VARIABLE REGION SEQUENCES

C2N Grafted V Region Sequences

Light Chain

	CDR1	CDR2	CDR3	
MuVL	DIVLTQSPASLAVSLGQRATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPARFSGSGSGTDFTLNIHPLEEEDAATYYCHHSWEIPLTFGAGTKLELK			(SEQ ID NO:11)
VK1	DIVLTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDFTLNIHPLEEEDFATYYCHHSWEIPLTFGQGTKLEIK			(6) (SEQ ID NO:1)
VK2	DIVLTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDFTLNIHPLEPEDFATYYCHHSWEIPLTFGQGTKLEIK			(7) (SEQ ID NO:2)
VK3	DIVLTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHHSWEIPLTFGQGTKLEIK			(11) (SEQ ID NO:3)
VK4	DIVMTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCHHSWEIPLTFGQGTKLEIK			(12) (SEQ ID NO:4)

Heavy Chain

	CDR1	CDR2	CDR3	
MuVH	EVKVEESGGGLVQPGGSMKLSCVVSGFTFSNYWVNWVRQSPEKGLEWVAQIRLKSDNYATHYEESVKGRFTISRDDSKSSVYLQMNNLRAEDSGIYYCTNWEDYWGQGTTLTVSSASTKGPSVF			
	(SEQ ID NO:12)			
VH1	EVKVVESGGGLVQPGGSMKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRDDSKSSVYLQMNNLRAEDSGIYYCTNWEDYWGQGTTVTVSSASTKGPSVF			(4)
	(SEQ ID NO:5)			
VH2	EVKVVESGGGLVQPGGSLKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRDDSKSSVYLQMNSLRAEDTGIYYCTNWEDYWGQGTTVTVSSASTKGPSVF			(7)
	(SEQ ID NO:6)			
VH3	EVQVVESGGGLVQPGGSLKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRDDSKNSVYLQMNSLRAEDTAIYYCTNWEDYWGQGTTVTVSSASTKGPSVF			(10)
	(SEQ ID NO:7)			
VH4	EVQLVESGGGLVQPGGSLKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRDDSKNSLYLQMNSLRAEDTAIYYCTNWEDYWGQGTTVTVSSASTKGPSVF			(12)
	(SEQ ID NO:8)			

Key:

Mu: Denotes the murine sequence
VH: Denotes the humanized variable heavy chain
VL: Denotes the humanized variable light chain
CDR: Complementary determining region
AAA: Framework changes proposed by Antitope

FIG. 1

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variants

Grafted Heavy Chain Variants

gVH1 Theoretical pI/Mw: 6.80 / 48822.90

EVKVVESGGGLVQPGGSMKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRD
DSKSSVYLQMNNLRAEDSGIYYCTNWEDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
(SEQ ID NO:13)

FIG. 2A

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variants

Grafted Heavy Chain Variants

gVH2 Theoretical pI/Mw: 6.80/48791.87

EVKVVESGGGLVQPGGSLKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRD
DSKSSVYLQMNSLRAEDTGIYYCTNWEDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
(SEQ ID NO:14)

FIG. 2B

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variants

Grafted Heavy Chain Variants

gVH3 Theoretical pI/Mw: 6.51 /48832.88

EVQVVESGGGLVQPGGSLKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRD
DSKNSVYLQMNSLRAEDTAIYYCTNWEDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
(SEQ ID NO:15)

FIG. 2C

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variants

Grafted Heavy Chain Variants

gVH4 Theoretical pI/Mw: 6.51 /48860.93

EVQLVESGGGLVQPGGSLKLSCVVSGFTFSNYWVNWVRQAPGKGLEWVAQIRLKSDNYATHYEESVKGRFTISRD
DSKNSLYLQMNSLRAEDTAIYYCTNWEDYWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPE
PVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCP
APEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK
(SEQ ID NO:16)

FIG. 2D

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variant

Grafted Light Chain Variants

gVL1 Theoretical pI/Mw: 6.08 / 24100.81

DIVLTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDF
TLNIHPLEEEDFATYYCHHSWEIPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:17)

FIG. 2E

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variant

Grafted Light Chain Variants

gVL2 Theoretical pI/Mw: 6.33 / 24068.81

DIVLTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDF
TLNIHPLEPEDFATYYCHHSWEIPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:18)

FIG. 2F

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variant

Grafted Light Chain Variants

gVL3 Theoretical pI/Mw: 6.57 / 23994.72

DIVLTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCHHSWEIPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:19)

FIG. 2G

Full Amino Acid Sequences of C_2N Heavy Chain and Light Chain Variant

Grafted Light Chain Variants

gVL4 Theoretical pI/Mw: 6.57 / 24012.76

DIVMTQSPDSLAVSLGERATISCRASQSVSTSRYSYIHWYQQKPGQPPKLLIKYASNLESGVPSRFSGSGSGTDF
TLTISSLQPEDFATYYCHHSWEIPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV
QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO:20)

FIG. 2H

Transient expression data from humanized variants

Humanized Variant	Transient expression Round 1 (ug/mL)	Transient expression Round 2 (ug/mL)
VH1/VK1	9.0	
VH1/VK2	13.4	13.1
VH1/VK3	22.2	20.2
VH1/VK4	12.4	
VH2/VK1	6.5	
VH2/VK2	11.3	6.2
VH2/VK3	13.7	13.8
VH2/VK4	9.3	
VH3/VK1	7.3	
VH3/VK2	12.0	8.1
VH3/VK3	15.2	15.9
VH3/VK4	6.4	
VH4/VK3	3.6	

