Lund, J., et al (1992) *Mol. Immunol.*, 29,53-59; ; Tamm and Schmidt, 1997, *Int Rev Immunol* 16(1-2):57-85). Fc variant L234A/L235A (LALA) (numbering according to EU nomenclature) mutant has been shown to have either eliminated or reduced interaction with FcγRs (FcγRIa, FcγRIIa, FcγRIIa and FcγRIIIa) and exhibit significantly reduced effector function. Additionally, Fc LALA mutants have also been shown to have diminished interaction with complement proteins resulting in reduced complement-dependent cytotoxicity (CDC).

[0747] hu11M14 H1b heavy chain (SEQ ID NO:250), hu8H24 H1 heavy chain (SEQ ID NO:251), and hu5E20 H7 heavy chain (SEQ ID NO:252) incorporate LALA mutations in the human IgG1 heavy chain constant region.

[0748] Table 34: Antibodies with LALA mutations

Name	SEQ ID NO of heavy chain :	SEQ ID NO of light chain
hu11M14 H1bL3b_ IgG1 LALA_	250	245
hu8H24 H1L2_ IgG1 LALA_	251	247
hu5E20 H7L4_ IgG1 LALA_	252	249

[0749] Additionally, to engineer these antibodies to have improved pharmacokinetics,YTE (M252T/S254T/T256E) (numbering according to EU nomenclature) mutations in the Fc region (W.F. Dall'Acqua *et al.* 2006 *J. Biol. Chem.* 281:23514-23) were incorporated. YTE mutant has been shown to have enhanced binding/interaction to the neonatal Fc receptor (FcRn); thereby leading to antibody half-life extension in circulation. YTE mutations were generated simultaneously along with LALA mutations during de novo DNA synthesis at Atum CRO.

Modified Fc region was subcloned into an expression vector placing modified Fc region 3' to the variable heavy domain to express full length heavy chain incorporating LALA and YTE mutations.

[0750] hu11M14 H1b heavy chain (SEQ ID NO:244), hu8H24 H1 heavy chain (SEQ ID NO:246), and hu5E20 H7 heavy chain (SEQ ID NO:248) incorporate LALA and YTE mutations in the human IgG1 heavy chain constant region.

[0751] Table 35: Antibodies with LALA/YTE mutations

Name	SEQ ID NO of heavy chain :	SEQ ID NO of light chain
hu11M14 H1bL3b_IgG1 LALA_YTE	244	245
hu8H24 H1L2_IgG1 LALA_YTE	246	247
hu5E20 H7L4_IgG1 LALA_YTE	248	249

[0752] **Example 21. Multiple dose pharmacokinetic and pharmacodynamic studies in non-human Primates**

[0753] “Multiple dose pharmacokinetic and pharmacodynamic studies were conducted in non-human Primates. Cynomolgus monkey (*Macaca fascicularis*) [Males: 36-42 months of age, weight 2.6-3.1 kg; Females: 32-47 months of age, weight 2.1-3.2 kg] were administered test compound according to the protocol below. Cynomolgus monkeys were treated with hu8H24 H1L2_IgG1_LALA, hu5E20 H7L4_IgG1_LALA, and hu11M14 H1bL3b_IgG1 LALA at doses of 30 mg/kg and 60 mg/kg.

[0754] **A. Multiple Dose Studies: Dosing**

[0755] Table 36: Dosing in Multiple dose pharmacokinetic and pharmacodynamic studies

Group No.	Test Material	Day of Dosing	Dose Level (mg/kg)	Dose Volume (mL/kg) ^a	Dose Concentration (mg/mL)	No. of Females
1	hu8H24 H1L2_IgG1_LALA heavy chain SEQ ID NO:252 light chain SEQ ID NO:247	1	30	6	5	3
		50	60	10	6	
2	hu5E20 H7L4_IgG1_LALA heavy chain SEQ ID NO:252 light chain SEQ ID NO:249	1	30	6	5	3
		50	60	10	6	
3	hu11M14 H1bL3b_IgG1 LALA heavy chain SEQ ID NO:250 light chain SEQ ID NO:245	1	30	6	5	3
		50	60	10	6	

^a Based on the most recent body weight measurement.**[0756]** Dose Route: Intravenous (slow Bolus) injection

Frequency & Duration: Once on Day 1 and Day 50

[0757] Method: The first day of dosing was designated as Day 1. The animals were temporarily restrained for dose administration and were not sedated. The test articles were administered to the appropriate animals via a suitable peripheral vein. The dose volume for each animal was based on the most recent body weight measurement.

[0758] B. Multiple Dose Studies: Pharmacokinetic Evaluation

[0759] Blood and CSF were drawn from the animals at multiple time-points after dosing (see Tables 37-38) to obtain anti-Sortilin antibody concentrations in plasma and cerebrospinal fluid (CSF), which are measurements of anti-Sortilin antibody pharmacokinetics. Sample collection was as in Table 37 and methods, target volum, and anticoagulant as in Table 38.

[0760] Table 37: Bioanalytical sample collection for Multiple Dose Studies,
Pharmacokinetics

Sample Collection Time Points		
Group Nos.	Study Day	Time Points (Relative to Dosing)
1 to 3	Day 1	Pre; Day 1, 0.25 and 8 hr post
	Day 2	Day 1, 24 hr post
	Day 4	Day 1, 72 hr post
	Day 8	Day 1, 168 hr post
	Day 15	Day 1, 336 hr post
	Day 29	Day 1, 672 hr post
	Day 50	Pre; Day 50, 0.25 and 8 hr post
	Day 51	Day 50, 24 hr post
	Day 53	Day 50, 72 hr post
	Day 57	Day 50, 168 hr post
	Day 64	Day 50, 336 hr post
	Day 78	Day 50, 672 hr post

[0761] Table 38: Methods, Target Volume, Anticoagulant for Multiple Dose Studies,
Pharmacokinetics

Method/Comments:	Venipuncture
Target Volume (mL)	0.5
Anticoagulant:	K3EDTA

[0762] 1. Pharmacokinetic Analysis, Multiple Dose Studies

[0763] Anti-Sortilin antibody concentrations were assayed using a plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 96-well MSD plates were coated with 2ug/mL human Sortilin protein (R&D Biosystems) in PBS overnight at 4°C. plates were blocked in 3% MSD buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 50 uL of appropriately diluted CSF or Plasma was added to

the wells and incubated at room temperature for 2 hours. Plates were then washed as above and 50uL of 1ug/mL goat anti-human IgG-ST (Bethyl labs) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1 hour at room temperature and then washed as above. 140uL 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and antibody levels were quantitated against a standard curve using 4-fold serial dilutions starting at 0.5 ug/mL of each Anti-sortilin antibody used in the study.

[0764] 2. Results from Pharmacokinetic Analysis, Multiple Dose Studies

Rapid antibody clearance was observed with all three antibodies, hu11M14 H1bL3b_IgG1 LALA, hu8H24 H1L2_IgG1_LALA, and hu5E20 H7L4_IgG1_LALA, with a single dose of 30mg/kg. 60mg/kg only slightly increased exposure. (Table 39 and Figure 14)

[0765] Table 39

Plasma Anti-Sortilin Antibody Levels in Cynomolgus Monkeys

Time (hr)	8H24-H1L2 (mg/ml)		5E20-H7L4 (mg/ml)		11M14-H1bL3b (mg/ml)	
	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg
0.25	619 ± 84	ND	1348 ± 156	ND	921 ± 69	ND
8	647 ± 42	ND	545 ± 72	ND	615 ± 110	ND
24	631 ± 120	ND	351 ± 23	ND	401 ± 107	ND
72	251 ± 39	ND	162 ± 21	ND	274 ± 59	ND
168	0.01 ± 0.01	19 ± 33	44 ± 19	186 ± 27	213 ± 33	182 ± 160
336	BLQ	BLQ	BLQ	4.7 ± 1.5	1.7 ± 1.5	30 ± 47
672	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ

Data represents Mean ± SD, n = 3/group

ND = No Data

BLQ = Below limit of quantification

[0766] C. Multiple Dose Studies: Pharmacodynamic Evaluation

[0767] Progranulin (PGRN) levels, which are a measurement of pharmacodynamics, were also determined from Plasma and cerebrospinal fluid (CSF). Sample collection was as in Table 40 and methods, target volum, and anticoagulant as in Table 41.

**[0768] 1. Pharmacodynamic Sample collection for Multiple Dose Studies,
Plasma**

[0769] Table 40 Pharmacodynamic Sample collection for Multiple Dose Studies, Plasma

Sample Collection Time Points		
Group Nos.	Study Day/Week	Time Points (Relative to Dosing)
All animals	Week -1	-
1 to 3	Day 8	Day 1, 168 hr post
	Day 15	Day 1, 336 hr post
	Day 29	Day 1, 672 hr post
	Day 48	-
	Day 57	Day 50, 168 hr post
	Day 64	Day 50, 336 hr post
	Day 78	Day 50, 672 hr post

[0770] Table 41: Methods, Target Volume, Anticoagulant for Multiple Dose Studies, Pharmacodynamics, Plasma

Method/Comments:	Venipuncture
Target Volume (mL):	Week -1 to Day 29: 0.5 mL Day 48 to Day 78: 1.0 mL
Anticoagulant:	K3EDTA

[0771] 2. Pharmacodynamic Sample collection for Multiple Dose Studies, CSF

Timepoints: All animals: Week -1

Groups 1 to 3: Days 15, 29, 48, 57, 64, and 78

Target volume: Approximately 0.5 mL

Procedure The animals were given Buprenorphine SR (0.20mg/kg) SQ SID and Meloxicam SR (0.60mg/kg) SQ SID pre-procedure.

Animals were pre-anesthetized with Ketamine HCl (10-15 mg/kg) IM. Dexdomitor 0.06 mg/kg IM was administered for additional anesthesia.

[0772] 3. Pharmacodynamic Sample Analysis Multiple Dose Studies

[0773] Progranulin levels were measured by plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 384-well MSD plates were coated with 1ug/mL goat anti-huPGRN (R&D Biosystems) in PBS overnight at 4°C. plates were blocked in 3% MSD

buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 20uL of appropriately diluted CSF or Plasma was added to the wells and incubated at room temperature for 2 hours. Plates were then washed as above and 20uL of 1ug/mL biotinylated goat anti-huPGRN (R&D Biosystems) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1 hour at room temperature. Plates were washed as above and 05.ug/mL SULFO-TAG-Streptavidin (MSD) in 1% MSD buffer A in PBS was added the wells and plates were incubated for 30min to 1 hour at room temperature and then washed as above. 40uL 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and were quantitated against a standard curve using 3-fold serial dilutions starting at 200ng/mL of recombinant huPGRN (R&D Biosystems). Fold PGRN levels over controls were calculated using Microsoft Excel.

[0774] 4. Results from Pharmacodynamic Analysis Multiple Dose Studies

For all three antibodies, hu11M14 H1bL3b_IgG1 LALA, hu8H24 H1L2_IgG1_LALA, and hu5E20 H7L4_IgG1_LALA, the levels of PGRN in the plasma (Table 42 and Figure 15) increased in a dose dependent manner and showed a greater than 2 fold increase in PGRN levels with the 60mg/kg dose at Day7 post dose. PGRN levels in the CSF (Table 43 and Figure 16) show a ≥ 1.5 fold increase on day 7 with the 60mg/kg dose.

[0775] Table 42

Change in Plasma Progranulin from Pre-dose Baseline in Cynomolgus Monkeys

	8H24-H1L2		5E20-H7L4		11M14-H1bL3b	
	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg
Day 7	1.26 $\pm$ 0.19	2.43 $\pm$ 0.75	1.57 $\pm$ 0.14	2.43 $\pm$ 0.32	1.70 $\pm$ 0.10	2.10 $\pm$ 0.46
Day 14	0.78 $\pm$ 0.06	1.37 $\pm$ 0.25	0.75 $\pm$ 0.11	2.07 $\pm$ 0.32	1.26 $\pm$ 0.35	1.30 $\pm$ 0.44
Day 28	0.71 $\pm$ 0.10	1.23 $\pm$ 0.25	0.60 $\pm$ 0.03	1.17 $\pm$ 0.25	0.77 $\pm$ 0.10	0.87 $\pm$ 0.15

Data represents Mean $\pm$ SD fold change over pre-dose baseline levels of progranulin, n = 3/group

[0776] Table 43

Change in CSF Progranulin from Pre-dose Baseline in Cynomolgus Monkeys

	8H24-H1L2		5E20-H7L4		11M14-H1bL3b	
	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg	30 mg/kg	60 mg/kg

Day 7	ND	1.30 ± 0.40	ND	1.10 ± 0.40	ND	1.63 ± 0.29
Day 14	0.94 ± 0.02	1.10 ± 0.26	0.91 ± 0.02	0.80 ± 0.20	1.24 ± 0.22	1.23 ± 0.31
Day 28	0.81 ± 0.03	1.13 ± 0.35	0.83 ± 0.07	0.80 ± 0.26	1.00 ± 0.12	1.00 ± 0.26

Data represents Mean ± SD fold change over pre-dose baseline levels of progranulin, n = 3/group

ND = No Data

[0777] Example 22. Repeat dose pharmacokinetic and pharmacodynamic studies in non-human Primates

[0778] Repeat dose pharmacokinetic and pharmacodynamic studies were conducted in non-human Primates. Cynomolgus monkey (*Macaca fascicularis*) [Males: 36-42 months of age, weight 2.6-3.1 kg; Females: 32-47 months of age, weight 2.1-3.2 kg] were administered test compound according to the protocol below. Cynomolgus monkeys were treated with hu11M14 H1bL3b_ IgG1 LALA or hu11M14 H1bL3b_ IgG1 LALA_YTE at 4 weekly repeat doses of 60 mg/kg.

[0779] A. Repeat Dose Studies: Dosing

[0780] Table 44 Repeat dose pharmacokinetic and pharmacodynamic studies

Group No.	Test Material	Dose Level (mg/kg) ^a	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No. of Animals	
					Males	Females
1	hu11M14H1bL3b IgG1 LALA heavy chain SEQ ID NO:250 light chain SEQ ID NO:245	60	10	6	2	2
2	hu11M14H1bL3b IgG1 LALA YTE heavy chain SEQ ID NO:244 light chain SEQ ID NO:245	60	10	6	2	2

TBD = to be determined.

^a Based on the most recent body weight measurement.**[0781] B. Pharmacokinetic Evaluation, Repeat Dose Studies**

[0782] Blood and CSF were drawn from the animals at multiple time-points after dosing (see Tables 45-46) to obtain anti-Sortilin antibody concentrations in plasma and cerebrospinal fluid (CSF), which are measurements of anti-Sortilin antibody pharmacokinetics

[0783] 1. Pharmacokinetic Sample collection for Repeat Dose Studies, Plasma

[0784] Table 45: Bioanalytical sample collection for Repeat Dose Studies, Pharmacokinetics

Sample Collection Time Points		
Group Nos.	Study Day/Week	Time Points (Relative to Dosing)
All animals	Day 1	Day 1: pre
1 and 2	Day 1	Day 1: 0.25 and 8 hr post
	Day 2	Day 1: 24 hr post
	Day 4	Day 1: 72 hr post
	Day 6	Day 1: 120 hr post
	Day 8	Day 1: 168 hr post (prior to dosing on Day 8)
	Day 9	Day 8: 24 hr post
	Day 11	Day 8: 72 hr post
	Day 13	Day 8: 120 hr post
	Day 15	Day 8: 168 hr post (prior to dosing on Day 15)
	Day 16	Day 15: 24 hr post
	Day 18	Day 15: 72 hr post
	Day 20	Day 15: 120 hr post
	Day 22	Day 15: 168 hr post (prior to dosing on Day 22)
	Day 22	Day 22: 0.25 and 8 hr post
	Day 23	Day 22: 24 hr post
	Day 25	Day 22: 72 hr post
	Day 27	Day 22: 120 hr post
	Day 29	Day 22: 168 hr post
	Day 32	Day 22: 240 hr post
	Day 36	Day 22: 336 hr post

[0785] Table 46; Methods, Target Volume, Anticoagulant for Repeat Dose Studies, Pharmacokinetics

Method/Comments:	Venipuncture
Target Volume (mL)a:	0.5
Anticoagulant:	K3EDTA
X = sample to be collected; - = not applicable; hr= hour; min = minute. a Unless otherwise stated, additional collection of protocol-specified blood samples are permitted (e.g., due to unacceptable sample quality) since the total blood collected is below the Testing Facility IACUC recommended maximum blood volume limit.	

