SUPPORTING INFORMATION

Identification of Alzheimer's Disease Autoantibodies and Their Target Biomarkers by Phage Microarrays

Pablo San Segundo-Acosta[†,‡,#], Ana Montero-Calle[‡,#], Manuel Fuentes[¶,ç], Alberto Rábano[§], Mayte Villalba[†], Rodrigo Barderas[‡,*]

[#]These authors share authorship.

[†]Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, E-28040 Madrid, Spain
[‡]Chronic Disease Programme (UFIEC), Instituto de Salud Carlos III, Majadahonda E-28220, Madrid, Spain
[¶]Proteomics Unit, Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), 37007, Salamanca, Spain.
[ç]Department of Medicine and General Cytometry Service-Nucleus, CIBERONC CB16/12/00400, Cancer Research Centre (IBMCC/CSIC/USAL/IBSAL), 37007, Salamanca, Spain.
[§]Alzheimer Disease Research Unit, CIEN Foundation, Queen Sofia Foundation Alzheimer Center, Madrid, Spain

[*]To whom correspondence should be addressed:
Rodrigo Barderas.
Chronic Disease Programme (UFIEC), Instituto de Salud Carlos III, E-28222 Majadahonda, Madrid, Spain; Tel.: 34-91-8223231; E-mail: r.barderasm@isciii.es

CONTENTS **PAGE/S**

Table S1	**S3-S7**
Table S2	**S8**
Table S3	**S9-S11**
Figure S1	**S12**
Figure S2	**S13**

Table S1-1. Information on Alzheimer Disease (AD) patients used for validation.

Code Number	Age (years)	Braak stage	Gender
AD1	62	V	Male
AD2	80	IV	Male
AD3	85	V	Female
AD4	92	V	Male
AD5	85	III	Male
AD6	85	VI	Female
AD7	94	III	Female
AD8	92	IV	Female
AD9	82	V	Female
AD10	87	III	Female
AD11	86	V	Male
AD12	90	III	Female
AD13	80	V	Male
AD14	91	V	Female
AD15	94	VI	Male
AD16	91	VI	Female
AD17	92	VI	Female
AD18	94	V	Female
AD19	75	V	Female
AD20	82	III	Female
AD21	87	IV	Female
AD22	94	V	Female
AD23	92	VI	Female
AD24	78	V	Female
AD25	85	V	Male
AD26	83	VI	Female
AD27	101	VI	Female
AD28	82	V	Female
AD29	86	IV	Male
AD30	84	VI	Female
AD31	82	V	Female
AD32	87	V	Female
AD33	85	V	Female
AD34	91	V	Female
AD35	86	V	Female
AD36	89	VI	Female
AD37	92	V	Female
AD38	89	V	Female
AD39	80	V	Female
AD40	80	V	Female
AD41	80	V	Female
AD42	80	V	Female
AD43	80	V	Female
AD44	78	V	Female
AD45	80	V	Male
AD46	95	V	Female
AD47	89	V	Female
AD48	84	V	Female

AD49	68	V	Male
AD50	75	V	Female
AD51	85	V	Female
AD52	78	V	Female
AD53	75	V	Female
AD54	75	V	Female
AD55	76	V	Male
AD56	74	V	Female
AD57	84	V	Female
AD58	96	V	Male
AD59	89	V	Female
AD60	80	V	Female
AD61	85	V	Female
AD62	88	V	Female
AD63	83	V	Female
AD64	80	V	Female
AD65	78	V	Female
AD66	80	V	Female
AD67	84	V	Female
AD68	83	V	Male
Age average ± SD		84±6.8	
Age range		62-101	
Braak III	**Braak IV**	**Braak V**	**Braak VI**
5 (7.4%)	4 (5.9%)	50 (73.5%)	9 (13.2%)

Table S1-2. Information on the AD patients used in phage array experiments.

Code Number	Gender	Age (years)	Braak
AD69 (Alzheimer 4*)	Female	92	IV
AD70 (Alzheimer 6*)	Female	74	V
AD71 (Alzheimer 8*)	Female	77	VI
AD72 (Alzheimer 10*)	Female	83	V
AD73 (Alzheimer 12*)	Female	91	IV
AD74 (Alzheimer 14*)	Female	88	V
AD75 (Alzheimer 16*)	Female	82	V
AD76 (Alzheimer 18*)	Male	78	V
Age average ± SD		84.23±6.7	
Age range		74.88±3.14	
Braak III	**Braak IV**	**Braak V**	Braak VI
-	2 (25%)	5 (62.5%)	1 (12.5%)

*, Nomenclature according to Figure 2.

Table S1-2. Information on the healthy individuals used as controls in phage array experiments and validation steps.