FIG. 3

Potency assay

Antibody	IC50 (relative to chimera)		
	C_2N run 1	C_2N run 2	External
Chimera	1.00	1.00	1.00
VH1/VK1	1.49	1.11	0.84
VH1/VK2	1.34	0.91	0.78
VH1/VK3.1	1.58	0.92	0.79
VH1/VK3.2	1.93	1.07	1.02
VH1/VK4	2.05	1.02	1.41
VH2/VK1	1.43	1.02	1.02
VH2/VK2	1.37	0.85	1.12
VH2/VK3	1.17	0.97	0.94
VH2/VK4	1.36	1.03	1.37
VH3/VK1	1.16	1.42	1.04
VH3/VK2	0.81	1.28	0.94
VH3/VK3	0.84	1.34	0.93
VH3/VK4	0.89	1.22	1.51
VH4/VK3	1.79	1.9	1.31
HJ8.5	0.79	0.99	1.07

FIG. 4

Antibody binding kinetics against human tau

Humanized Variant	Association constant K_a (1/Ms)	Dissociation constant K_d (1/s)	Affinity K_D (M)
Chimera	7.21E+05	1.43E-03	1.99E-09
VH1/VK2	3.02E+05	6.21E-04	2.06E-09
VH1/VK4	2.87E+05	5.99E-04	2.09E-09
VH2/VK3	2.73E+05	6.08E-04	2.23E-09
VH2/VK4	2.80E+05	5.93E-04	2.13E-09
VH3/VK3	2.76E+05	5.15E-04	1.88E-09
VH3/VK4	2.65E+05	5.07E-04	1.92E-09

FIG. 5

Binding of humanized antibo Binding of humanized

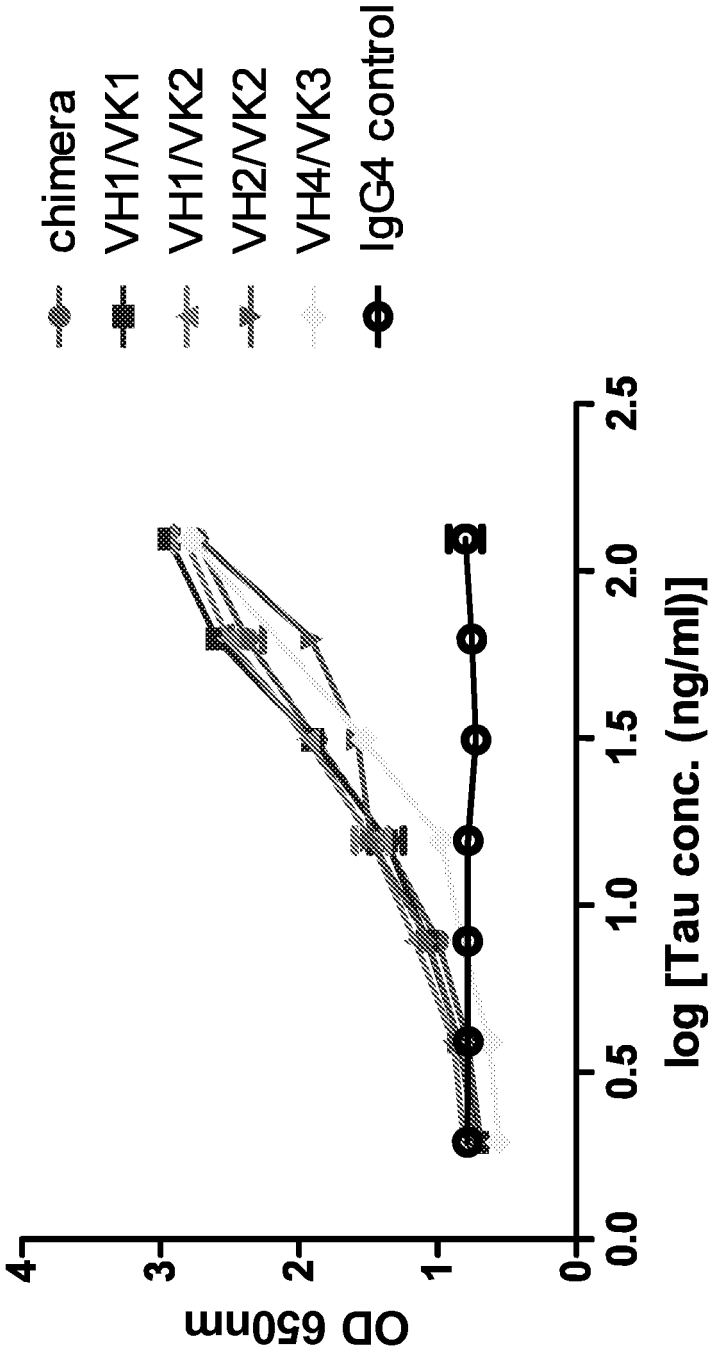
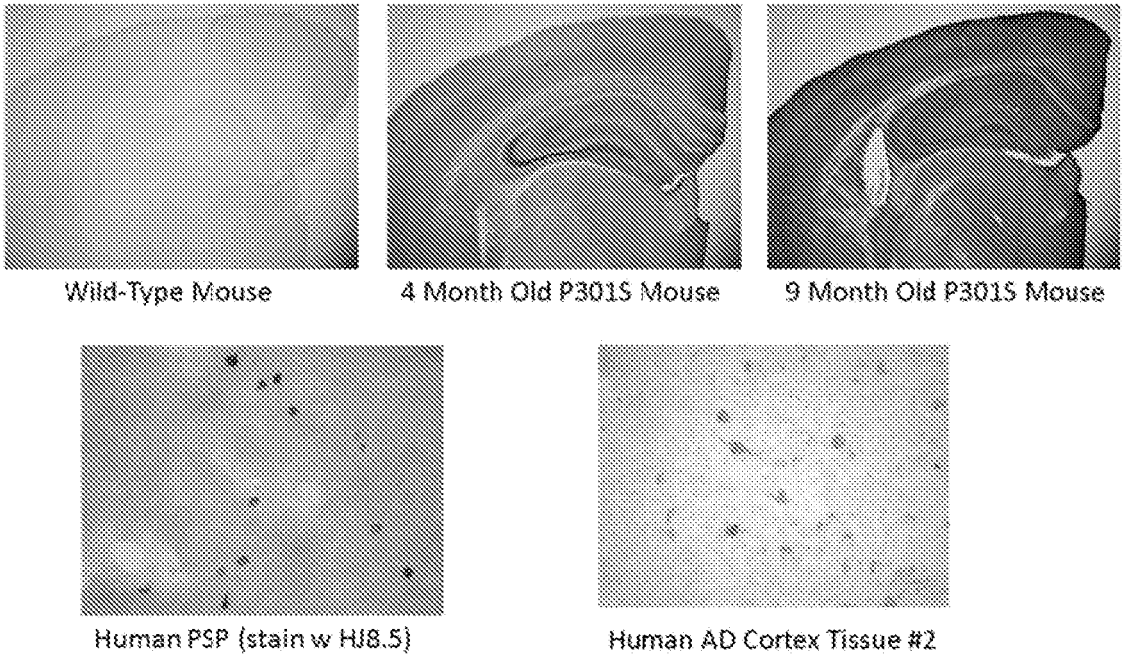
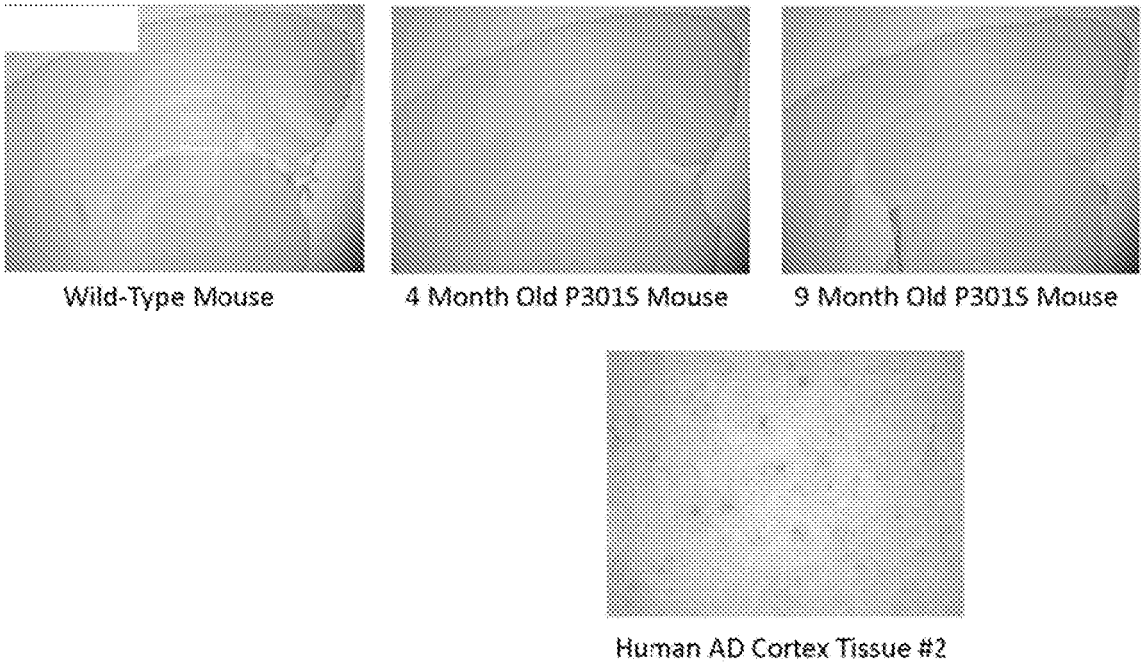