[0786] 2. Pharmacokinetic Analysis, Repeat Dose Studies

[0787] Anti-Sortilin antibody (hu11M14 H1bL3b_ IgG1 LALA and hu11M14 H1bL3b_ IgG1 LALA_YTE) concentrations were assayed using a plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 96-well MSD plates were coated with 2ug/mL human Sortilin protein (R&D Biosystems) in PBS overnight at 4°C. plates were blocked in 3% MSD buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 50 uL of appropriately diluted CSF or Plasma was added to the wells and incubated at room temperature for 2 hours. Plates were then washed as above and 50uL of 1ug/mL goat anti-human IgG-ST (Bethyl labs) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1 hour at room temperature and then washed as above. 140uL 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and antibody levels were quantitated against a standard curve using 4-fold serial dilutions starting at 0.5 ug/mL of each Anti-sortilin antibody used in the study.

[0788] Table 47 and Figure 17 show hu11M14 H1bL3b_ IgG1 LALA and hu11M14 H1bL3b_ IgG1 LALA_YTE levels in plasma with 4 weekly repeat doses of 60 mg/kg of hu11M14 H1bL3b_ IgG1 LALA and hu11M14 H1bL3b_ IgG1 LALA_YTE.

[0789] Table 47

Plasma Anti-Sortilin Antibody Levels Following 4 Weekly Doses in Cynomolgus Monkeys

	11M14-H1bL3b LALA	11M14-H1bL3b LALA YTE
Time (hr)	Antibody [mg/ml]	Antibody [mg/ml]
0.25	1992 ± 145	1609 ± 277
8	1552 ± 233	1265 ± 113
24	989 ± 164	878 ± 167
72	611 ± 76	571 ± 44
120	506 ± 139	416 ± 34
168	354 ± 63	472 ± 42
192	1437 ± 285	1350 ± 152
240	1056 ± 152	1211 ± 23
288	807 ± 115	774 ± 113
336	519 ± 78	953 ± 159
360	1819 ± 412	2054 ± 146
408	1287 ± 75	1578 ± 144
456	990 ± 152	1137 ± 148
504	973 ± 296	1178 ± 267
504.25	2540 ± 313	2974 ± 448
512	2339 ± 187	2364 ± 602
528	1730 ± 261	1943 ± 113
576	1167 ± 294	1794 ± 413
624	1623 ± 1107	1277 ± 115
672	1687 ± 1324	1666 ± 461
744	589 ± 501	789 ± 125
840	370 ± 457	663 ± 68

Data represents Mean ± SD, n = 4/group

Animals were dosed with 60 mg/kg antibody at 0.25, 168, 336, and 504 hrs

[0790] Table 48 includes the plasma mean C_{max} , mean $AUC^{0-168hr}$ after the first and fourth dose and total $AUC^{0-840hr}$ for each of the antibodies tested, hu11M14 H1bL3b_IgG1 LALA and hu11M14 H1bL3b_IgG1 LALA_YTE. N=4 for each Ab.

[0791] Table 48

	hu11M14 H1bL3b_IgG1 LALA	hu11M14 H1bL3b_ IgG1 LALA_YTE.
Day 1 : AUC⁰⁻¹⁶⁸ ug*h/mL	119,618	108,027
Day 22: AUC⁰⁻¹⁶⁸ ug*h/mL	267,353	289,150
Total AUC^{0-840hr}	877,775	972,313
Day 1: C_{max} ug/mL	1,922	1,609
Day 22: C_{max} ug/mL	2,540	2,974

[0792] Table 49 and Figure 18 show hu11M14 H1bL3b_IgG1 LALA and hu11M14 H1bL3b_IgG1 LALA_YTE CSF levels with 4 weekly repeat doses of 60mg/kg of hu11M14 H1bL3b_IgG1 LALA or hu11M14 H1bL3b_IgG1 LALA_YTE.

[0793] Table 49

CSF Anti-Sortilin Antibody Levels Following 4 Weekly Doses
in Cynomolgus Monkeys

	11M14-H1bL3b LALA	11M14- H1bL3b LALA YTE
Time (hr)	Antibody [mg/ml]	Antibody [mg/ml]
24	0.11 ± 0.06	0.43 ± 0.58
72	0.10 ± 0.04	0.18 ± 0.16
120	0.07 ± 0.03	0.14 ± 0.05
168	0.13 ± 0.12	0.12 ± 0.08
192	0.47 ± 0.36	0.26 ± 0.14
240	0.24 ± 0.16	0.37 ± 0.27
288	0.15 ± 0.10	0.43 ± 0.38
336	0.24 ± 0.25	0.20 ± 0.22
360	0.80 ± 0.99	0.85 ± 1.03
408	0.43 ± 0.28	0.54 ± 0.47
456	0.18 ± 0.13	0.52 ± 0.57
504	0.18 ± 0.12	0.49 ± 0.58
528	0.23 ± 0.13	0.75 ± 0.84
576	0.26 ± 0.19	0.57 ± 0.66
624	0.11 ± 0.06	0.59 ± 0.72
672	0.10 ± 0.10	1.16 ± 1.56
744	0.13 ± 0.17	0.83 ± 1.20
840	0.10 ± 0.15	1.16 ± 1.81

Data represents Mean ± SD, n = 3-4/group

Animals were dosed with 60 mg/kg antibody at 0.25, 168, 336,
and 504 hrs

[0794] 3. Results from Pharmacokinetic Analysis, Repeat Dose Studies

Results: Repeat dose with either hu11M14 H1bL3b_ IgG1 LALA or hu11M14 H1bL3b_ IgG1 LALA_YTE showed increased antibody exposure in the plasma and CSF. hu11M14 H1bL3b_ IgG1 LALA_YTE variant showed greater accumulation of antibody over time and a slightly more sustained response.

[0795] C. Repeat Dose Studies: Pharmacodynamic Evaluation

[0796] Progranulin (PGRN) levels, which is a measurement of pharmacodynamics, were also Determined from Plasma and CSF. Samples were collected as in Table 50.

[0797] 1. Pharmacodynamic Sample collection for Repeat Dose Studies, Plasma and CSF

[0798] Table 50 Bioanalytical sample collection for Repeat Dose Studies,
Pharmacodynamics, Plasma & CSF

Sample Collection Time Points		
Group Nos.	Study Day/Week	Time Points (Relative to Dosing
All animals	Day -4	-
All animals	Day -2	-
1 and 2	Day 2	Day 1: 24 hr post
	Day 4	Day 1: 72 hr post
	Day 6	Day 1: 120 hr post
	Day 8	Day 1: 168 hr post (prior to dosing on Day 8)
	Day 9	Day 8: 24 hr post
	Day 11	Day 8: 72 hr post
	Day 13	Day 8: 120 hr post
	Day 15	Day 8: 168 hr post (prior to dosing on Day 15)
	Day 16	Day 15: 24 hr post
	Day 18	Day 15: 72 hr post
	Day 20	Day 15: 120 hr post
	Day 22	Day 15: 168 hr post (prior to dosing on Day 22)
	Day 23	Day 22: 24 hr post
	Day 25	Day 22: 72 hr post
	Day 27	Day 22: 120 hr post
	Day 29	Day 22: 168 hr post
	Day 32	Day 22: 240 hr post
	Day 36	Day 22: 336 hr post

[0799] 2. Pharmacodynamic Sample Analysis Repeat Dose Studies, CSF & Plasma PGRN Levels

[0800] Progranulin levels were measured by plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 384-well MSD plates were coated with 1ug/mL goat anti-huPGRN (R&D Biosystems) in PBS overnight at 4°C. plates were blocked in 3% MSD buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 20uL of appropriately diluted CSF or Plasma was added to the wells and incubated at room temperature for 2 hours. Plates were then washed as above and 20uL of 1ug/mL biotinylated goat anti-huPGRN (R&D Biosystems) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1 hour at room temperature. Plates were washed as above and 05.ug/mL SULFO-TAG-Streptavidin (MSD) in 1% MSD buffer A in PBS was added the wells and plates were incubated for 30min to 1 hour at room temperature and then washed as above. 40uL 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and were quantitated against a standard curve using 3-fold serial dilutions starting at 200ng/mL of recombinant huPGRN (R&D Biosystems). Fold PGRN levels over controls were calculated using Microsoft Excel.

[0801] 3. Results from Pharmacodynamics Analysis for Plasma and CSF Progranulin, Repeat Dose Studies:

Plasma PGRN levels increase over 2-fold that of baseline in plasma (Table 51 and Figure 19) in animals treated with either hu11M14 H1bL3b_ IgG1 LALA or hu11M14 H1bL3b_ IgG1 LALA_YTE. Both Antibody groups (hu11M14 H1bL3b_ IgG1 LALA or hu11M14 H1bL3b_ IgG1 LALA_YTE) show a sustained PGRN levels greater than baseline two weeks after final dose. Overall Plasma PGRN levels were higher with hu11M14 H1bL3b_ IgG1 LALA_YTE. As seen in Table 52 and Figure 20, CSF PGRN levels show a ≥ 1.5 fold increase which do not drop to baseline levels 2 weeks after the final dose.

[0802] Table 51
**Changes in Plasma Progranulin Levels Following 4 Weekly
Doses of Anti-Sortilin Antibody in Cynomolgus Monkeys**

	11M14-H1bL3b LALA	11M14- H1bL3b LALA YTE
Time (Days)	Fold Change Over Pre- Dose	Fold Change Over Pre- Dose
1	1.54 ± 0.09	1.76 ± 0.30
3	1.77 ± 0.24	1.92 ± 0.23
5	1.89 ± 0.40	2.12 ± 0.35
7	1.86 ± 0.45	2.19 ± 0.36
8	1.80 ± 0.41	2.27 ± 0.40
10	1.96 ± 0.54	2.28 ± 0.28
12	1.91 ± 0.53	2.10 ± 0.19
14	1.83 ± 0.50	2.06 ± 0.25
15	1.65 ± 0.36	2.12 ± 0.22
17	1.74 ± 0.23	2.10 ± 0.26
19	1.70 ± 0.43	1.92 ± 0.15
21	1.69 ± 0.37	2.07 ± 0.26
22	1.61 ± 0.22	2.15 ± 0.18
24	1.66 ± 0.27	2.16 ± 0.17
26	1.57 ± 0.31	1.92 ± 0.17
28	1.58 ± 0.24	2.04 ± 0.28
31	1.53 ± 0.23	1.95 ± 0.31
35	1.56 ± 0.24	1.79 ± 0.20

Data represents Mean ± SD, n =

4/group

Animals were dosed with 60 mg/kg antibody on days 0, 7, 14,
and 21

[0803] Table 52
**Changes in CSF Progranulin Levels Following 4 Weekly
 Doses of Anti-Sortilin Antibody in Cynomolgus Monkeys**

	11M14-H1bL3b LALA	11M14- H1bL3b LALA YTE
Time (Days)	Fold Change Over Pre- Dose	Fold Change Over Pre- Dose
1	1.13 ± 0.25	1.40 ± 0.20
3	1.20 ± 0.35	1.30 ± 0.26
5	1.70 ± 0.44	1.37 ± 0.57
7	1.20 ± 0.20	1.23 ± 0.38
8	1.43 ± 0.31	1.50 ± 0.72
10	1.70 ± 0.62	1.37 ± 0.35
12	1.90 ± 0.78	1.70 ± 0.36
14	1.90 ± 1.21	1.33 ± 0.25
15	2.07 ± 1.50	1.30 ± 0.17
17	1.90 ± 1.22	1.43 ± 0.25
19	1.83 ± 0.87	1.43 ± 0.46
21	1.73 ± 0.75	1.33 ± 0.25
22	1.87 ± 0.76	1.30 ± 0.35
24	1.53 ± 0.67	1.07 ± 0.21
26	2.20 ± 0.62	1.57 ± 0.49
28	1.53 ± 0.59	1.27 ± 0.06
31	1.23 ± 0.51	1.23 ± 0.12
35	1.23 ± 0.42	1.20 ± 0.28

Data represents Mean ± SD, n =

3/group

Animals were dosed with 60 mg/kg antibody on days 0, 7, 14,
 and 21

[0804] 4. Pharmacodynamic Sample Analysis Repeat Dose Studies, Sortilin
 Levels in White Blood Cells

[0805] Peripheral Blood Mononuclear cells (PBMCs) isolated from whole blood were lysed in cell lysis buffer. Sortilin levels were measured by plate-based electrochemiluminescence using Meso Scale Discovery (MSD). Standard binding 384-well MSD plates were coated with 1 µg/mL goat anti-huSortilin (R&D Biosystems) in PBS overnight at 4°C. plates were blocked in 3% MSD buffer A in PBS for 1 hour at room temperature. Plates were then washed 3 times in TBST. 20 µL diluted lysates were added to the wells and incubated at room temperature for 2 hours. Plates were then washed as above and 20 µL of 1 µg/mL biotinylated goat anti-huSortilin (R&D Biosystems) in 1% MSD buffer A in PBS was added to the wells and plates were incubated for 1

hour at room temperature. Plates were washed as above and 0.5ug/mL SULFO-TAG-Streptavidin (MSD) in 1% MSD buffer A in PBS was added the wells and plates were incubated for 30min to 1 hour at room temperature and then washed as above. 40uL 2x MSD read buffer T was added to the wells and the electrochemiluminescence signal was read using the Meso Sector S 600. Data was analyzed using the Discovery workbench software (MSD) and were quantitated against a standard curve using 3-fold serial dilutions starting at 100ng/mL of recombinant huSortilin.

[0806] 5. Results from Pharmacodynamics Analysis for Sortilin Levels in White Blood Cells

[0807] As shown in Table 53 and Figure 21, sortilin protein levels in PBMCs decrease after treatment with both antibodies, hu11M14 H1bL3b_IgG1 LALA and hu11M14 H1bL3b_IgG1 LALA_YTE.

[0808] Table 53

Changes in Sortilin Levels in PBMCs from Cynomolgus Monkeys Treated with Anti-Sortilin Antibodies

	11M14-H1bL3b LALA	11M14-H1bL3b LALA YTE
Time (Days)	% Change from Pre-Dose	% Change from Pre-Dose
1	82 ± 13	117 ± 51
7	25 ± 9	51 ± 28
8	21 ± 6	36 ± 20
14	22 ± 11	32 ± 8
15	22 ± 6	30 ± 5
21	23 ± 12	41 ± 20
22	26 ± 8	36 ± 22
28	13 ± 6	23 ± 5
31	28 ± 4	33 ± 20
35	22 ± 10	25 ± 8

Data represents Mean ± SD, n = 4/group

Animals were dosed with 60 mg/kg antibody on days 0, 7, 14, and 21

Sortilin levels were normalized to total protein and expressed as a percent of pre-dose baseline values

[0809] 6. Overall Results from Repeat Dose Studies:

[0810] Taken together, *in vivo* non-human primate results show a sustained response. Weekly 60mg.kg doses increase antibody exposure and lead to increased PGRN levels over baseline. A roughly 75% sustained decrease in Sortilin levels is observed in PMBCs beginning after the first

dose. hu11M14 H1bL3b_ IgG1 LALA_YTE variant shows slightly better PK and Plasma PD profile over hu11M14 H1bL3b_ IgG1 LALA , potentially due to Ab half-life extension properties of hu11M14 H1bL3b_ IgG1 LALA_YTE variant.

[0811] Listing of Sequences

[0812] SEQ ID NO:1> Amino acid sequence of human sortilin extracellular domain
 MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWG
 LRAAAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRGVSLSW
 VGDSTGVILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPE
 NSGKVVLTAEVSGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTE
 NGLWVSKNFGGKWEIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLG
 KSFKTIGVKIYSFGLGGRFLFASVMADKDTTRRIHVSTDQGDWWSMAQLPSVGQEQFYS
 ILAANDDMVFMHVDEPGDTGFGTIFTSDDRGIYVSKSLDRHLYTTTGGETDFTNVTSLR
 GVIYITSVLSEDNSIQTMITFDQGGRWTHLRKPENSECDATAKNKNECSLHIHASYSISQK
 LNVPMAPLSEPNVAVGIVIAHGSVGDASVMVPDVYISDDGGYSWTKMLEGPHYTYTILDS
 GGIIVAEIHSSRPINVIKVFSTDEGQCWQTYTFTTRDPIYFTGLASEPGARSMNISIWGFTEFSL
 TSQWVSYTIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILGYKEQFLRLRKSSVCQN
 GRDYVVTQKPSICLCSLEDFLCDFGYRPENDSKCVEQPELKGHDLEFCLYGREEHLTT
 NGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNS

[0813] SEQ ID NO:2> Murine 5E20VH nucleotide sequence (mIgG1):
 ATGAACCTCGGGCTCAGCTTGATTTTCCTTGCCCTATTTTAAAAGGTGTCCAGTGTG
 AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
 CTCTCCTGTGCAGCCCCTGGATTCACTTTCAGTACTTATGGCATGTCTTGGGTTTCGCC
 AGACTCCAGACAAGAGGCTGGAGTGGGTCGCAATCATTAGTAGTGGTGGTAGTTAC
 ACCTACTATTACAGACACTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAA
 GAACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATT
 ATTGTTCAAGGAGTAGTAGCCACTGGTATTTCGATGTCTGGGGCACAGGGACCACGG
 TCACCGTCTCCTCA

[0814] SEQ ID NO:3 > amino acid sequence for signal peptide for murine 5E20VH
 MNFGLSLIFLALILKGVQC

[0815] SEQ ID NO: 4 > Murine >5E20VH amino acid sequence
 EVQLVESGGDLVKPGGSLKLSAAPGFTFSTYGMSWVRQTPDKRLEWVAIISGGSYTY
 YSDTVKGRFTISRDNKNTLYLQMSSLKSEDTAMYYCSRSSSHWYFDVWGTGTTVTVS
 S

[0816] SEQ ID NO:5> Murine 5E20_Kabat Chothia Composite CDR-H1 amino acid sequence:
 GFTFSTYGMS

[0817] SEQ ID NO:6> Murine 5E20_Kabat Chothia Composite CDR-H2 amino acid sequence:
 IISGGSYTYYS DTVKG

[0818] SEQ ID NO:7> Murine 5E20_Kabat Chothia Composite CDR-H3 amino acid sequence:
 SSSHWYFDV

[0819] SEQ ID NO:8 >Murine 5E20VL nucleotide sequence (kappa):
ATGGTCCTTGCTCAGTTTCTTGCAATCCTTGTTGCTTTGGTTTCCAGGTGCAAGATGTG
ACATCCTGATGACCCAATCTCCATCCTCCATGTCTGTATCTCTGGGAGACACAGTCA
GCATCACTTGCCATGCAAGTCAGGGCATTAGCAGTAATATAGGGTGGTTGCAGCAG
AAACCAGGGAAATCATTTAAGGGCCTGATCTATCATGGAACCAACTTGAAAGATGG
AGTTCCATCAAGGTTCAAGTGGCAGTGGATCTGGAGCAGATTTTCTCTCACCATCAG
CAGCCTGGAATCTGAAGATTTTGCAGACTATTTCTGTGTTTCAGTATGCTCAGTTTCCG
TACACGTTCCGAGGGGGGACCAAGCTGGAAAAAAGA

[0820] SEQ ID NO:9 >amino acid sequence for signal peptide for murine 5E20VL
MVLAQFLAFLLLWFPGARC

[0821] SEQ ID NO:10>Murine 5E20VL Vk amino acid sequence
DILMTQSPSSMSVSLGDTVSITCHASQGISSNIGWLQQKPGKSFKGLIYHGTNLKDGVP
RFSGSGSGADFSLTISSEDFADYFCVQYAQFPYTFGGGTKLEKR

[0822] SEQ ID NO:11 >Murine 5E20_ Kabat Chothia Composite CDR-L1 amino acid
sequence
HASQGISSNIG

[0823] SEQ ID NO:12 >Murine 5E20_ Kabat Chothia Composite CDR-L2 amino acid
sequence
HGTNLKD

[0824] SEQ ID NO:13>Murine 5E20_ Kabat Chothia Composite CDR-L3 amino acid sequence
VQYAQFPYT

[0825] SEQ ID NO:14 >Kabat CDR-H1 of mouse 5E20 antibody

TYGMS

[0826] SEQ ID NO:15 >Chothia CDR-H1 of mouse 5E20 antibody

GFTFSTY

[0827] SEQ ID NO:16 >Chothia CDR-H2 of mouse 5E20 antibody

SSGGSY

[0828] SEQ ID NO:17 >AbM CDR-H2 of mouse 5E20 antibody

IISGGSYTY

[0829] SEQ ID NO:18 >Contact CDR-H1 of mouse 5E20 antibody

STYGMS

[0830] SEQ ID NO:19 >Contact CDR-H2 of mouse 5E20 antibody

WVAIISSGGSYTY

[0831] SEQ ID NO:20 >Contact CDR-H3 of mouse 5E20 antibody

SRSSSHWYFD

[0832] SEQ ID NO:21 >Contact CDR-L1 of mouse 5E20 antibody

SSNIGWL

[0833] SEQ ID NO:22 >Contact CDR-L2 of mouse 5E20 antibody

GLIYHGTNLK

[0834] SEQ ID NO:23 >Contact CDR-L3 of mouse 5E20 antibody

VQYAQFPY

[0835] SEQ ID NO:24 > amino acid sequence of a chimeric 5E20 heavy chain

EVQLVESGGDLVKPGGSLKLSCAAPGFTFSTYGMWVRQTPDKRLEWVAIISSGGSYTYYSDTV
KGRFTISRDNKNTLYLQMSSLKSEDTAMYYCSRSSSHWYFDVWGTGTTVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKHTCTCPPEAPEAAGGPSVFLFPPKPKDTLMISR
TPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK

[0836] SEQ ID NO:25 > amino acid sequence of a chimeric 5E20 light chain

DILMTQSPSSMSVSLGDTVSITCHASQGISSNIGWLQQKPGKSKFKGLIYHGTNLKDGVP
RFSGSGSGADFLSTISSLESEDFADYFCVQYAQFPYTFGGGTKLEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0837] SEQ ID NO: 26 > Murine 8H24VH nucleotide sequence (IgG2c):

ATGGGATTACAGCAGGATCTTTCTCTTCCTCCTGTCAGTAACTACAGGTGTCCACTCCC
AGGCTTATCTACAGCAGTCTGGGACTGAGCTGGTGAGGCCTGGGGCCTCAGTGAAG
ATGTCCTGCAAGGCTTCTGGCTACACATTTACCAGTTACAGTATGCACTGGGTAAAG
CAGACACCTAGACAGGGCCTGGAATGGATTGGAGCTATTTATCCAGGAAATGATGC
TACTTCCTACAATCAGAAGTTCAAGGGCAAGGCCACACTGACTGTAGACAAATCCTC
CAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAAGACTCTGCGGTCTATTT
CTGTGCAAGAGAGGGCTACTACGGTAGTAGCTTCGAAGCCTGGTTTGCTTCTTGGGG
CCAAGGGACTCTGGTCACTGTCTCTGCA

[0838] SEQ ID NO:27 >Murine > amino acid sequence for murine 8H24VH signal peptide
MGFSRIFLFLLSVTTGVHS

[0839] SEQ ID NO:28 >Murine 8H24Vh amino acid sequence
QAYLQQSGTELVRPGASVKMSCKASGYTFTSYSMHVVKQTPRQGLEWIGAIYPGNDAT
SYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCAREGYYGSSFEAWFASWGQG
TLVTVSA

[0840] SEQ ID NO:29 >Murine 8H24_ Kabat Chothia Composite CDR-H1 amino acid
sequence:
GYTFTSYSMH

[0841] SEQ ID NO:30 >Murine 8H24_ Kabat Chothia Composite CDR-H2 amino acid
sequence:
GAIYPGNDATSYNQKFKG

[0842] SEQ ID NO:31 >Murine 8H24_ Kabat Chothia Composite CDR-H3 amino acid
sequence:
EGYYGSSFEAWFAS

[0843] SEQ ID NO:32 >Murine 8H24VL nucleotide sequence (kappa):
ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGGTTCCAGCAGTG
ATGTTTTGATGACCCAACTCCACTTTCCTGCCTGTCAGTCTTGGAGATCAAGCCTC
CATCTCTGCAGATCTAGTCAGAGCATTGTACATAGTAATGGAAACACCTATTTAGA
ATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCAA
CCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGATTTAC
ACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGG
TTCACATGTTCTTCCGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAA

[0844] SEQ ID NO:33 >amino acid sequence for murine 8H24VL signal peptide
MKLPVRLLVLMFWIPGSSS

[0845] SEQ ID NO:34 >Murine 8H24Vk amino acid sequence
DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNR
SGVPDRFSGSGGTDFTLKISRVEAEDLGVYYCFQGSHVLPFTFGGGTKLEIK

[0846] SEQ ID NO:35 >Murine 8H24_ Kabat Chothia Composite CDR-L1 amino acid
sequence:
RSSQSIVHSNGNTYLE

[0847] SEQ ID NO:36>Murine 8H24_ Kabat Chothia Composite CDR-L2 amino acid sequence:
KVSNRFS

[0848] SEQ ID NO:37 >Murine 8H24_ Kabat Chothia Composite CDR-L3 amino acid
sequence:

FQGSHVLPT

[0849] SEQ ID NO:38 >Kabat CDR-H1 of mouse 8H24 antibody

SYSMH

[0850] SEQ ID NO:39 >Chothia CDR-H1 of mouse 8H24 antibody

GYTFTSY

[0851] SEQ ID NO:40 >Chothia CDR-H2 of mouse 8H24 antibody

YPGNDA

[0852] SEQ ID NO:41 >AbM CDR-H2 of mouse 8H24 antibody

AIYPGNDATS

[0853] SEQ ID NO:42 >Contact CDR-H1 of mouse 8H24 antibody

TSYSMH

[0854] SEQ ID NO:43 > Contact CDR-H2 of mouse 8H24 antibody

WIGAIYPGNDATS

[0855] SEQ ID NO:44 > Contact CDR-H3 of mouse 8H24 antibody

AREGYYGSSFEAWFA

[0856] SEQ ID NO:45> Contact CDR-L1 of mouse 8H24 antibody

NTYLEWY

[0857] SEQ ID NO:46 >Contact CDR-L2 of mouse 8H24 antibody

LLIYKVS NRF

[0858] SEQ ID NO:47 > Contact CDR-L3 of mouse 8H24 antibody

FQGSHVLP

[0859] SEQ ID NO:48 > amino acid sequence of a chimeric 8H24 heavy chain

QAYLQQSGTELVRPGASVKMSCKASGYTFTSYSMHWVKQTPRQGLEWIGAIYPGNDAT
SYNQKFKGKATLTVDKSSSTAYMQLSSLTSEDSAVYFCAREGYYGSSFEAWFASWGQG
TLVTVSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF

PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCP
 APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0860] SEQ ID NO:49 > amino acid sequence of a chimeric 8H24 light chain
 DVLMTQTPLSLPVSLGDQASISCRSSQIVHSNGNTYLEWYLQKPGQSPKLLIYKVSNRF
 SGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVLPFTFGGGTKLEIKRTVAAPSV
 FIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSTYS
 LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[0861] SEQ ID NO:50>Murine 11M14VH nucleotide sequence (IgG1):
 ATGAACCTTCGGGCTCAGCTTGATTTTCCTTGCCCTTATTTTAAAAGGTGTCCAGTGTG
 AGGTACAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
 CTCTCCTGTGCAGCCTCTGGATTCACTTTCAATATCTATGGCATGTCTTGGGTTCGCC
 AGACTCCAGACAAGAGGCTGGAGTGGGTCGCAACCATTAGTTCTGGTGGTATTTATA
 CCTACTATCCAGACATTTTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAAGA
 ACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATTACT
 GTGCAAGACATCCGGGTGGGGCTATGGACTACTGGGGTCAAGGAACCTCAGTCACC
 GTCTCCTCA

[0862] SEQ ID NO:51 >amino acid sequence for murine 11M14VH signal peptide
 MNFGLSLIFLALILKGVQC

[0863] SEQ ID NO:52 >Murine 11M14Vh amino acid sequence
 EVQLVESGGDLVKPGGSLKLSAASGFTFNIYGMSWVRQTPDKRLEWVATISSGGIYTY
 YPDILKGRFTISRDNANKNTLYLQMSSLKSEDTAMYYCARHPGGAMDYWGQGTSTVTVSS

[0864] SEQ ID NO:53 >Murine 11M14_ Kabat Chothia Composite CDR-H1 amino acid
 sequence:
 GFTFNIYGMS

[0865] SEQ ID NO:54 >Murine 11M14_ Kabat Chothia Composite CDR-H2 amino acid
 sequence:
 TISSGGIYTYPDILKG

[0866] SEQ ID NO:55 >Murine 11M14_ Kabat Chothia Composite CDR-H3 amino acid
 sequence:
 HPGGAMDY

[0867] SEQ ID NO:56 >Murine 11M14Vk nucleotide sequence (kappa)
ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATGCCAGA
TGTGACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGTATCTGTGGGAGAACT
GTCACCATCACATGTCGAGTAAGTGAGAATATTTACAGTAATTTAGCATGGTATCAG
CAGAAACAGGGAAAATCTCCTCACCTCCTGGTCTATGCTGCAACAACTTAGCAGAT
GGTGTGCCATCAAGGTTTCAGTGGCAGTGGATCAGGCACACAGTATTCCCTCAAGATC
AACAGCCTGCAGTCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTGGGGTACT
CCTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAA

[0868] SEQ ID NO:57 >amino acid sequence for murine 11M14Vk signal peptide
MSVPTQVLGLLLLWLT DARC

[0869] SEQ ID NO:58 >Murine 11M14Vk amino acid sequence
DIQMTQSPASLSVSVGETVTITCRVSENIYSNLAWYQQKQKSPHLLVYAATNLADGVP
SRFSGSGSGTQYSLKINSLSQSEDFGSYYCQHFWGTPPWTFGGGTKLEIK

[0870] SEQ ID NO:59 >Murine 11M14_ Kabat Chothia Composite CDR-L1 amino acid
sequence:
RVSENIYSNLA

[0871] SEQ ID NO:60 >Murine 11M14_ Kabat Chothia Composite CDR-L2 amino acid
sequence:
AATNLAD

[0872] SEQ ID NO:61 >Murine 11M14_ Kabat Chothia Composite CDR-L3 amino acid
sequence:
QHFWGTPPWT

[0873] SEQ ID NO:62 > Kabat CDR-H1 of mouse 11M14 antibody
IYGMS

[0874] SEQ ID NO:63: Chothia CDR-H1 of mouse 11M14 antibody
GFTFNIY

[0875] SEQ ID NO:64: Chothia CDR-H2 of mouse 11M14 antibody
SSGGIY

[0876] SEQ ID NO:65 AbM CDR-H2 of mouse 11M14 antibody
TISSGGIYTY

[0877] SEQ ID NO:66: Contact CDR-H1 of mouse 11M14 antibody
NIYGMS

[0878] SEQ ID NO:67: Contact CDR-H2 of mouse 11M14 antibody

WVATISSGGIYTY

[0879] SEQ ID NO:68: Contact CDR-H3 of mouse 11M14 antibody

ARHPGGAMD

[0880] SEQ ID NO:69: Contact CDR-L1 of mouse 11M14 antibody

YSNLAWY

[0881] SEQ ID NO:70:: Contact CDR-L2 of mouse 11M14 antibody

LLVYAATNLA

[0882] SEQ ID NO:71 Contact CDR-L3 of mouse 11M14 antibody

QHFWGTPPW

[0883] SEQ ID NO:72 Alternate Kabat-Chothia CDR-L2 (present in Hu11M14VLv3b, SEQ ID NO:198)