FIG. 6

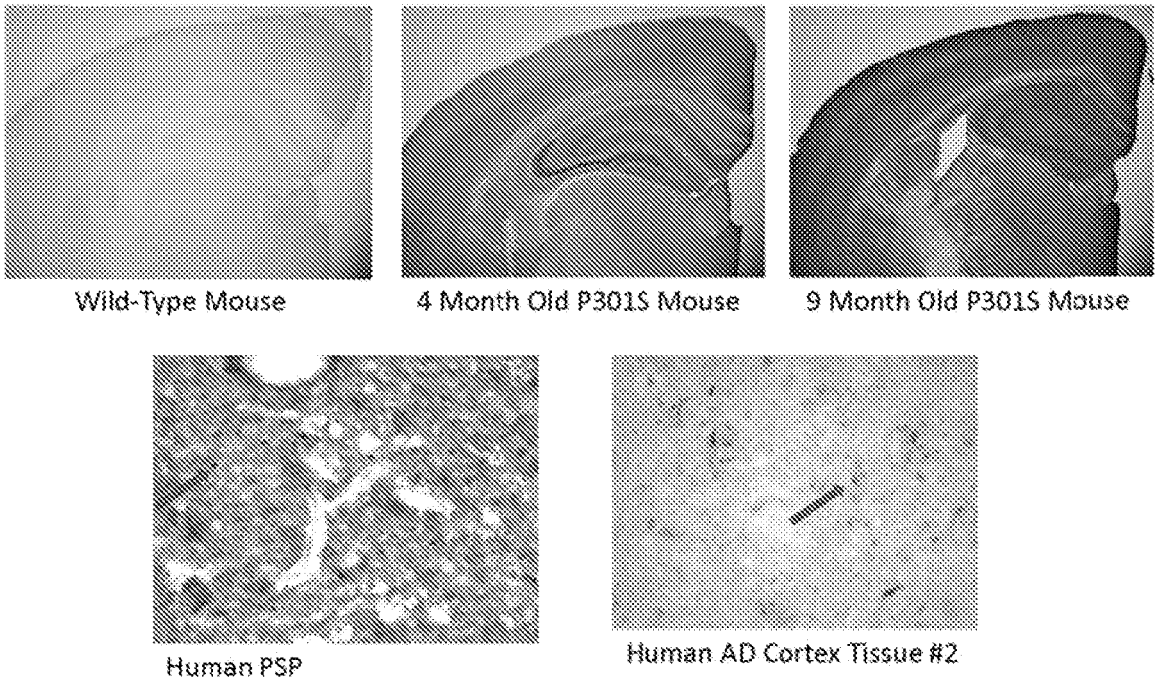
Binding of humanized antibodies to human tau in human and mouse brain