AATNGAD

[0884] SEQ ID NO:73: Alternate Kabat-Chothia CDR-L2 (present in Hu11M14VLv4b, SEQ ID NO:199)

AATNIAD

[0885] SEQ ID NO:74: Alternate Contact CDR-L2 (present in Hu11M14VLv3b, SEQ ID NO:198)

LLVYAATNGA

[0886] SEQ ID NO:75: Alternate Contact CDR-L2 (present in Hu11M14VLv4b, SEQ ID NO:199)

LLVYAATNIA

[0887] SEQ ID NO:76 >Murine 5M13VH nucleotide sequence (IgG1):

GATGTGCAGCTTCAGGAGTCGGGACCTGGCCTGGTGAAACCTTCTCAGTCTCTGTCC
CTCACCTGCACTGTCACTGGCTATTCAATCACCAGTGATTATGCCTGGAACCTGGATC
CGGCAGTTTCCAGGAAACAACTGGAGTGGATGGGCTACATAAGCTCCAGTGGTAG
CACTAGCTACAACCATCTCTCAAAAGTCGAATCTCTGTCACTCGAGACACATCCAA
GAACCAGTTCTTCCTGCAGTTGAATTTTGTGACTGCTGAGGACACAGCCACATATTA

CTGTGCATTTGTCACTACGACTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGT
CTCCTCA

[0888] SEQ ID NO:77 >amino acid sequence for Murine 5M13VH signal peptide
MRVLILLWLFTAAPGILS

[0889] SEQ ID NO:78 >Murine 5M13VH amino acid sequence
DVQLQESGPGLVKPSQSLSLTCTVTGYSDYAWNWIQFPGNKLEWMGYISSSGSTS
YNPSLKSRLSVTRDTSKNQFFLQLNFVTAEDTATYYCAFVTTTMDYWGQGTSVTVSS

[0890] SEQ ID NO:79 >Murine 5M13_ Kabat Chothia Composite CDR-H1 amino acid
sequence:
GYSITS DYAWN

[0891] SEQ ID NO:80 >Murine 5M13_ Kabat Chothia Composite CDR-H2 amino acid
sequence:
YISSSGSTSYNPSLKS

[0892] SEQ ID NO:81 >Murine 5M13_ Kabat Chothia Composite CDR-H3 amino acid
sequence:
VTTTMDY

[0893] SEQ ID NO: 82>Murine 5M13VL nucleotide sequence (kappa):
ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCTGCTTCCAGCAGTG
ATGTTGTGATGACCCAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCT
CCATCTCTTGAGATCTAGTCAGAGCCTTGACACAGTAATGGAAACACCTATTTAC
ATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATCTACACAGTTTCCA
ACCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGATTTCA
CACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTTCTGCTCTCAAA
GTACACATGTTCCATTCACGTTTCGGCTCGGGGACAAAGTTGGAAATAAAA

[0894] SEQ ID NO:83 >amino acid sequence for Murine 5M13VL signal peptide
MKLPVRLLVLMFWIPASSS

[0895] SEQ ID NO:84 >Murine 5M13VL Vk amino acid sequence:
DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWY LQKPGQSPKLLIYTVSNRF
SGVPDRFSGSGSTDFTLKISRVEAEDLGVYFCSQSTHVPFTFGSGTKLEIK

[0896] SEQ ID NO:85 >Murine 5M13_ Kabat Chothia Composite CDR-L1 amino acid
sequence:
RSSQSLVHSNGNTYLH

[0897] SEQ ID NO:86 >Murine 5M13_ Kabat Chothia Composite CDR-L2 amino acid
sequence:
TVSNRFS

[0898] SEQ ID NO:87 >Murine 5M13_ Kabat Chothia Composite CDR-L3 amino acid sequence:
SQSTHVPFT

[0899] SEQ ID NO:88>Murine 2F18VH nucleotide sequence (mIgG1):
ATGAAC TTCGGGCTCAGCTTGATTTTCCTTGCCCTTATTTTACAAGGTGTCCAGTGTG
AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCACTTTCAATATCTATGGCATGTCTTGGGTTCGCC
AGACTCCAGACAAGAGGCTGGAGTGGGTCGCAACCATTAGTACTGGTGGTATTTAC
ACCTACTATCCAGACAGTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAA
GAACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATT
ATTGTGCAAGACATCCGGTTGGGGCTCTGGACTACTGGGGTCAAGGAACCTCAGTCA
CCGTCTCCTCAGCCAAAACGACACCCCATCTGTCTATCCACTGGCCCCTGGATCTG
CTGCCCAAATACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCCTG
AGCCAGTGACAGTGACCTGGAACCTCTGGATCCCTGTCCAGCGGT

[0900] SEQ ID NO:89 >amino acid sequence for murine 2F18VH signal peptide
MNFGLSLIFLALILQGVQC

[0901] SEQ ID NO:90 >Murine > amino acid sequence 2F18VH
EVQLVESGGDLVKPGGSLKLSAASGFTFNIGMSWVRQTPDKRLEWVATISTGGIYTY
YPDSVKGRFTISRDNKNTLYLQMSSLKSEDTAMYYCARHPVGALDYWGQGTSVTVSS

[0902] SEQ ID NO:91 >Murine 2F18_ Kabat Chothia Composite CDR-H1 amino acid sequence:
GFTFNIGMS

[0903] SEQ ID NO:92 >Murine 2F18_ Kabat Chothia Composite CDR-H2 amino acid sequence:
TISTGGIYTYYPDSVKG

[0904] SEQ ID NO:93 >Murine 2F18_ Kabat Chothia Composite CDR-H3 amino acid sequence:
HPVGALDY

[0905] SEQ ID NO:94 >Murine 2F18VL nucleotide sequence (kappa)
ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGATGCCAGA
TGTGACATCCAGGTGACTCAGTCTCCAGCCTCCCTATCTGTATCTGTGGGAGAACT
GTCACCATCACATGTCGAGCAAGTGAGAATATTTACAGTAATTTAGCATGGTATCAG
CAGAAACAGGGAAAATCTCCTCACCTCCTGGTCTATACTGCAACAACCTTAGCAGAT
GGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGCACACAGTATTCCTCAAGATC
AACAGCCTGCAGTCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTGGGGTACT
CCTCCGTGGACGTTTCGGTGGAGGCACCAAGCTGGAAATCAAACGGGCTGATGCTGC
ACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTC
AGTCGTGTGCTTCTTGAACAACCTTACCCAGAGACATCAATGTCAAGTGGAAGAT
TGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG

[0906] SEQ ID NO: 95 >amino acid sequence for murine 2F18VL signal peptide
MSVPTQVLGLLLLWLTDA RC

[0907] SEQ ID NO:96 >Murine Vk_2F18VL amino acid sequence
DIQVTQSPASLSVSVGETVTITCRASENIYSNLAWYQQKQGKSPHLLVYTATTLADGVPS
RFSGSGSGTQYSLKINSLSQSEDFGSYYCQHFWGTPPWTFGGGTKLEIK

[0908] SEQ ID NO:97 >Murine 2F18_ Kabat Chothia Composite CDR-L1 amino acid
sequence:
RASENIYSNLA

[0909] SEQ ID NO:98 >Murine 2F18_ Kabat Chothia Composite CDR-L2 amino acid
sequence:
TATTLAD

[0910] SEQ ID NO:99 >Murine 2F18_ Kabat Chothia Composite CDR-L3 amino acid
sequence:
QHFWGTPPW T

[0911] SEQ ID NO:100 >Murine 2P22VH nucleotide sequence (IgG2b):
ATGAACCTTCGGGCTCAGCTTGATTTTCCTTGCCCTATTTTAAAAGGTGTCCAGTGTG
AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTATCTATGGCATGTCTTGGGTTCCGCC
AGACTCCAGACAAGAAGCTGGAGTGGGTTCGAGCCATTAGTAGTGGTGGTATTTAT
ACCTATTATCCAGACAGTGTGAAGGGGCGATTACCATCTCCAGAGACAACGCCAA
GAACACCCTGTACCTGCAAATGAGCAGTCTGAAGTCTGCGGACACAGCCATGTATTA
CTGTACAAGAAATGATTACGACTGGTTTGCTTACTGGGGCCAAGGGACTCTGGTCAC
TGTCTCTGCAGCCAAAACAACACCCCCATCAGTCTATCCACTGGCCCCCTGGGTGTGG
AGATACAACTGGTTCCTCTGTGACTCTGGGATGCCTGGTCAAGGGCTACTTCCCTGA
G

[0912] SEQ ID NO:101 >amino acid sequence for murine 2P22VH signal peptide
MNFGLSLIFLALILKGVQC

[0913] SEQ ID NO:102 >Murine 2P22VH amino acid sequence
EVQLVESGGDLVKPGGSLKLSAASGFTFSIYGMSWVRQTPDKKLEWVAAISSGGIYTY
YPDSVKGRFTISRDNKNTLYLQMSSLKSADTAMYYCTRNDYDWFAYWGQGLTVTS
A

[0914] SEQ ID NO:103 >Murine 2P22_ Kabat Chothia Composite CDR-H1 amino acid
sequence
GFTFSIYGMS

[0915] SEQ ID NO:104 >Murine 2P22_ Kabat Chothia Composite CDR-H2 amino acid
sequence

AISSGGIYTYYPDSVKG

[0916] SEQ ID NO:105 >Murine 2P22_ Kabat Chothia Composite CDR-H3 amino acid sequence
NDYDWFAY

[0917] SEQ ID NO:106 >Murine 2P22VL nucleotide sequence (kappa)
ATGAGGGCTCCTGCACAGATTTTTGGCTTCTTGTTGCTCTTGTTTCCAGGTACCAGAT
GTGACATCCAGATGACCCAGTCTCCATCCTTATCTGCCTCTCTGGGAGAAAGAG
TCAGTCTCACTTGTCGGGCAAGTCAGGACATTGGTAGTAGCTTAAACTGGCTTCAGC
AGGAACCAGATGGAACATTTAAACGCCTGATCTACGCCACATCCAGTTTAGATTCTG
GTGTCCCCAAAAGGTTTCAGTGGCAGTAGGTCTGGGTCAGATTATTCTCTCACCATCA
GCAGCCTTGAGTCTGAAGATTTTGTAGACTATTACTGTCTACAATATGCTAGTTCTCC
GTACACGTTTCGGAGGGGGGACCAAGCTGGAAATAAAGCGGGCTGATGCTGCACCAA
CTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG
TGTGCTTCTTGAACAACTTCTACCCCAGAGACATCAATGTCAAGTGGAAGATTGATG
GCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG

[0918] SEQ ID NO:107 >amino acid sequence for 2P22VL signal peptide
MRAPAQIFGLLLLFPGTRC

[0919] SEQ ID NO:108 >Murine >Vk_2P22VL amino acid sequence
DIQMTQSPSSLSASLGERVSLTCRASQDIGSSLNWLQQEPDGTIKRLIYATSSLDSGVPKR
FSGSRSGSDYSLTISSESEDFVDYYCLQYASSPYTFGGGTKLEIK

[0920] SEQ ID NO:109 >Murine 2P22_ Kabat Chothia Composite CDR-L1 amino acid sequence:
RASQDIGSSLN

[0921] SEQ ID NO:110 >Murine 2P22_ Kabat Chothia Composite CDR-L2 amino acid sequence:
ATSSLDS

[0922] SEQ ID NO:111 >Murine 2P22_ Kabat Chothia Composite CDR-L3 amino acid sequence:
LQYASSPYT

[0923] SEQ ID NO:112 >Murine 6B15VH nucleotide sequence (IgG1):
ATGAAC TTCGGGCTCAGCTTGATTTTCCTTGCCCTTATTTTAAAAGGTGTCCAGTGTG
AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCCCTGGATTCACTTTCAGTACTTATGGCATGTCTTGGGTTCCGC
AGACTCCAGACAAGAGGCTGGAGTGGGTCGCAATCATTAGTAGTGGTGGTAGTTAC
ACCTACTATTTCAGACACTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAA
GAACACCCTGAACCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTACT
TTTGTTC AAGGAGTAGTAGCCACTGGTATTTTCGATGTCTGGGGCACGGGGACACGG
TCACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCCTGGAT

CTGCTGCCCAAATACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCC
CTGAGCCAGTGACAGTGACCTGGAACCTCTGGATCCCTGTCCAGCGGT

[0924] SEQ ID NO:113 >amino acid sequence for 6B15VH signal peptide
MNFGLSLIFLALILKGVQC

[0925] SEQ ID NO:114 >Murine 6B15VH amino acid sequence
EVQLVESGGDLVKPGGSLKLSAAPGFTFSTYGMSWVRQTPDKRLEWVAIISSGGSYTY
YSDTVKGRFTISRDNKNTLNLQMSSLKSEDTAMYFCRSSHWHYFDVWGTGTTVTVS
S

[0926] SEQ ID NO:115 >Murine 6B15_ Kabat Chothia Composite CDR-H1 amino acid
sequence:
GFTFSTYGMS

[0927] SEQ ID NO:116 >Murine 6B15_ Kabat Chothia Composite CDR-H2 amino acid
sequence:
IISSGGSYTYYSDDTVKG

[0928] SEQ ID NO:117 >Murine 6B15_ Kabat Chothia Composite CDR-H3 amino acid
sequence:
SSSHWHYFDV

[0929] SEQ ID NO:118 >Murine 6B15VL nucleotide sequence (kappa)
ATGGTCCTTGCTCAGTTTCTTGCAATCTTGTTGCTTTGGTTTCCAGGTGCAAGATGTG
ACATCCTGATGACCCAATTTCCATCCTCCATGTCTGTATCTCTGGGAGACACAGTCA
GCATCACTTGCCATGCAAGTCAGGGCATTAGCAGTAATATAGGGTGGTTGCAGCAG
AAACCAGGGAATCATTTAAGGGCCTGATCTATCATGGAACCAACTTGAAAGATGG
AGTTCCATCAAGGTTTCAGTGGCAGTGGATCTGGAACAGATTTTTCTCTCACCATCAG
CAGCCTGGAATCTGAAGATTTTGCACTATTACTGTGTACAGTATGCTCAGTTTCC
GTACACGTTTCGGAGGGGGGACCAAGCTGGAAAAAAGACGGGCTGATGCTGCACCAA
CTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG
TGTGCTTCTTGAACAACCTTACCCAGAGACATCAATGTCAAGTGGAAGATTGATG
GCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG

[0930] SEQ ID NO:119 >amino acid sequence for 6B15VL signal peptide
MVLAQFLAFLLLWFPGARC

[0931] SEQ ID NO:120 >Murine 6B15VL Vk_6B15 amino acid sequence
DILMTQFPSSMSVSLGDTVSI THASQGISSNIGWLQKPGKSFKGLIYHGTNLKDGVP
S RFSGSGSGTDFSLTISSLESEDFADYYCVQYAQFPYTFGGGTKLEKR

[0932] SEQ ID NO:121 >Murine 6B15_ Kabat Chothia Composite CDR-L1 amino acid
sequence:
HASQGISSNIG

[0933] SEQ ID NO:122 >Murine 6B15_Kabat Chothia Composite CDR-L2 amino acid sequence:
HGTNLKD

[0934] SEQ ID NO:123 >Murine 6B15_Kabat Chothia Composite CDR-L3 amino acid sequence:
VQYAQFPYT

[0935] SEQ ID NO:124 >Murine 2C14VH nucleotide sequence (IgG1):
ATGAAC TTCGGGCTCAGCTTGATTTTCCTTGCCCTTATTTTAAAAGGTGTCCAGTGTG
AGGTGCAGCTGGTGGAGTCTGGGGGAGACTTAGTGAAGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCACTTTCAATACCCATGGCATGTCTTGGGTTCGCC
AGACTCCGGACAAGAGTCTGGAGTGGGTCGCAACCATTAGTACTGGTGGTTTTTACA
CCTCCTATCCAGACAGTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAAG
AACACCCTGTTCTGCAAATGAGCAGTCTGAAGTCTGAGGACACAGCCATGTATTAC
TGTGCAAAGAGTAGTAGCCACTGGTACTTCGATGTCTGGGGCACAGGGACCACGGT
CACCGTCTCCTCAGCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCCTGGATC
TGCTGCCCCAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAGGGCTATTTCCC
TGAGCCAGTGACAGTGACCTGGAACCTCTGGATCCCTGTCCAGCGGT

[0936] SEQ ID NO:125 >amino acid sequence for 2C14VH signal peptide
MNFGSLIFLALILKGVQC

[0937] SEQ ID NO:126 >Murine 2C14VH amino acid sequence
EVQLVESGGDLVKPGGSLKLSAASGFTFNTHGMSWVRQTPDKSLEWVATISTGGFYT
SYPDSVKGRFTISRDNKNTLFLQMSSLKSEDTAMYYCAKSSSHWYFDVWGTGTTVTV
SS

[0938] SEQ ID NO:127 >Murine 2C14_Kabat Chothia Composite CDR-H1 amino acid sequence:
GFTFNTHGMS

[0939] SEQ ID NO:128 >Murine 2C14_Kabat Chothia Composite CDR-H2 amino acid sequence:
TISTGGFYTSYPDSVKG

[0940] SEQ ID NO:129 >Murine 2C14_Kabat Chothia Composite CDR-H3 amino acid sequence:
SSSHWYFDV

[0941] SEQ ID NO:130 >Murine 2C14VL nucleotide sequence (kappa)
ATGGTCCTTGCTCAGTTTCTTGCACTTCTGTTGCTTTGGTTTCCAGGTGCAAGATGTG
ACATCCTGATGACCCAATCTCCATCCTCCATGTCTGTATCTCTGGGAGACACAGTCA
GCATCACTTGCCATGCAAGTCAGGGCATCAGTAGTAATATAGGGTGGTTGCAGCAG
AAACCAGGGAAATCATTTAAGGGCCTGATCTATCATGGAACCAACTTGAAGATGG
AGTTCCATCAAGGTTCAAGTGGCAGTGGATCTGGAGCAGATTATTCTCTCACCATCAG

TAGCCTGGAATATGAAGATTTTGCAGACTATTACTGTGTACAGTATGCTCACTTTCC
 GTACACGTTTCGGAGGGGGGACCAAGCTGGAAATAAAAACGGGCTGATGCTGCACCAA
 CTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTCG
 TGTGCTTCTTGAACAACTTCTACCCCAGAGACATCAATGTCAAGTGGAAGATTGATG
 GCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG

[0942] SEQ ID NO:131 >amino acid sequence for 2C14VL signal peptide
 MVLAQFLAFLLLWFPGARC

[0943] SEQ ID NO:132 >Murine 2C14VL Vk_2C14 amino acid sequence
 DILMTQSPSSMSVSLGDTVSITCHASQGISSNIGWLQKPGKSFKGLIYHGTNLEDGVPSR
 FSGSGSGADYSLTISSLEYEDFADYYCVQYAHFPYTFGGGTKLEIK

[0944] SEQ ID NO:133 >Murine 2C14_ Kabat Chothia Composite CDR-L1 amino acid
 sequence:
 HASQGISSNIG

[0945] SEQ ID NO:134 >Murine 2C14_ Kabat Chothia Composite CDR-L2 amino acid
 sequence:
 HGTNLED

[0946] SEQ ID NO:135 >Murine 2C14_ Kabat Chothia Composite CDR-L3 amino acid
 sequence:
 VQYAHFPYT

[0947] SEQ ID NO:136 >Murine 9N18VH nucleotide sequence (IgG2b):
 ATGGGATGGAAGCTGGATCTTTATTTTAATCCTGTCAGTAACTACAGGAGTCCACTCT
 GAGATCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAA
 GATATCCTGCAAGGCTTCTGGTTACTCATTCACTGGCTACTACATGAACTGGGTGAA
 GCAAAGTCCTGAAAAGAGCCTTGAGTGGATTGGAGAGATTAATCCTATCACTGGTG
 GTACTACCTACAACCAGAATTTCAAGGCCAAGGCCACATTGACTGTAGACAAATCCT
 CCAGCACAGCCTACCTGCAGCTCAGGAGCCTGACATCTGAGGACTCTGCAGTCTATT
 ACTGTGCATCCGATTACTTCGGTAGTAATCCTGGTTTGCTTACTGGGGCCAAGGGA
 CTCTGGTCACTGTCTCTGCAGCCAAAACAACACCCCATCAGTCTATCCACTGGCCC
 CTGGGTGTGGAGATACAACTGGTTCCTCTGTGACTCTGGGATGCCTGGTCAAGGGCT
 ACTTCCCTGAG

[0948] SEQ ID NO:137 >amino acid sequence for 9N18VH signal peptide
 MGWNWIFILISVTTGVHS

[0949] SEQ ID NO:138 >Murine 9N18VH amino acid sequence
 EIQLQQSGPELVKPGASVKISCKASGYSFTGYMNVWKQSPEKSLEWIGEINPITGGTTY
 NQNFKAKATLTVDKSSSTAYLQLRSLTSEDSAVYYCASDYFGSNSWFAYWGQGLVTV
 SA

[0950] SEQ ID NO:139 >Murine 9N18_ Kabat Chothia Composite CDR-H1 amino acid sequence:
GYSFTGYVMN

[0951] SEQ ID NO:140 >Murine 9N18_ Kabat Chothia Composite CDR-H2 amino acid sequence:
EINPITGGTTYNQNFKA

[0952] SEQ ID NO:141 >Murine 9N18_ Kabat Chothia Composite CDR-H3 amino acid sequence:
DYFGSNSWFAY

[0953] SEQ ID NO:142 >Murine 9N18VL nucleotide sequence (kappa)
ATGAAGTTGCCTGTTAGGCTGTTGGTGGTGTGTTCTGGATTCTGCTTCCAGCAGTG
ATGTTTTGATGACCCAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTC
CATCTCTTGAGATCTAGTCAGAGCATTGTACATAGAAATGGAAACACCTATTTAGA
ATGGTACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAA
CCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGATTTTCA
ACTCAGGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCTTTCAAGG
TTCACATGTTCCGTACACGTTCCGAGGGGGGACCAAGCTGGAAATAAAACGGGGCTG
ATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAG
GTGCCTCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAGAGACATCAATGTCAAGT
GGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG

[0954] SEQ ID NO:143 >amino acid sequence for 9N18VL signal peptide
MKLPVRLLVVMFWIPASS

[0955] SEQ ID NO:144 >Murine 9N18VL Vk_9N18 amino acid sequence
DVLMTQTPLSLPVSLGDQASISCRSSQSIVHRNGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLRISRVEAEDLGVYYCFQGSHVPYTFGGGKLEIK

[0956] SEQ ID NO:145 >Murine 9N18_ Kabat Chothia Composite CDR-L1 amino acid sequence:
RSSQSIVHRNGNTYLE

[0957] SEQ ID NO:146 >Murine 9N18_ Kabat Chothia Composite CDR-L2 amino acid sequence:
KVSNRFS

[0958] SEQ ID NO:147 >Murine 9N18_ Kabat Chothia Composite CDR-L3 amino acid sequence:
FQGSHPYTF

[0959] SEQ ID NO:148 >Murine 4N2VH nucleotide sequence (IgG3):
ATGAACCTCGGGCTCAGCTTGATTTTCTTGTCTTAATTTTAAAGGTGTCCAGTGTG
AAGTGAAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCTGGAGGGTCCCTGAAA

CTCTCCTGTGGAGCCTCTGGATTCACTTTCAGTAACTATGTCATGTCTTGGGTTCGCC
AGACTCCGGAGAGGAGGCTGGAGTGGGTCGCAACCATTAGTGGTGGTGGTCGTTAC
TCCTACTATCCAGACAGTGTGAAGGGGCGATTACCATCTCCAGAGACAATGCCAA
GAACAACCTGTACCTACAAATGAGCAGTCTGAGGTCTGAGGACACGGCCTTATATTA
CTGTGGAAGACAGGATGATTACGACTCTTTTCCTTACTGTGGCCAAGGGACTCTGGT
CACTGTCTCTGTAGCTACAACAACAGCCCCATCTGTCTATCCCTTGGTCCCTGGCTGC
GGTGACACA

[0960] SEQ ID NO:149 >amino acid sequence for murine 4N2VH signal peptide
MNFGSLIFLVILKGVQC

[0961] SEQ ID NO:150 >Murine 4N2VH Vh_4N2 amino acid sequence
EVKLVESGGGLVKPGGSLKLSCGASGFTFSNYVMSWVRQTPERRLEWVATISGGGRYS
YYPDSVKGRFTISRDNAKNNLYLQMSSLRSEDALYYCGRQDDYDSFPYCGQGLVTV
SV

[0962] SEQ ID NO:151 >Murine 4N2_ Kabat Chothia Composite CDR-H1 amino acid
sequence:
GFTFSNYVMS

[0963] SEQ ID NO:152 >Murine 4N2_ Kabat Chothia Composite CDR-H2 amino acid
sequence:
TISGGGRYSYYPDSVKG

[0964] SEQ ID NO:153 >Murine 4N2_ Kabat Chothia Composite CDR-H3 amino acid
sequence:
QDDYDSFPY

[0965] SEQ ID NO:154 >Murine 4N2VL nucleotide sequence (kappa)
ATGGTATCCACACCTCAGTTCCTTGTATTTTGGCTTTTCTGGATTCCAGCCTCCAGAG
GTGACATCTTGCTGACTCAGTCTCCAGCCATCCTGTCTGTGAGTCTAGGAGAAAGAG
TCAGTTTCTCCTGCAGGGCCAGTCAGAGCATTGGCACAAGCATACTGGTTTCAGC
TAAGAACAAATGGTTCTCCAAGGCTTCTCATAAAGTATGCTTCTGAGCCCATCTCTG
GGATCCCTTCCAGGTTTAGTGGCAGTGGATCAGGGACAGATTTTACTCTTAGCATCA
ATAGTGTGGAATCTGAAGATATTGCAGATTATTTCTGTCAACAAAGTAAGAGCTGGC
CAATCACGTTCCGGTACTGGGACCAAGCTGGAGCTGAAACGGGCTGATGCTGCACCA
ACTGTATCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCCTCAGTC
GTGTGCTTCTTGAACAACCTTACCCAGAGACATCAATGTCAAGTGGAAGATTGAT
GGCAGTGAACGACAAAATGGCGTCCTGAACAGTTGGACTGATCAG

[0966] SEQ ID NO:155 >amino acid sequence for murine 4N2VL signal peptide
MVSTPQFLVLLFWIPASRG

[0967] SEQ ID NO:156 >Murine > Murine 4N2VL Vk_4N2 amino acid sequence
DILLTQSPAILSVSLGERVSFSCRASQSIGTSIHWFQLRTNGSPRLLIK YASEPISGIPSRFSG
SGSGTDFTL SINSVE SEDIADYFCQSKSWPITFGTGTKLELK

[0968] SEQ ID NO:157 >Murine 4N2_ Kabat Chothia Composite CDR-L1 amino acid sequence:
RASQSIGTSIH

[0969] SEQ ID NO:158 >Murine 4N2_ Kabat Chothia Composite CDR-L2 amino acid sequence:
YASEPIS

[0970] SEQ ID NO:159 >Murine 4N2_ Kabat Chothia Composite CDR-L3 amino acid sequence:
QQSKSWPIT

[0971] SEQ ID NO:160 >3V6F-VH_mSt
EVQLVESGGDLVKPGGSLRLSCAASGFTFSSYGMSWVRQTPDKRLEWVATISSGGNYIYPDTVKGRTISR
NAKNTLYLQMSSLKSEDTAMYYCTREGAYSGSSSYPMDYWGQGTSVTVSS

[0972] SEQ ID NO:161 > AEX29086-VH_huFrwk
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGGGSTYYADSVRGRFTISR
DNSKNTLYLQMNSLRAEDTAVYYCAKSGTPWYFDYWGQGTSLTVTVSS

[0973] SEQ ID NO:162 >IGHV3-21*01
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSSYIYADSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARDADFVWGQGTMTVTVSS

[0974] SEQ ID NO:163 >h5E20VH version1 (87.8% human)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQAPGKGLEWVAIISGGGSYTYSDTVKGRFTISR
NSKNTLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTSLTVTVSS

[0975] SEQ ID NO:164 >h5E20VH version2 (89.8% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQAPGKGLEWVAIISGGGSYTYSDTVKGRFTISR
DNSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTSLTVTVSS

[0976] SEQ ID NO:165 >h5E20VH version3 (86.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQAPGKLEWVAIISGGGSYTYSDTVKGRFTISR
DNSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTSLTVTVSS

[0977] SEQ ID NO:166 >h5E20VH version4 (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQAPDKRLEWVAIISGGGSYTYSDTVKGRFTISR
NSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTSLTVTVSS

[0978] SEQ ID NO:167 >h5E20VH version5 (84.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQAPDKRLEWVAIISGGGSYTYSDTVKGRFTISR
NSKNSLYLQMNSLKAEDTAVYYCSRSSSHWYFDVWGQGTSLTVTVSS

[0979] SEQ ID NO:168 >h5E20VH version6 (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQTPGKRLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLTVSS

[0980] SEQ ID NO:169 >h5E20VH version7 (84.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFSTYGMSWVRQTPDKRLEWVAIISSGGSYTYSDTVKGRFTISRD
NSKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLTVSS

[0981] SEQ ID NO:170 >3V6F-VL_mSt
NIMMTQSPSSLAVSAGEKVTMNCKSSQSVLYSSNQKNYLAWYQQKPGQSPKLLIYWASTRESGPDRFTGS
GSGTDFTLTISVQTEDLAVYYCHQYLSSMYTFGGGTKEIK

[0982] SEQ ID NO:171 >BAH04687-VL_huFrwk
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLT
SSLQPEDFATYYCQQSYSTPLTFGGGTKEIR

[0983] SEQ ID NO:172 >IGKV1-12*01
DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTL
TISSLQPEDFATYYCQANSFPYTFGGGTKEIR

[0984] SEQ ID NO:173 >h5E20VL version1 (78.9% human measured against IGKV2-29*02)
DIQMTQSPSSLSASVGDRVTITCHASQGISSNIGWLQQKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFTL
TISSLQPEDFATYFCVQYAQFPYTFGGGTKEIR

[0985] SEQ ID NO:174 >h5E20VL version2 (80.0% human)
DIQMTQSPSSVSASVGDRVTITCHASQGISSNIGWLQQKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFT
LTISLQPEDFATYFCVQYAQFPYTFGGGTKEIR

[0986] SEQ ID NO:175 >h5E20VL version3 (80.0% human)
DIQMTQSPSSVSASVGDRVTITCHASQGISSNIGWLQQKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFT
LTISLQPEDFATYFCVQYAQFPYTFGGGTKEIR

[0987] SEQ ID NO:176 >h5E20VL version4 (78.9% human)
DIQMTQSPSSVSASVGDRVTITCHASQGISSNIGWLQQKPGKAFKGLIYHGTNLKDGVPSPRFSGSGSGADFT
LTISLQPEDFADYFCVQYAQFPYTFGGGTKEIR

[0988] SEQ ID NO:177 >1MRC-VH_mSt
QVQLQQSGAELVKPGASVKLSCKASGYTFTSYWMQWVKQRPQGQGLEWIGEIDPSDSYTNYNQFKGKATL
TVDTSSSTAYMQLSSLTSEDSAVYYCANLRGYFDYWGQGTTLVSS