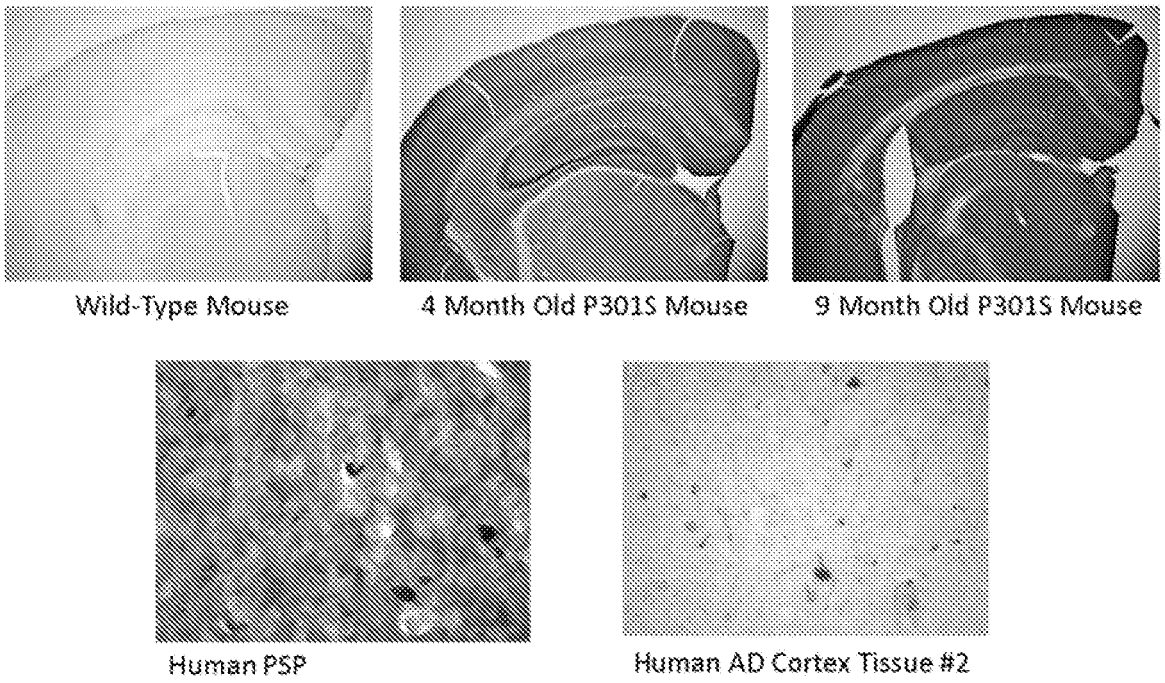
Binding of chimera (positive control) to mouse and human tissue
FIG. 7A



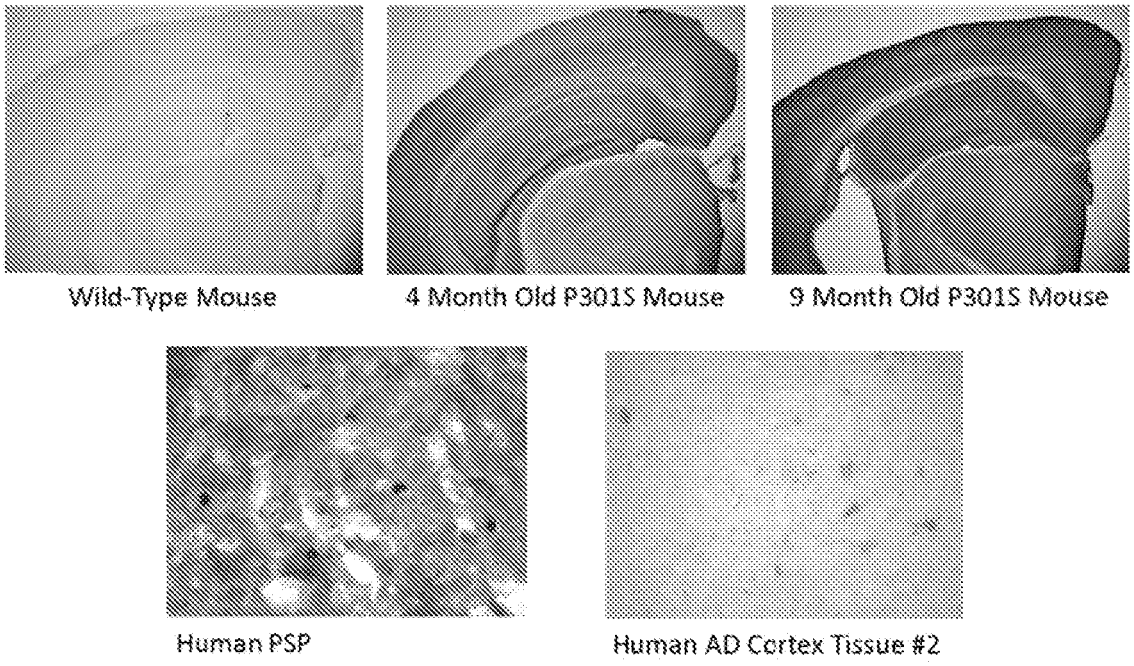
Binding of non-specific human IgG4 (negative control) to mouse and human tissue
FIG. 7B