[0989] SEQ ID NO:178 >AAC51714-VH_huFrwk
QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAIWVRQAPGQGLEWMGGIPIFGTANYAQKFQGRVTIT
ADKSTSTAYMELSSLRSEDTAVYYCARAYCSSTSCYKTFVWGQGTLTVSS

[0990] SEQ ID NO:179 >IGHV1-69*08_IGHJ1*01
QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYTISWVRQAPGQGLEWMGRIIPILGTANYAQKFQGRVTITA
DKSTSTAYMELSSLRSEDTAVYYCARAEYFQHWGQGTLTVSS

[0991] SEQ ID NO:180 >h8H24VH version1 (81.6.% human)
QAQLVQSGAEVKKPGSSVKVSKASGYTFTSYSMHWVRQAPGQGLEWIGAIYPGNDATSYNQKFKGRATL
TVDKSTSTAYMELSSLRSEDTAVYFCAREGGYGSFEAWFASWGQGTTTVSS

[0992] SEQ ID NO:181 >h8H24VH version2 (80.6% human)
QAQLVQSGAEVKKPGSSVKVSKASGYTFTSYSMHWVRQAPGQGLEWIGAIYPGNDATSYNQKFKGRATL
TVDKSTSTAYMELSSLRSEDTAVYFCAREGGYGSFEAWFASWGQGTTTVSS

[0993] SEQ ID NO:182 >1MRC -VL_mSt
DVVMTQTPLSLPVSLGDQASISCRSSQSLVHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSG
TDFTLKISRVEAEDLGVYFCSQSTHVPRTFGGGTKLEIK

[0994] SEQ ID NO:183 > ABC66914-VL_huFrwk
DIVMTQTPLSLPVTGPGEASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSG
TDFTLKISRVEAEDVGVYYCMQALQTPLTFGGGKVEIK

[0995] SEQ ID NO:184 >IGKV2-40*01
DIVMTQTPLSLPVTGPGEASISCRSSQSLDSDGNTYLDWYLQKPGQSPQLLIYLSYRASGVPDRFSGSGSG
TDFTLKISRVEAEDVGVYYCMQRIEFPLTFGGGKVEIK

[0996] SEQ ID NO:185 >h8H24VL version1 (88.4% human)
DVVMTQTPLSLPVTGPGEASISCRSSQIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSG
TDFTLKISRVEAEDVGVYYCFQGSHVLPTFGGGKVEIK

[0997] SEQ ID NO:186 >h8H24VL version2 (86.3% human)
DVVMTQTPLSLPVTGPGEASISCRSSQIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSG
TDFTLTISRVEAEDVGVYYCFQGSHVLPTFGGGKVEIK

[0998] SEQ ID NO:187>1MQK-VH_mSt
EVKLQESGGDLVQPGGSLKLSAASGFTFSSYTMSWVRQTPEKRLEWVASINNGGGRTYYPDTVKGRTISR
DNAKNTLYLQMSSLKSEDTAMYYCVRHEYYYAMDYWGQGTTTVSS

[0999] SEQ ID NO:188> ACS96198-VH_huFrwk
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYEMNWVRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARENIAAFDYWGQGTLTVSSGS

[1000] SEQ ID NO:189>IGHV3-48*03
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYEMNWVRQAPGKGLEWVSYISSSGSTIYYADSVKGRFTISR
NAKNSLYLQMNSLRAEDTAVYYCARYFDYWGQGTLTVSS

[1001] SEQ ID NO:190>h11M14VH version1b (86.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNIIYGMWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRD
NAKNSLYLQMNSLRAEDTAVYYCARHPGGAMDYWGQGTLLTVSS

[1002] SEQ ID NO:191>h11M14VH version2b (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNIIYGMWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRD
NAKNSLYLQMNSGRAEDTAVYYCARHPGGAMDYWGQGTLLTVSS

[1003] SEQ ID NO:192>h11M14VH version3b (85.7% human)
EVQLVESGGGLVQPGGSLRLSCAASGFTFNIIYGMWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRD
NAKNSLYGQMNSLRAEDTAVYYCARHPGGAMDYWGQGTLLTVSS

[1004] SEQ ID NO:193>1MQK -VL_mSt
DIELTQTPVSLASVGETVTITCRASENIYSYLAWYQQKQKGKSPQFLVYNAKTLGEGVPSRFSGSGSGTQFSLKI
NSLLPEDFGSYQCQHHYGTPLTFGGGKLEIK

[1005] SEQ ID NO:194> CBZ39892-VL_huFrwk
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLT
ISLTQPEDFATYYCQSYSTPPYTFGGGKLEIK

[1006] SEQ ID NO:195>IGKV1-39*01
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFTLT
ISLTQPEDFATYYCQSYSTPPYTFGGGKLEIK

[1007] SEQ ID NO:196>h11M14VL version1b (83.2% human)
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKAPKLLVYAATNLADGVPSRFSGSGSGTDYTL
TISLTQPEDFATYYCQHFWGTPPWTFGGGKLEIK

[1008] SEQ ID NO:197>h11M14VL version2b (83.2% human)
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKSPKLLVYAATNLADGVPSRFSGSGSGTDYTL
TISSLQPEDFATYYCQHFWGTPPWTFGGGKLEIK

[1009] SEQ ID NO:198>h11M14VL version3b (82.1% human)
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKSPKLLVYAATNGADGVPSRFSGSGSGTDYTL
TISSLQPEDFATYYCQHFWGTPPWTFGGGKLEIK

[1010] SEQ ID NO:199>h11M14VL version4b (82.1% human)
DIQMTQSPSSLSASVGDRVTITCRVSENIYSNLAWYQQKPGKSPKLLVYAATNIADGVPSRFSGSGSGTDYTL
TISSLQPEDFATYYCQHFWGTPPWTFGGGKLEIK

[1011] SEQ ID NO:200 HA peptide
YPYDVDPYAG

[1012] SEQ ID NO:201 c-Myc peptide
EQKLISEEDL

[1013] SEQ ID NO:202 consensus motif of a peptide bound by antibody 5E20
FTESFLT

[1014] SEQ ID NO:203: consensus motif of a peptide bound by antibody 5E20
ESFL

[1015] SEQ ID NO:204 peptide bound by antibody 5E20
DGCILGYKEQFL

[1016] SEQ ID NO:205 peptide bound by antibody 5E20
PSICLCSLEDFL

[1017] SEQ ID NO:206 sequence motif bound by antibody 5E20
E(S/Q/D)FL

[1018] SEQ ID NO:207 Linker sequence
GSGSGSG

[1019] SEQ ID NO:208 amino acid sequence of chimeric 11M14 heavy chain
EVQLVESGGDLVKPGGSLKLSAASGFTFNIYGMSWVRQTPDKRLEWVATISSGGIYTY
YPDILKGRFTISRDNKNTLYLQMSSLKSEDTAMYYCARHPGGAMDYWGQGTSTVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVD
KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[1020] SEQ ID NO:209 amino acid sequence of chimeric 11M14 light chain
DIQMTQSPASLSVSVGETVTITCRVSENIYSNLAWYQQKQKSPHLLVYAATNLADGVP
SRFSGSGSGTQYSLKINSLSQSEDFGSYYCQHFHWGTPPWTFGGGKLEIKRTVAAPSVFIFP
PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC

[1021] SEQ ID NO:210 amino acid sequence of residues 523-610 of sortilin
HYYTILDSGGIIVAIEHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGLASEPGARSMNISI
WGFTESFLTSQWVSYTIDFKDILER

[1022] SEQ ID NO:211 amino acid sequence of a sortilin peptide

FTESFLTSQW

[1023] SEQ ID NO:212 amino acid sequence of a sortilin peptide

LTSQW

[1024] SEQ ID NO:213 amino acid sequence of a peptide bound by antibody 8H24

RTEFGMAIGP

[1025] SEQ ID NO:214 amino acid sequence of a peptide bound by antibody 11M14

WGFTESFLTS

[1026] SEQ ID NO:215; Amino acid sequence of human sortilin extracellular domain without signal peptide

QDRLDAPPPPAAPLPRWSGPIGVSWGLRAAAAGGAFPRGGRWRRSAPGEDEECGRVRD
FVAKLANNTHQHVFDLRLGSLVSWVGDSTGVILVLTTFHVPLVIMTFGQSKLYRSEDY
GKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEVSGGSRGGRIFRSSDFAKNFVQTD
LPFHPLTQMMYSPQNSDYLLALSTENGLWVSKNFGGKWEEIHKAVCLAKWGSNTIFF
TTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFASVMADKDTTRRIH
VSTDQGDTWSMAQLPSVGQEYFYSILAANDDMVFMHVDEPGDTGFGTIFTSDDRGIVY
SKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRKPENS
ECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIIVIAHGSVGDASVMVPDV
YISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDP
IYFTGLASEPGARSMNISIWGFTESFLTSQWVSYTIDFKDILERNCEEKDYTIWLAHSTDP
EDYEDGCILGYKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDS
KCVEQPELKGHDLEFCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNF
LSPEKQNSKNS

[1027] SEQ ID NO:216 amino acid sequence of hSORT1_ECD_Emut1:

MERPWGAADGLSRWPHGLLLLLLQLLPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQAVFDAAAGSVLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWVSKNFGGKWE
EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEYFYSILAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIIVIAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPYFTGL
ASEPGARSMNISIWGFTESFLTSQWVSYTIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDLE

FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKNSGGSGGS
GGSHHHHHHHH

[1028] SEQ ID NO:217 amino acid sequence of hSORT1_ECD_Emut2a:

MERPWGAADGLSRWPHGLLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTAAVPLAIATFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEVS
GGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWEE
IHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFAS
VMADKDTTTRRIHVSTDQGDWMAQLPSVGQEQFYLSILAANDDMVFMHVDEPGDTGFGTIFT
SDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRK
PENSECDAKKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIHAGSVGDAISVMVPDVYI
SDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKSTDEGQCWQTYTFTRDPIYFTGL
ASEPGARSMNISIWGFTESFLTSQWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVTKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGDHLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKNSGGSGGS
GGSHHHHHHHH

[1029] SEQ ID NO:218 amino acid sequence of hSORT1_ECD_Emut2b:

MERPWGAADGLSRWPHGLLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTAGASALARSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEVS
GGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWEE
IHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFAS
VMADKDTTTRRIHVSTDQGDWMAQLPSVGQEQFYLSILAANDDMVFMHVDEPGDTGFGTIFT
SDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRK
PENSECDAKKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIHAGSVGDAISVMVPDVYI
SDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKSTDEGQCWQTYTFTRDPIYFTGL
ASEPGARSMNISIWGFTESFLTSQWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVTKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGDHLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKNSGGSGGS
GGSHHHHHHHH

[1030] SEQ ID NO:219 amino acid sequence of hSORT1_ECD_Emut3:

MERPWGAADGLSRWPHGLLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKAITALINAAFIRTEFGMAIGPENSGKVVLTAEVS
GGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWEE
IHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFAS
VMADKDTTTRRIHVSTDQGDWMAQLPSVGQEQFYLSILAANDDMVFMHVDEPGDTGFGTIFT
SDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRK
PENSECDAKKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIHAGSVGDAISVMVPDVYI
SDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKSTDEGQCWQTYTFTRDPIYFTGL
ASEPGARSMNISIWGFTESFLTSQWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVTKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGDHLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKNSGGSGGS
GGSHHHHHHHH

[1031] SEQ ID NO:220 amino acid sequence of hSORT1_ECD_Emut4:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEA
 AGGAAGGAIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEQFYSLAANDDMVMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVDPVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
 ASEPARGSMNISIWGFTESFLT SQWVS YTIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREV KDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GGSHHHHHHHH

[1032] SEQ ID NO:221 amino acid sequence of hSORT1_ECD_Emut5:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVATLPAFLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEQFYSLAANDDMVMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVDPVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
 ASEPARGSMNISIWGFTESFLT SQWVS YTIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREV KDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GGSHHHHHHHH

[1033] SEQ ID NO:222 amino acid sequence of hSORT1_ECD_Emut6N:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTANGLAVSKNFGGKWE
 AIAAAVCLAKWGSNDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEQFYSLAANDDMVMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVDPVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
 ASEPARGSMNISIWGFTESFLT SQWVS YTIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREV KDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GGSHHHHHHHH

[1034] SEQ ID NO:223 amino acid sequence of hSORT1_ECD_Emut8N:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNDNTIFFTTAANGSCAADAGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA

SVAAAAATTRRIHVSTDQGDTWSMAQLPSVGAAQAYSILAANDDMVFMHVDEPGDTGFGTIFT
 SDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLRK
 PENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVIGIVIAHGSVGDASVMVPDVYI
 SDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTRDPIYFTGL
 ASEPGRASMNISIWGFTEFSLTSQWVSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSGSHHHHHHHH

[1035] SEQ ID NO:224 amino acid sequence of hSORT1_ECD_Emut11N:

MERPWGAADGLSRWPHGLGLLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVGDDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEQFYLSILAANDDMVFMHVAEPGAAGFGTIF
 TSDRGIVYSKSLDRHLATAAGGETDFTNVTSLRGVYITSVASADASAQTMITFDQGGRWTHL
 RKPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVIGIVIAHGSVGDASVMVPD
 VYISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTRDPIYFT
 GLASEPGARSMNISIWGFTEFSLTSQWVSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCI
 LGYKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 LEFCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSG
 GSGSHHHHHHHH

[1036] SEQ ID NO:225 amino acid sequence of hSORT1_ECD_Emut14N:

MERPWGAADGLSRWPHGLGLLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVGDDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEQFYLSILAANDDMVFMHVDEPGDTGFGTIF
 TSDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISAKAAVPMAPLSEPNAVIGIVIAHGSVGAAASVMAPAV
 YISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTRDPIYFTG
 LASEPGARSMNISIWGFTEFSLTSQWVSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCIL
 GYKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 EFCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGG
 GSGSHHHHHHHH

[1037] SEQ ID NO:226 amino acid sequence of hSORT1_ECD_Emut16:

MERPWGAADGLSRWPHGLGLLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVGDDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDTWSMAQLPSVGQEQFYLSILAANDDMVFMHVDEPGDTGFGTIF
 TSDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVIGIVIAHGSVGDASVMVPDVY
 ISDDGGYSWTAMLAGPAYYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTRDPIYFTGL
 ASEPGRASMNISIWGFTEFSLTSQWVSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG

YKEQFLRLRKSSVCQNGRDYVVKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSHHHHHHHH

[1038] SEQ ID NO:227 amino acid sequence of hSORT1_ECD_Emut17:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNDTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEQFYSLAANDDMVMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQMTITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVIAHASAPIAVIKFSTDEGQCWQTYTFTRDPIYFTGL
 ASEPGRSMNISIWGFTEFSLTSQWWSYTIIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSHHHHHHHH

[1039] SEQ ID NO:228 amino acid sequence of hSORT1_ECD_Emut18:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNDTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEQFYSLAANDDMVMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQMTITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVIAIEHSSRPINVIKFSSTDEGQCWQTYAFTADPIYFTGL
 ASEPGRSMNISIWGFTEFSLTSQWWSYTIIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSHHHHHHHH

[1040] SEQ ID NO:229 amino acid sequence of hSORT1_ECD_Emut19:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNDTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEQFYSLAANDDMVMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQMTITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVIAIEHSSRPINVIKFSSTDEGQCWQTYTFTRDPIYFTGL
 ASEPGRSMNISIWGFTEAAAATSQWWSYTIIDFKDILERNCEEKDYTIWLAHSTDPEYEDGCIL
 GYKEQFLRLRKSSVCQNGRDYVVKQPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 EFCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGG
 SGGSHHHHHHHH