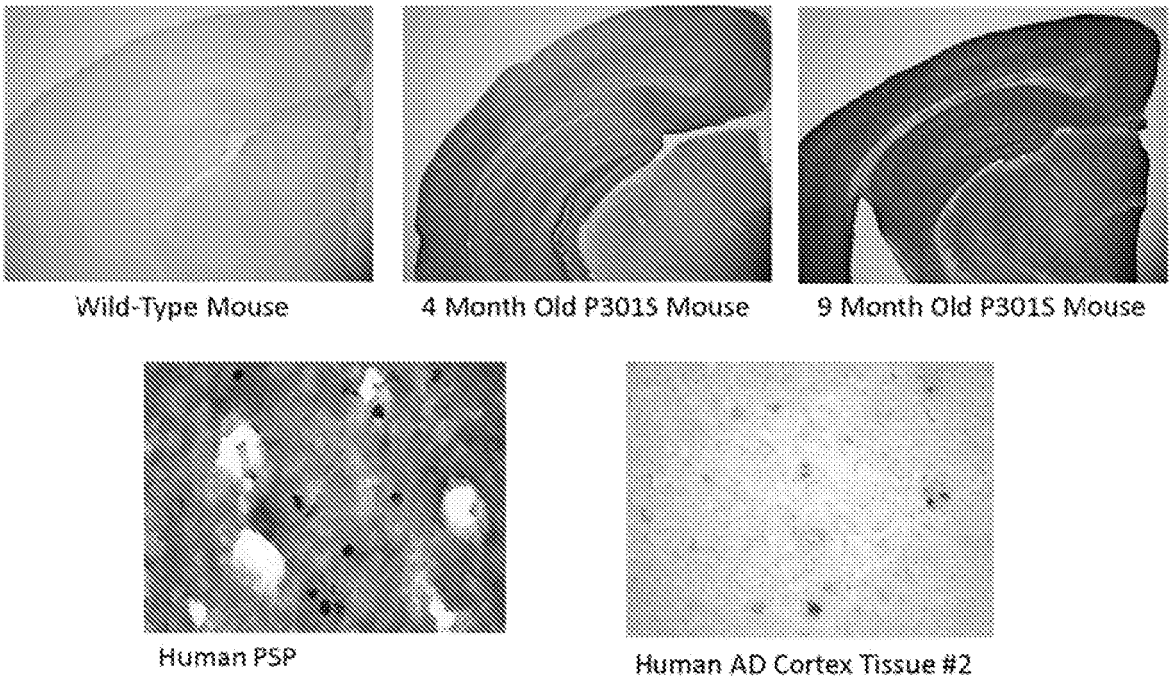
Binding of VH1/VK2 to mouse and human tissue
FIG. 7C



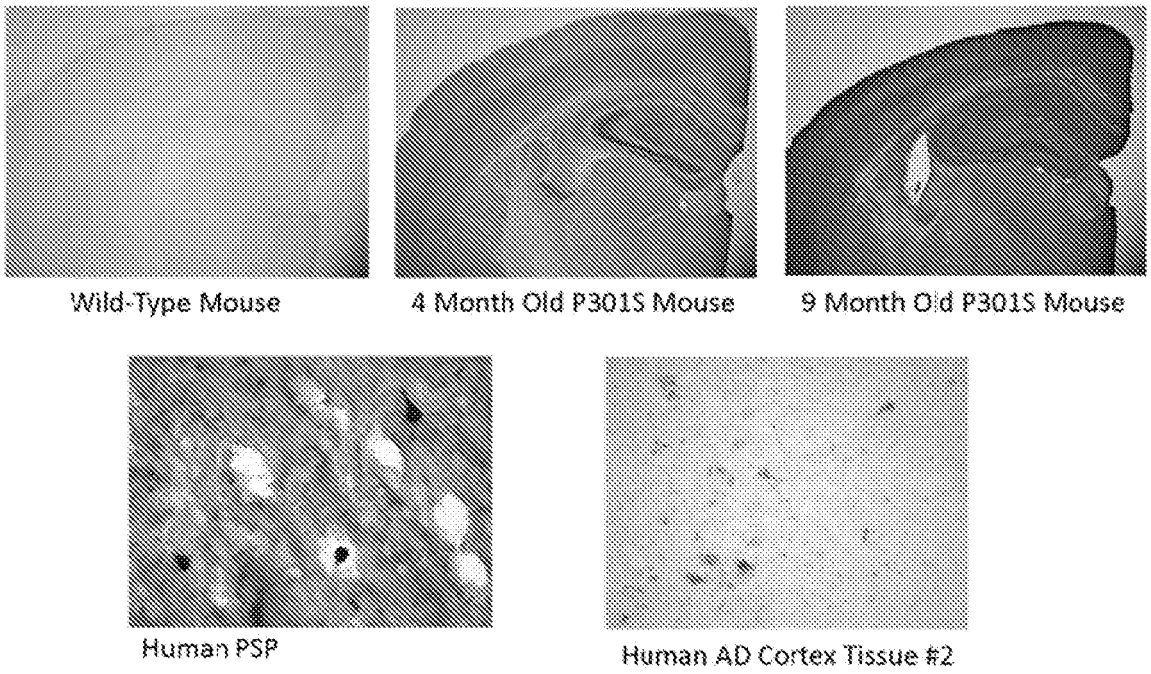
Binding of VH1/VK3 to mouse and human tissue
FIG. 7D



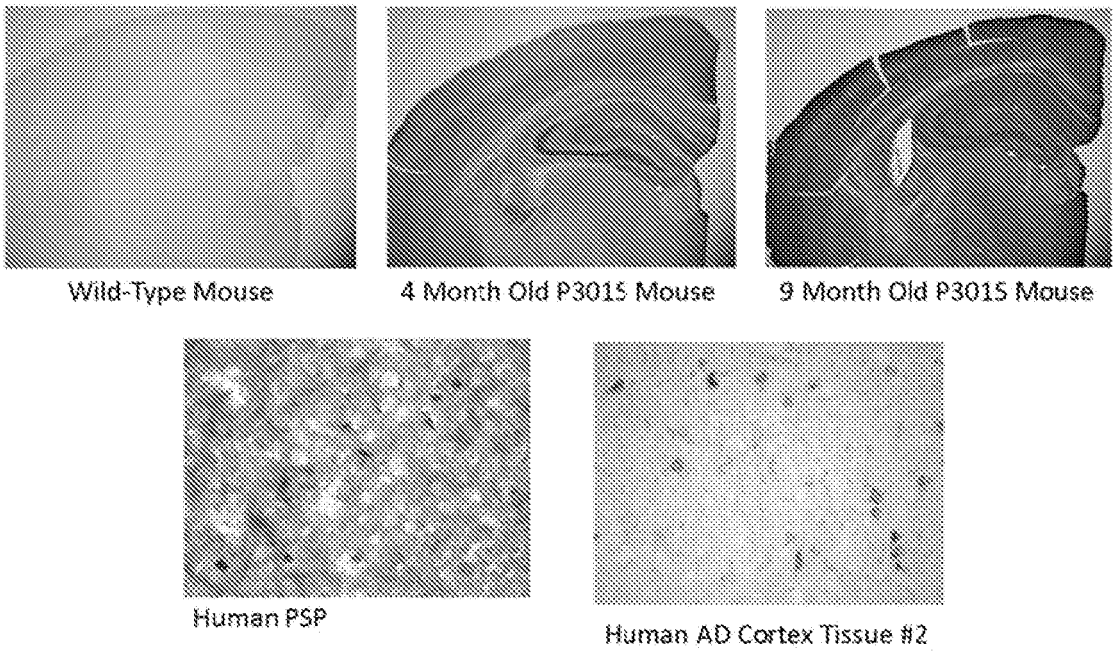
Binding of VH2/VK2 to mouse and human tissue
FIG. 7E



Binding of VH2/VK3 to mouse and human tissue
FIG. 7F



Binding of VH3/VK2 to mouse and human tissue
FIG. 7G



Binding of VH3/VK3 to mouse and human tissue
FIG. 7H

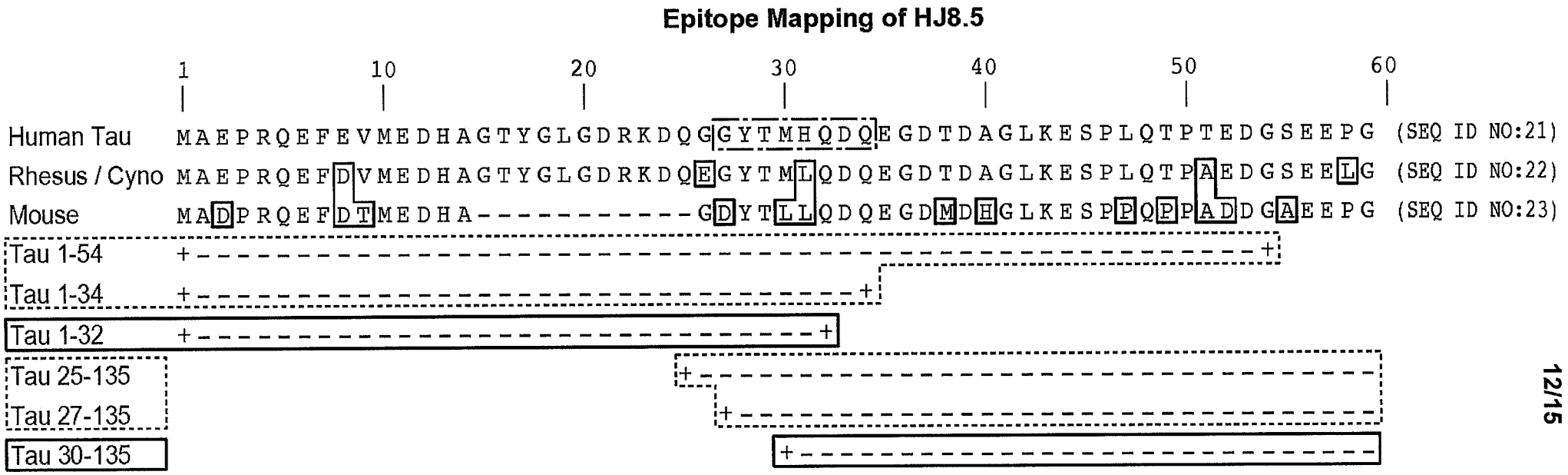

Epitope Mapping of HJ8.5
1
10
20
30
40
50
60
Human Tau MAEPRQEFEVMEDHAGTYGLGDRKDQGGYTMHQDQEGDTDAGLKESPLQTPTEDGSEEPG (SEQ ID NO:21)
Rhesus / Cyno MAEPRQEFDVMEDHAGTYGLGDRKDQEGYTMLQDQEGDTDAGLKESPLQTPAEDGSEELG (SEQ ID NO:22)
Mouse MADPRQEFDTMEDHA----------GDYTLLQDQEGDMDHGLKESPPQPPADDGAEEPG (SEQ ID NO:23)
Tau 1-54
Tau 1-34
Tau 1-32
Tau 25-135
Tau 27-135
Tau 30-135

FIG. 8

Epitope Mapping of C_2N-8E12 and HJ8.5

Antibodies	Core Epitopes
HJ8.5 and C_2N-8E12	$_{25}$DQGGYT$_{30}$ (SEQ ID NO:9)