[1041] SEQ ID NO:230 amino acid sequence of hSORT1_ECD_Emut20:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVF DALRGSVLSWVG DSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGS DNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGD TWSMAQLPSVGQE QFYSLAANDDMVMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGD AISVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESFLT SQWVS YTIDFKDILERNCEEKD YTIWLAHSTD PEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVT KQPSICLCSLEDFLCDFGYR PENDSKC VEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREV KDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1042] SEQ ID NO:231 amino acid sequence of hSORT1_ECD_Emut21:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLAGSVLSWVG DSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGS DNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGD TWSMAQLPSVGQE QFYSLAANDDMVMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGD AISVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESFLT SQWVS YTIDFKDILERNCEEKD YTIWLAHSTD PEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVT KQPSICLCSLEDFLCDFGYR PENDSKC VEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREV KDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1043] SEQ ID NO:232 amino acid sequence of hSORT1_ECD_Emut22:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDL RGSVLSWVG DSTG
VILVLTTAHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGS DNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGD TWSMAQLPSVGQE QFYSLAANDDMVMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGD AISVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESFLT SQWVS YTIDFKDILERNCEEKD YTIWLAHSTD PEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVT KQPSICLCSLEDFLCDFGYR PENDSKC VEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREV KDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1044] SEQ ID NO : 233 amino acid sequence of hSORT1_ECD_Emut23:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDL RGSVLSWVG DSTG
VILVLTTFHVPLVIMTFGQSALYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV

SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDAISVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAEIHSSRPINVIKSTDEGQCWQTYTFTRDPIYFTGL
 ASEPARGSMNISIWGFTEFSLTSQWWSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSHHHHHHHH

[1045] SEQ ID NO:234 amino acid sequence of hSORT1_ECD_Emut24:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVG DSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDAISVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAEIHSSRPINVIKSTDEGQCWQTYTFTRDPIYFTGL
 ASEPARGSMNISIWGFTEFATSQWWSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSHHHHHHHH

[1046] SEQ ID NO: 235 amino acid sequence of hSORT1_ECD_Emut25:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVG DSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDAISVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAEIHSSRPINVIKSTDEGQCWQTYTFTRDPIYFTGL
 ASEPARGSMNISIWGFTEFLASQWWSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDL
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
 GSHHHHHHHH

[1047] SEQ ID NO: 236 amino acid sequence of hSORT1_ECD_Emut26:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
 AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVLSWVG DSTG
 VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
 SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EIHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
 SVMADKDTTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDAISVMVPDVY

ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESFLTSAWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1048] SEQ ID NO:237 amino acid sequence of hSORT1_ECD_Emut27:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGSNDTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEYFYSILAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIIVAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRAINVIKFSSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESFLTSQWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1049] SEQ ID NO:238 amino acid sequence of hSORT1_ECD_Emut28:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGSNDTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEYFYSILAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIIVAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSSTDEGQCWQTYTFTTRDAIYFTGL
ASEPGARSMNISIWGFTESFLTSQWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1050] SEQ ID NO:239 amino acid sequence of hSORT1_ECD_Emut29:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGSNDTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDWMAQLPSVGQEYFYSILAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGIIVAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSSTDEGQCWQTYTFTTRDPIAFTGL
ASEPGARSMNISIWGFTESFLTSQWWSYIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCVEQPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1051] SEQ ID NO:240 amino acid sequence of hSORT1_ECD_Emut30:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGSNDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTASFLTSQWWSYITDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1052] SEQ ID NO:241 amino acid sequence of hSORT1_ECD_Emut31:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGSNDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESALTSQWWSYITDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1053] SEQ ID NO:242 amino acid sequence of hSORT1_ECD_Emut32:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEFGMAIGPENSGKVVLTAEV
SGGSRGGRIFRSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
EIHKAVCLAKWGSNDNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRFLFA
SVMADKDTTRRIHVSTDQGDWWSMAQLPSVGQEQFYSLAANDDMVFMHVDEPGDTGFGTIF
TSDDRGIVYSKSLDRHLYTTTGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAIHSSRPINVIKFSTDEGQCWQTYTFTTRDPIYFTGL
ASEPGARSMNISIWGFTESALTSQWWSYITDFKDILERNCEEKDYTIWLAHSTDPEYEDGCILG
YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVVDLKKKCTSNFLSPEKQNSKSNSSGGSGGS
GGSHHHHHHHH

[1054] SEQ ID NO: 243 amino acid sequence of hSORT1_ECD_Emut33:

MERPWGAADGLSRWPHGLGLLLLLQLLPPSTLSQDRLDAPPPPAAPLPRWSGPIGVSWGLRA
AAAGGAFPRGGRWRRSAPGEDEECGRVRDFVAKLANNTHQHVFDDLGRSVSLSWVGDSTG
VILVLTTFHVPLVIMTFGQSKLYRSEDYGKNFKDITDLINNTFIRTEAGMAAGPENSGKVVLTAEV

SGGSRGGRIFRSSDFAKNFVQTDLPFHPLTQMMYSPQNSDYLLALSTENGLWWSKNFGGKWE
 EHKAVCLAKWGSNTIFFTTYANGSCKADLGALELWRTSDLGKSFKTIGVKIYSFGLGGRLFA
 SVMADKDTTTRRIHVSTDQGDWWSMAQLPSVGQEYFYSILAANDDMVFMHVDEPGDTGFGTIF
 TSDDRGIVYSKSLDRHLYTTTGGGETDFTNVTSLRGVYITSVLSEDNSIQTMITFDQGGRWTHLR
 KPENSECDATAKNKNECSLHIHASYSISQKLNVPMAPLSEPNAVGVIAHGSVGDASVMVPDVY
 ISDDGGYSWTKMLEGPHYTYTILDSGGIIVAEIHSSRPINVIKFSTDEGQCWQTYTFTRDPIYFTGL
 ASEPGARSMNISIWGFTEFSLTSQWWSYITIDFKDILERNCEEKDYTIWLAHSTDPEDYEDGCILG
 YKEQFLRLRKSSVCQNGRDYVVTQKPSICLCSLEDFLCDFGYRPPENDSKCQEPELKGHDLE
 FCLYGREEHLTTNGYRKIPGDKCQGGVNPVREVKDLKKKCTSNFLSPEKQNSKNSNGSGSGS
 GSHHHHHHHH

[1055] SEQ ID NO:244 hu11M14_H1b_IgG1 LALA_YTE – Heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFNIGMSWVRQAPGKGLEWVATISSGGIYTY
 YPDILKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARHPGGAMDYWGQGLTVTVSS
 ASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
 GLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAG
 GPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
 YNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
 REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVD
 KSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[1056] SEQ ID NO:245 hu11M14_L3b – Light chain amino acid sequence:

DIQMTQSPSSLSASVGDRTITCRVSENIYSNLAWYQQKPGKSPKLLVYAATNGADGVP
 SRFSGSGSGTDYTLTISSLQPEDFATYYCQHFVGTTPPWTFGQGTKLEIKRTVAAPSVFIFP
 PSDEQLKSGTASVVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKDYSLST
 LTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[1057] SEQ ID NO:246 hu8H24_H1_IgG1 LALA_YTE – Heavy chain amino acid sequence:

QAQLVQSGAEVKKPGSSVKVSCKASGYTFTSYSMHWVRQAPGQGLEWIGAIYPGND
 TSYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYFCAREGYGSSFEAWFASWGQ
 GTTVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHT
 FPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPC
 PAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
 TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
 VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFL
 YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[1058] SEQ ID NO:247 hu8H24_L2 – Light chain amino acid sequence:

DVVMTQTPSSLPVTPGEPASISCRSSQIVHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFS
 GVPDRFSGSGSGTDFTLTISRVEAEDVGVYYCFQGSFVLPFTFGGGTKVEIKRTVAAPSVF
 IFPPSDEQLKSGTASVVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKDYSL
 SSTLTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[1059] SEQ ID NO:248 hu5E20_H7_IgG1 LALA_YTE – Heavy chain amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFTSTYGMWVRQTPDKRLEWVAIISGGSYTY
 YSDTVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGLTVTVS

SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLYITREPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[1060] SEQ ID NO:249 hu5E20_L4 – Light chain amino acid sequence:

DIQMTQSPSSVSASVGDRVTITCHASQGISNIGWLQQKPGKAFKGLIYHGTNLKDGVPSTRFSGSGSGADFTLTISLQPEDFADYFCVQYAQFPYTFGQGTKVEKRRRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSLSTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[1061] SEQ ID NO:250 hu11M14VHv1b_IgG1_LALA heavy chain amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFNIGMSWVRQAPGKGLEWVATISSGGIYTYYPDILKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCARHPGGAMDYWGQGTLLTVVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[1062] SEQ ID NO:251 hu8H24 H1_IgG1_LALA heavy chain amino acid sequence

QAQLVQSGAEVKKPGSSVKVCSKASGYTFTSYSMHWVRQAPGQGLEWIGAIYPGNDATSYNQKFKGRATLTVDKSTSTAYMELSSLRSEDTAVYFCAREGYYGSSFEAWFASWGQGTITVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK

[1063] SEQ ID NO:252 hu5E20 H7_IgG1_LALA heavy chain amino acid sequence

EVQLVESGGGLVQPGGSLRLSCAASGFTFTSYGMSWVRQTPDKRLEWVAIISGGSYTYYSDTVKGRFTISRDNANKNSLYLQMNSLRAEDTAVYYCSRSSSHWYFDVWGQGTLLTVVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS

SLGTQTYICNVNHHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

WHAT IS CLAIMED IS:

1. An isolated monoclonal antibody that competes for binding to human sortilin with antibody 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2.
2. The antibody of claim 1 that binds to the same epitope on human sortilin as antibody 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2.
3. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 5E20, wherein 5E20 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:4 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:10.
4. The antibody of claim 3, wherein the antibody is a humanized antibody.
5. The humanized antibody of claim 4, wherein the CDRs are of a definition selected from the group of Kabat, Chothia, Kabat/Chothia Composite, AbM and Contact.
6. The humanized antibody of claim 5, wherein the humanized mature heavy chain variable region comprises the three Kabat/Chothia Composite heavy chain CDRs of 5E20 (SEQ ID NOS:5-7) and the humanized mature light chain variable region comprises the three Kabat/Chothia Composite light chain CDRs of 5E20 (SEQ ID NOS:11-13).
7. The humanized antibody of claim 5 wherein the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 5E20 (SEQ ID NO:14, SEQ ID NO:6, and SEQ ID NO:7) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 5E20 (SEQ ID NOS:11-13).
8. The humanized antibody of claim 5 wherein the humanized mature heavy chain variable region comprises the three Chothia heavy chain CDRs of 5E20 (SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:7) and the humanized mature light chain variable region comprises the three Chothia light chain CDRs of 5E20 (SEQ ID NOS:11-13).
9. The humanized antibody of claim 5, wherein the humanized mature heavy chain variable region comprises the three AbM heavy chain CDRs of 5E20 (SEQ ID NO:5, SEQ

ID NO:17, and SEQ ID NO:7) and the humanized mature light chain variable region comprises the three AbM light chain CDRs of 5E20 (SEQ ID NOS:11-13).

10. The humanized antibody of claim 5 wherein the humanized mature heavy chain variable region comprises the three Contact heavy chain CDRs of 5E20 (SEQ ID NOS:18-20) and the humanized mature light chain variable region comprises the three Contact light chain CDRs of 5E20 (SEQ ID NOS:21-23).

11. The humanized antibody of any one of claims 4-10 comprising a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:163-169, and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:173-176.

12. The humanized antibody of claim 11 wherein at least one of the following positions in the VH region is occupied by the amino acid as specified: H5 is occupied by L or V, H40 is occupied by A or T, H42 is occupied by G or D, H44 is occupied by G or R, H49 is occupied by A, H77 is occupied by T or S, H83 is occupied by R or K, H93 is occupied by S, H94 is occupied by R.

13. The humanized antibody of claim 12, provided positions H49, H93, and H94 in the VH region are occupied by A, S, and R, respectively.

14. The humanized antibody of claim 12, provided positions H5, H49, H77, H93, and H94 in the VH region are occupied by V, A, S, S, and R, respectively.

15. The humanized antibody of claim 12, provided positions H5, H44, H49, H77, H93, and H94 in the VH region are occupied by V, R, A, S, S, and R, respectively.

16. The humanized antibody of claim 12, provided positions H5, H42, H44, H49, H77, H93, and H94 in the VH region are occupied by V, D, R, A, S, S, and R, respectively.

17. The humanized antibody of claim 12, provided positions H5, H42, H44, H49, H77, H83, H93, and H94 in the VH region are occupied by V, D, R, A, S, K, S, and R, respectively.

18. The humanized antibody of claim 12, provided positions H5, H40, H44, H49, H77, H93, and H94 in the VH region are occupied by V, T, R, A, S, S, and R, respectively.

19. The humanized antibody of claim 12, provided positions H5, H40, H42, H44, H49, H77, H93, and H94 in the VH region are occupied by V, T, D, R, A, S, S, and R, respectively.

20. The humanized antibody of claim 11, wherein at least one of the following positions in the VL region is occupied by the amino acid as specified: L11 is L or V, L36 is L, L44 is F, L46 is G, L69 is A, L85 is T or D, L87 is F, L100 is G or Q, L106 is I or K.

21. The humanized antibody of claim 26, provided positions L36, L44, L46, L69, and L87 in the VL region are occupied by L, F, G, A, and F, respectively.

22. The humanized antibody of claim 26, provided positions L11, L36, L44, L46, L69, and L87 in the VL region are occupied by V, L, F, G, A, and F, respectively.

23. The humanized antibody of claim 26, provided positions L11, L36, L44, L46, L69, L87, L100, and L106 in the VL region are occupied by V, L, F, G, A, F, Q, and K, respectively.

24. The humanized antibody of claim 26, provided positions L11, L36, L44, L46, L69, L85, L87, L100, and L106 in the VL region are occupied by V, L, F, G, A, D, F, Q, and K, respectively.

25. The humanized antibody of claim 11, comprising a mature heavy chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NO: 163-169 and a mature light chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NOS:173-176.

26. The humanized antibody of claim 25, comprising a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:163-169 and a mature light chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:173-176.

27. The humanized antibody of claim 26 wherein the mature heavy chain variable region has an amino acid sequence of any one of SEQ ID NOS:163-169, and the mature light chain variable region has an amino acid sequence of any one of SEQ ID NOS:173-176.

28. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:163 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

29. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:164 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

30. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:165 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

31. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:166 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

32. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:167 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

33. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:168 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

34. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:169 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:173.

35. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:163 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

36. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:164 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

37. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:165 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

38. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:166 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

39. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:167 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

40. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:168 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

41. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:169 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:174.

42. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO: 163 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

43. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO: 164 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

44. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:165 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

45. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:166 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

46. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:167 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

47. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:168 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

48. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:169 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:175.

49. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO: 163 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

50. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO: 164 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

51. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:165 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

52. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:166 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

53. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:167 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

54. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:168 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

55. The humanized antibody of claim 27, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:169 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:176.

56. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 8H24, wherein 8H24 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:28 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:34.

57. The antibody of claim 56, wherein the antibody is a humanized antibody.

58. The humanized antibody of claim 57, wherein the CDRs are of a definition selected from the group of Kabat, Chothia, Kabat/Chothia Composite, AbM and Contact.

59. The humanized antibody of claim 58 wherein the humanized mature heavy chain variable region comprises the three Kabat/Chothia Composite heavy chain CDRs of 8H24 (SEQ ID NOS:29-31) and the humanized mature light chain variable region comprises the three Kabat/Chothia Composite light chain CDRs of 8H24 (SEQ ID NOS:35-37).

60. The humanized antibody of claim 58 wherein the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 8H24 (SEQ ID NO:38, SEQ ID NO:30, and SEQ ID NO:31) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 8H24 (SEQ ID NOS:35-37).

61. The humanized antibody of claim 58 wherein the humanized mature heavy chain variable region comprises the three Chothia heavy chain CDRs of 8H24 (SEQ ID NO:39, SEQ ID NO:40, and SEQ ID NO:31) and the humanized mature light chain variable region comprises the three Chothia light chain CDRs of 8H24 (SEQ ID NOS:35-37).

62. The humanized antibody of claim 58 wherein the humanized mature heavy chain variable region comprises the three AbM heavy chain CDRs of 8H24 (SEQ ID NO:29, SEQ ID NO:41, and SEQ ID NO:31) and the humanized mature light chain variable region comprises the three AbM light chain CDRs of 8H24 (SEQ ID NOS:35-37).

63. The humanized antibody of claim 58 wherein the humanized mature heavy chain variable region comprises the three Contact heavy chain CDRs of 8H24 (SEQ ID NOS:42-44) and the humanized mature light chain variable region comprises the three Contact light chain CDRs of 8H24 (SEQ ID NOS:45-47).

64. The humanized antibody of any one of claims 57-63 comprising a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:180-181, and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:185-186.

65. The humanized antibody of claim 64 wherein at least one of the following positions in the VH region is occupied by the amino acid as specified: H2 is occupied by A, H12 is occupied by K or V, H48 is occupied by I, H67 is occupied by A, H71 is occupied by V, H91 is occupied by F, H108 is occupied by T.

66. The humanized antibody of claim 65, provided positions H2, H48, H67, H71, H91, and H108 in the VH region are occupied by A, I, A, V, F, and T, respectively.

67. The humanized antibody of claim 65, provided H2, H12, H48, H67, H71, H91, and H108 in the VH region are occupied by A, V, I, A, V, F, and T, respectively.

68. The humanized antibody of claim 64, wherein at least one of the following positions in the VL region is occupied by the amino acid as specified: L2 is V, L9 is L or S, L74 is K or T.

69. The humanized antibody of claim 68, provided position L2 in the VL region is occupied by V.

70. The humanized antibody of claim 68, provided positions L2, L9, and L74 in the VL region are occupied by V, S, and T, respectively.

71. The humanized antibody of claim 64, comprising a mature heavy chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NO: 180-181 and a mature light chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NOS:185-186.

72. The humanized antibody of claim 71, comprising a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:180-181 and a mature light chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:185-186.

73. The humanized antibody of claim 72 wherein the mature heavy chain variable region has an amino acid sequence of any one of SEQ ID NOS:180-181, and the mature light chain variable region has an amino acid sequence of any one of SEQ ID NOS:185-186.

74. The humanized antibody of claim 73, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:180 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:185.

75. The humanized antibody of claim 73, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:180 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:186.

76. The humanized antibody of claim 73, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:181 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:185.

77. The humanized antibody of claim 73, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:181 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:186.

78. An antibody specifically binding to sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 11M14, wherein 11M14 is a mouse

antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:52 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:58), except that position L54 can be L, G, or I.

79. The antibody of claim 78, wherein the antibody is a humanized antibody.

80. The humanized antibody of claim 79, wherein the CDRs are of a definition selected from the group of Kabat, Chothia, Kabat/Chothia Composite, AbM and Contact.

81. The humanized antibody of claim 80 wherein the humanized mature heavy chain variable region comprises the three Kabat/Chothia Composite heavy chain CDRs of 11M14 (SEQ ID NOS:53-55) and the humanized mature light chain variable region comprises the three Kabat/Chothia Composite light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I.

82. The humanized antibody of claim 80 wherein the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 11M14 (SEQ ID NO:62, SEQ ID NO:54, and SEQ ID NO:55) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I.

83. The humanized antibody of claim 80 wherein the humanized mature heavy chain variable region comprises the three Chothia heavy chain CDRs of 11M14 (SEQ ID NO:63, SEQ ID NO:64, and SEQ ID NO:55) and the humanized mature light chain variable region comprises the three Chothia light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I.

84. The humanized antibody of claim 80 wherein the humanized mature heavy chain variable region comprises the three AbM heavy chain CDRs of 11M14 (SEQ ID NO:53, SEQ ID NO:65, and SEQ ID NO:55) and the humanized mature light chain variable region comprises the three AbM light chain CDRs of 11M14 (SEQ ID NOS:59-61), except that position L54 can be L, G, or I.

85. The humanized antibody of claim 80 wherein the humanized mature heavy chain variable region comprises the three Contact heavy chain CDRs of 11M14 (SEQ ID NOS:66-68) and the humanized mature light chain variable region comprises the three Contact light chain CDRs of 11M14 (SEQ ID NOS:69-71), except that position L54 can be L, G, or I.

86. The humanized antibody of any one of claims 79-85 comprising a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:190-192, and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to any one of SEQ ID NOS:196-199.

87. The humanized antibody of claim 86 wherein at least one of the following positions in the VH region is occupied by the amino acid as specified: H49 is occupied by A, H80 is occupied by L or G, H82c is occupied by L or G.

88. The humanized antibody of claim 87, provided position H49 in the VH region is occupied by A.

89. The humanized antibody of claim 87, provided positions H49 and H82c in the VH region are occupied by A and G, respectively.

90. The humanized antibody of claim 87, provided positions H49 and H80 in the VH region are occupied by A and G, respectively.

91. The humanized antibody of claim 86, wherein at least one of the following positions in the VL region is occupied by the amino acid as specified: L43 is A or S, L48 is V, L54 is L, G, or I, L71 is Y, L76 is N or S.

92. The humanized antibody of claim 91, provided positions L48 and L71 in the VL region are occupied by V and Y respectively.

93. The humanized antibody of claim 91, provided positions L43, L48, L71, and L76 in the VL region are occupied by S, V, Y, and S, respectively.

94. The humanized antibody of claim 91, provided positions L43, L48, L54, L71, and L76 in the VL region are occupied by S, V, G, Y, and S, respectively.

95. The humanized antibody of claim 91 provided positions L43, L48, L54, L71, and L76 in the VL region are occupied by S, V, I, Y, and S, respectively.

96. The humanized antibody of claim 86, comprising a mature heavy chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NO: 190-192 and a mature light chain variable region having an amino acid sequence at least 95% identical to at least one of SEQ ID NOS:196-199.

97. The humanized antibody of claim 96, comprising a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:190-192 and a mature light chain variable region having an amino acid sequence at least 98% identical to SEQ ID NOS:196-199.

98. The humanized antibody of claim 97 wherein the mature heavy chain variable region has an amino acid sequence of any one of SEQ ID NOS:190-192, and the mature light chain variable region has an amino acid sequence of any one of SEQ ID NOS:196-199.

99. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:196.

100. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:197.

101. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:198.

102. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:190 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:199.

103. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:196.

104. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:197.

105. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:198.

106. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:191 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:199.

107. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:196.

108. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:197.

109. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:198.

110. The humanized antibody of claim 98, wherein the mature heavy chain variable region has an amino acid sequence of SEQ ID NO:192 and the mature light chain variable region has an amino acid sequence of SEQ ID NO:199.

111. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 5M13, wherein 5M13 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence

comprising SEQ ID NO:78 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:84.

112. The antibody of claim 111, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:79-81) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:85-87).

113. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 2F18, wherein 2F18 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:90 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:96.

114. The antibody of claim 113, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:91-93) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:97-99).

115. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 2P22, wherein 2P22 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:102 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:108.

116. The antibody of claim 115, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:103-105) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:109-111).

117. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 6B15, wherein 6B15 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:114 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:120.

118. The antibody of claim 117, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:115-117) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:121-123).

119. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 2C14, wherein 2C14 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:126 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:132.

120. The antibody of claim 119, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:127-129) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:133-135).

121. An antibody specifically binding to human sortilin, comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 9N18, wherein 9N18 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:138 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:144.

122. The antibody of claim 121, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:139-141) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:145-147).

123. An antibody specifically binding to human sortilin comprising three light chain CDRs and three heavy chain CDRs of monoclonal antibody 4N2, wherein 4N2 is a mouse antibody characterized by a heavy chain variable region having an amino acid sequence comprising SEQ ID NO:150 and a light chain variable region having an amino acid sequence comprising SEQ ID NO:156.

124. The antibody of claim 123, wherein the three heavy chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:151-153) and the three light chain CDRs are as defined by Kabat-Chothia Composite (SEQ ID NOS:157-159).

125. The antibody of any one of claims 111-124 that is a humanized antibody.
126. The antibody of any one of claims 1-125 that is an intact antibody.
127. The antibody of any one of claims 1-125 that is a binding fragment.
128. The antibody of any one of the preceding claims, wherein the isotype is human IgG1.
129. The antibody of any one of claims 1-125 wherein the mature light chain variable region is fused to a light chain constant region and the mature heavy chain variable region is fused to a heavy chain constant region.
130. The antibody of claim 129, wherein the heavy chain constant region is a mutant form of a natural human heavy chain constant region which has reduced binding to a Fc γ receptor relative to the natural human heavy chain constant region.
- 131.. The antibody of claim 129, wherein the heavy chain constant region is a mutant form of a natural human heavy chain constant region which has enhanced binding to a neonatal Fc γ receptor relative to the natural human heavy chain constant region.
132. The antibody of claim 129, comprising a heavy chain of SEQ ID NO:244 and a light chain of SEQ ID NO:245.
133. The antibody of claim 129, comprising a heavy chain of SEQ ID NO:246 and a light chain of SEQ ID NO:247.
134. The antibody of claim 129, comprising a heavy chain of SEQ ID NO:248 and a light chain of SEQ ID NO:249.
135. The antibody of claim 129, comprising a heavy chain of SEQ ID NO:250 and a light chain of SEQ ID NO:245.
136. The antibody of claim 129, comprising a heavy chain of SEQ ID NO:251 and a light chain of SEQ ID NO:247.

137. The antibody of claim 129, comprising a heavy chain of SEQ ID NO:252 and a light chain of SEQ ID NO:249.

138. The antibody of any one of claims 1-127 and 129-137 wherein the isotype is of human IgG2 or IgG4 isotype.

139. A pharmaceutical composition comprising an antibody as defined in any of claims 1-138 and a pharmaceutically-acceptable carrier.

140. A nucleic acid encoding the heavy chain and/or light chain of an antibody as described in any one of claims 1-139.

141. A method of humanizing a mouse antibody, the method comprising:

- (a) selecting one or more acceptor antibody sequences;
- (b) identifying amino acid residues of the mouse antibody to be retained;
- (c) synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and

(d) expressing the nucleic acids in a host cell to produce a humanized antibody;

wherein the mouse antibody is 5E20, wherein 5E20 is characterized by a mature heavy chain variable region of SEQ ID NO:4 and a mature light chain variable region of SEQ ID NO:10,

wherein the mouse antibody is 8H24, wherein 8H24 is characterized by a mature heavy chain variable region of SEQ ID NO:28 and a mature light chain variable region of SEQ ID NO:34,

wherein the mouse antibody is 11M14, wherein 11M14 is characterized by a mature heavy chain variable region of SEQ ID NO:52 and a mature light chain variable region of SEQ ID NO:58,

wherein the mouse antibody is 5M13, wherein 5M13 is characterized by a mature heavy chain variable region of SEQ ID NO:78 and a mature light chain variable region of SEQ ID NO:84,

wherein the mouse antibody is 2F18, wherein 2F18 is characterized by a mature heavy chain variable region of SEQ ID NO:90 and a mature light chain variable region of SEQ ID NO:96,

wherein the mouse antibody is 2P22, wherein 2P22 is characterized by a mature heavy chain variable region of SEQ ID NO:102 and a mature light chain variable region of SEQ ID NO:108,

wherein the mouse antibody is 6B15, wherein 6B15 is characterized by a mature heavy chain variable region of SEQ ID NO:114 and a mature light chain variable region of SEQ ID NO:120,

wherein the mouse antibody is 2C14, wherein 2C14 is characterized by a mature heavy chain variable region of SEQ ID NO:126 and a mature light chain variable region of SEQ ID NO:132,

wherein the mouse antibody is 9N18, wherein 9N18 is characterized by a mature heavy chain variable region of SEQ ID NO:138 and a mature light chain variable region of SEQ ID NO:144,

wherein the mouse antibody is 4N2, wherein 4N2 is characterized by a mature heavy chain variable region of SEQ ID NO:150 and a mature light chain variable region of SEQ ID NO:156.

142. A method of producing a humanized, chimeric, or veneered antibody, the method comprising:

(a) culturing cells transformed with nucleic acids encoding the heavy and light chains of the antibody, so that the cells secrete the antibody; and

(b) purifying the antibody from cell culture media;

wherein the antibody is a humanized, chimeric, or veneered form of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2.

143. A method of producing a cell line producing a humanized, chimeric, or veneered antibody, the method comprising:

(a) introducing a vector encoding heavy and light chains of an antibody and a selectable marker into cells;

(b) propagating the cells under conditions to select for cells having increased copy number of the vector;

(c) isolating single cells from the selected cells; and

(d) banking cells cloned from a single cell selected based on yield of antibody;

wherein the antibody is a humanized, chimeric, or veneered form of 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2.

144. The method of claim 143 further comprising propagating the cells under selective conditions and screening for cell lines naturally expressing and secreting at least 100 mg/L/ 10^6 cells/24 h.

145. A method of increasing progranulin levels in a subject having or at risk of developing a disease or disorder associated with changes in progranulin levels, comprising administering to the subject an effective dose of the antibody of any one of claims 1-140, thereby increasing progranulin levels in the subject.

146. A method of treating or effecting prophylaxis of a disease or disorder associated with changes in progranulin levels in a subject, comprising administering an effective dose of an antibody as defined by any one of claims 1-140 and thereby treating or effecting prophylaxis of the disease or disorder.

147. A method of claim 146, further comprising detecting progranulin levels in a subject.

148. A method of claim 147, further comprising monitoring progranulin levels in a subject.

149. A method of claim 146, wherein the disease or disorder associated with changes in progranulin levels is frontotemporal dementia, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, Batten disease, a neurodegenerative disorder, or a neurodegenerative disorder associated with aging.

150. A method of claim 149, wherein the disease or disorder associated with changes in progranulin levels is frontotemporal dementia.

151. A method of detecting sortilin in a subject having or at risk of a disease associated with a disease or disorder associated with changes in progranulin levels, comprising administering to a subject an antibody defined by any one of claims 1-140, and detecting the antibody bound to sortilin in the subject.

152. An isolated monoclonal antibody that specifically binds to a peptide consisting of residues FTESFLT (SEQ ID NO:202).

153. An isolated monoclonal antibody that specifically binds to a peptide consisting of residues ESFL (SEQ ID NO:203).

154. An isolated monoclonal antibody that specifically binds to human sortilin at an epitope within a motif of the formula E(S/Q/D)FL (SEQ ID NO:206).

155. An isolated monoclonal antibody that specifically binds to a peptide consisting of residues DGCILGYKEQFL (SEQ ID NO:204).

156. An isolated monoclonal antibody that specifically binds to a peptide consisting of residues PSICLCSLEDL (SEQ ID NO:205).

157. An isolated monoclonal antibody that specifically binds to a peptide consisting of residues RTEFGMAIGP (SEQ ID NO:213),

158. An isolated monoclonal antibody that specifically binds to a peptide consisting of residues WGFTEFLTS (SEQ ID NO:214).
159. An isolated monoclonal antibody that specifically binds an epitope defined by amino acid residues D74, R76, F97, K110, Y535, L560, and E557 of SEQ ID NO:215.
160. An isolated monoclonal antibody that specifically binds an epitope defined by amino acid residues K110, Y535, E557, T561, Q563, D74, P510, S558, F559, and L560 of SEQ ID NO:215.
161. An isolated monoclonal antibody that specifically binds an epitope defined by amino acid residues E557, S558, F559, L560, P510, and Y535 of SEQ ID NO:215.
162. A method of treating or effecting prophylaxis of a disease or disorder associated with changes in progranulin levels in a subject comprising administering an immunogen comprising a sortilin peptide of up to 20 contiguous amino acids of SEQ ID NO:1 to which antibody 5E20, 8H24, 11M14, 5M13, 2F18, 2P22, 6B15, 2C14, 9N18, or 4N2 specifically binds, wherein the peptide induces formation of antibodies specifically binding to sortilin in the subject.