PepLabData	SEQUENCE	Identifier	Label	HJ8.5 (Binding Signal)	C_2N-8E12 (Binding Signal)
PEP_2875800	DHAGTYGLGDRKDQG	SEQ ID NO:24	LIN	61	78
PEP_2875801	DHAGTYGLGAAKDQG	SEQ ID NO:25	LIN.AA	52	90
PEP_2875802	HAGTYGLGDRKDQGG	SEQ ID NO:26	LIN	63	65
PEP_2875803	HAGTYGLGDAADQGG	SEQ ID NO:27	LIN.AA	51	65
PEP_2875804	AGTYGLGDRKDQGGY	SEQ ID NO:28	LIN	427	1286
PEP_2875805	AGTYGLGDRAAQGGY	SEQ ID NO:29	LIN.AA	58	76
PEP_2875806	GTYGLGDRKDQGGYT	SEQ ID NO:30	LIN	2638	1714
PEP_2875807	GTYGLGDRKAAGGYT	SEQ ID NO:31	LIN.AA	110	114
PEP_2875808	TYGLGDRKDQGGYTM	SEQ ID NO:32	LIN	2640	2588
PEP_2875809	TYGLGDRKDAAGYTM	SEQ ID NO:33	LIN.AA	2814	2755
PEP_2875810	YGLGDRKDQGGYTMH	SEQ ID NO:34	LIN	2844	2671
PEP_2875811	YGLGDRKDQAAYTMH	SEQ ID NO:35	LIN.AA	1780	1876
PEP_2875812	GLGDRKDQGGYTMHQ	SEQ ID NO:36	LIN	2824	2729
PEP_2875813	GLGDRKDQGAATMHQ	SEQ ID NO:37	LIN.AA	80	65
PEP_2875814	LGDRKDQGGYTMHQD	SEQ ID NO:38	LIN	2835	2749
PEP_2875815	LGDRKDQGGAAMHQD	SEQ ID NO:39	LIN.AA	69	99
PEP_2875816	GDRKDQGGYTMHQDQ	SEQ ID NO:40	LIN	2647	2699
PEP_2875817	GDRKDQGGYAAHQDQ	SEQ ID NO:41	LIN.AA	2635	661
PEP_2875818	DRKDQGGYTMHQDQE	SEQ ID NO:42	LIN	2692	2788
PEP_2875819	DRKDQGGYTAAQDQE	SEQ ID NO:43	LIN.AA	2697	2585
PEP_2875820	RKDQGGYTMHQDQEG	SEQ ID NO:44	LIN	2699	2779
PEP_2875821	RKDQGGYTMAADQEG	SEQ ID NO:45	LIN.AA	2713	2791
PEP_2875822	KDQGGYTMHQDQEGD	SEQ ID NO:46	LIN	2701	2680
PEP_2875823	KDQGGYTMHAAQEGD	SEQ ID NO:47	LIN.AA	2707	2712
PEP_2875824	DQGGYTMHQDQEGDT	SEQ ID NO:48	LIN	2677	2648
PEP_2875825	DQGGYTMHQAAEGDT	SEQ ID NO:49	LIN.AA	2707	2585
PEP_2875826	QGGYTMHQDQEGDTD	SEQ ID NO:50	LIN	399	789
PEP_2875827	QGGYTMHQDAAGDTD	SEQ ID NO:51	LIN.AA	427	636
PEP_2875828	GGYTMHQDQEGDTDA	SEQ ID NO:52	LIN	109	110
PEP_2875829	GGYTMHQDQAADTDA	SEQ ID NO:53	LIN.AA	106	118
PEP_2875830	GYTMHQDQEGDTDAG	SEQ ID NO:54	LIN	77	97

FIG. 9

Binding of humanized anti-tau antibody to tau from rhesus monkeys

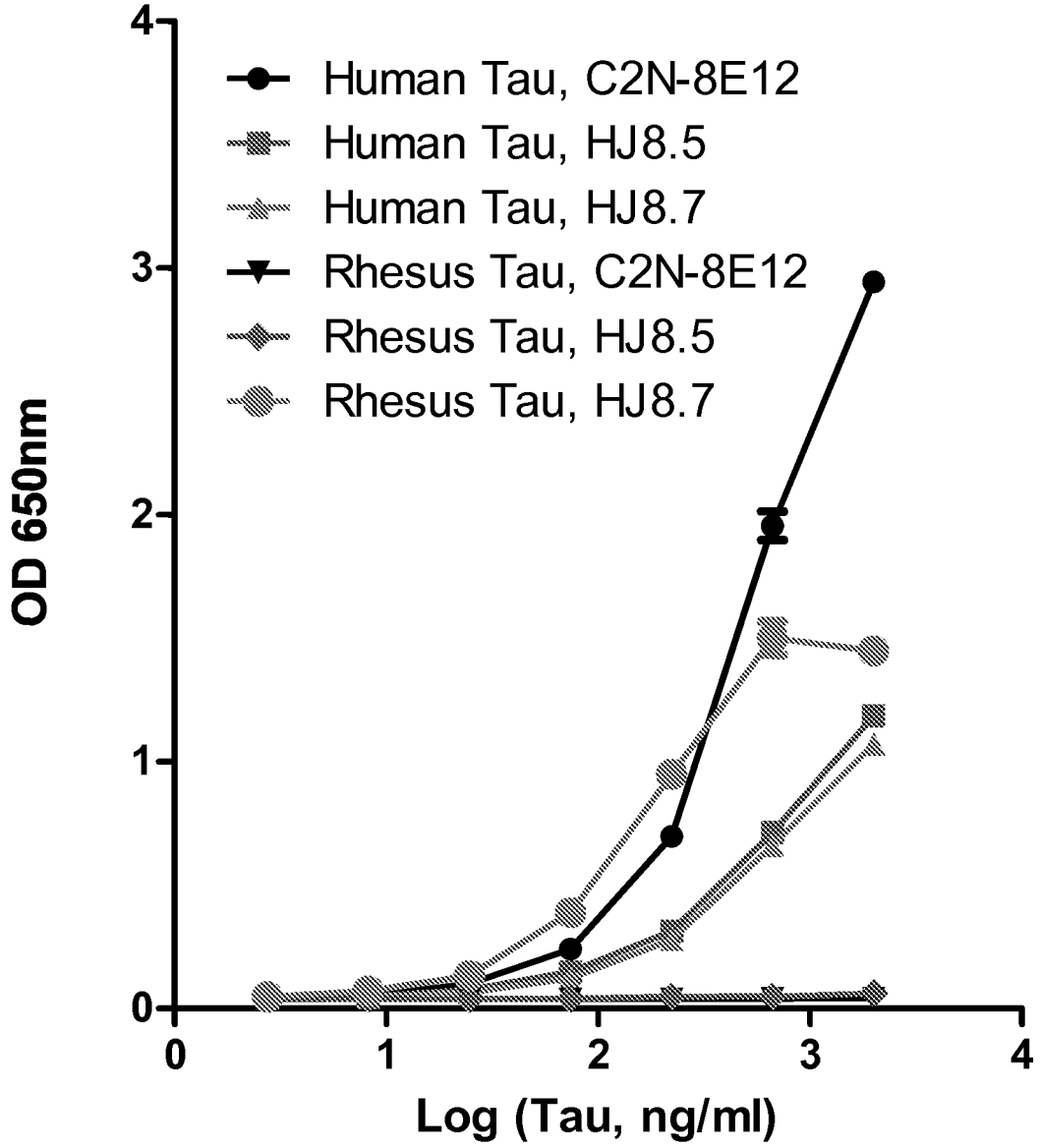


FIG. 10

Binding of humanized anti-tau antibody to tau in CSF from people with various tauopathies

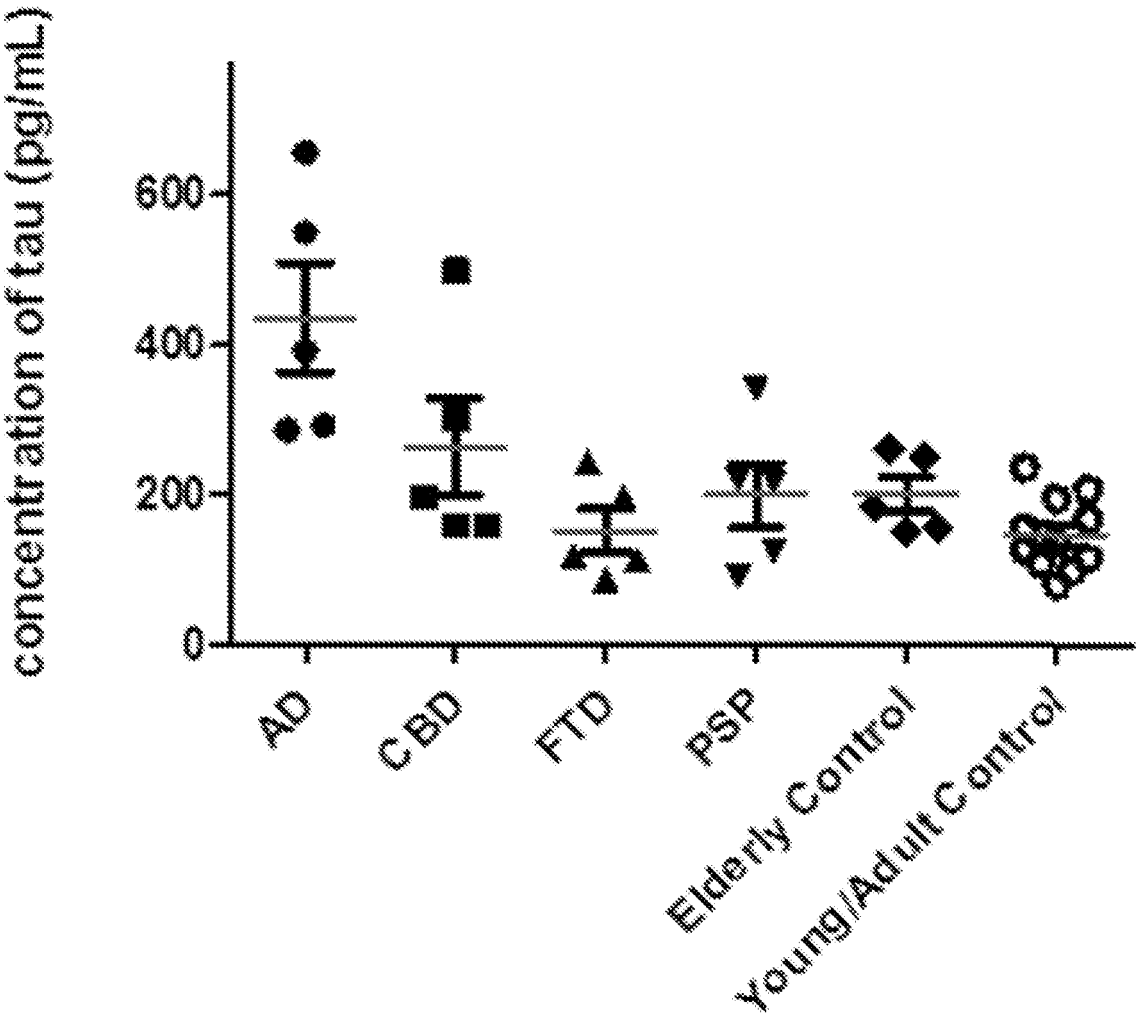


FIG. 11