Code Number	Age (years)	Gender
Control 1 (Control 5*)	82	Male
Control 2	80	Male
Control 3 (Control 7*)	74	Female
Control 4	75	Male
Control 5	73	Female
Control 6 (Control 9*)	75	Female
Control 7 (Control 11*)	73	Female
Control 8	72	Female
Control 9 (Control 13*)	74	Male
Control 10 (Control 15*)	73	Male
Control 11	80	Male
Control 12	82	Female
Control 13 (Control 17*)	74	Male
Control 14	73	Female
Control 15	71	Female
Control 16	76	Male
Control 17	71	Female
Control 18	72	Female
Control 19	76	Female
Control 20	78	Female
Control 21	83	Female
Control 22	77	Male
Control 23 (Control 19*)	76	Male
Control 24	84	Female
Control 25	82	Female
Control 26	51	Female
Control 27	77	Male
Control 28	66	Female
Control 29	63	Female
Control 30	80	Male
Control 31	80	Female
Control 32	52	Female
Control 33	80	Female
Control 34	46	Female
Control 35	40	Female
Control 36	43	Female
Control 37	48	Female
Control 38	41	Male
Control 39	36	Male
Control 40	41	Female
Control 41	84	Female
Control 42	87	Male
Control 43	83	Female
Control 44	75	Male
Control 45	82	Female
Control 46	82	Male
Control 47	88	Female
Control 48	90	Female

Control 49	80	Female
Control 50	86	Female
Control 51	82	Female
Control 52	73	Male
Age average ± SD	72.17±14.1	
Age range	36-90	

* Serum samples used in phage microarray and validation. In brackets, nomenclature used in Figure 2.

Table S2. Oligonucleotides used in the study.

pDEST-HisHALO vector assembly		
Template	**Primer Name**	**Sequence (5'-3')**
pET15b	**P1**	AAAGCCAGTACCGATTTCTGCCATGCTGCTGTGATGATGATGATGATGGCTGCT
	P2	AAGCCCGAAAGGAAGCTGAGTTGG
pFN18a	**P3**	ATGGCAGAAATCGGTACTGGCTTTCCA
	P4	GCCGGAAATCTCGAGCGTCGACAG
pDEST15	**P5**	GACGCTCGAGATTTCCGGCTCGGATCTGGTTCCGCGT
	P6	CCAACTCAGCTTCCTTTCGGGCTTTGT
Sequencing of phage inserted cDNA coding peptides		
Phage DNA	**T72_up2**	TGCTAAGGACAACGTTATCGG
	T7_down2	TTGATACCGGACGTTCAC
Cloning displayed peptides into pDEST-HisHALO		
Phage DNA	**AttbPhageF**	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGGGTCAGGTGTGATGCTCGGGGA
	AttbPhageR	GGGGACCACTTTGTACAAGAAAGCTGGGTCGGAGGTTCACCGATAGACGCCAGAAT

Supplementary table 3. Peptide sequences displayed by the most immunoreactive phages to AD sera after microarray analysis and the random selected ones after biopanning.

Phage Code	Amino Acid Sequence	Protein Name	Uniprot	NCBI Accession Number	p-Value	Score
4O4	NSSVSISNGLSGLP	Nuclear receptor ROR-beta Isoform 1	Q92753	NP_008845.2	3.7	25.7
4K16	NSCFRAVYSLVKDRNVSKENL	Glycogen phosphorylase, brain	P11216	NP_002853.2	3.3	26.1
3D6	NSSEMFSS	B-cell lymphoma/leukemia 10 isoform 1	O95999	NP_003912.1	2.8	23.5
4N8	NSSAGRPPPRSRPPPRPSTSPTR	Anthrax toxin receptor 1	Q9H6X2	NP_115584.1	0.001	93.3
3K15	NSSQIQRMASFPGGQHEGQ	Trafficking protein particle complex subunit 13, Isoform 7	A5PLN9	NP_001352272.1	14	24
3C19	NSGGXPLFITTKYLL	Thrombospondin type-1 domain-containing protein 1 isoform 2 precursor	Q9UPZ6	NP_954872.1	16	23.1
4F19	NSSGSGQNEV	F-box only protein 28 isoform a	Q9NVF7	NP_055991.1	0.032	29.5
3L13	NSFKRFIHWKGK	Nuclear pore complex protein Nup160 isoform 1	Q12769	NP_056046.1	0.78	26.1
3G19	NSSHEYFLCGK	Relaxin-3 receptor 1	Q9NSD7	NP_057652.1	1.8	39.9
4L5	NSCCIIF	Inhibin beta B chain preprotein	P09529	NP_002184.2	18	21
4D23	NSSVKKKKLGGKRPGLQ	Pleckstrin homology domain-containing family G member 7	Q6ZR37	NP_001004330.1	21	23.1
3J3	NSRVAEGGKGGKRGLGNGLT	Protein TANC1 isoform 2	Q9C0D5	NP_001139381.1	16	24
4B22 **4N10** **94AS (Random 6)**	NSERKKRGARR	Nuclear protein 1 (NUPR1) isoform a	O60356	NP_001035948.1	8e-05	37.1
4C1 **83AM (Random 1)**	NSPKQFSAG	Oxidative stresss-responsive serin-rich protein 1 (OSER1)	Q9NX31	NP_057554.4	2.5	24
3D8	NSPAXISRELVDKLAAALE	RING finger protein 39 isoform 2	Q9H2S5	NP_739575.2	7.1	24.8
3E13	NSSAXYPSTIC	UPF0488 protein C8orf33	Q9H7E9	NP_075568.1	11	22.7
4L15	NSSPRWRPRISSSAGSS	60S ribosomal protein L23	P62829	NP_000969.1	5.2	24.8

4O19	NSSLRLPGSRHSPASASRVVGT TGTHHHARLMFCIFSRDGVSLC	FAM122C, isoform 4	Q6P4D5	NP_001164252.1	1e-14	62.8
3P4						
3P21						
3F6						
34AM (Random 4)						
4B16	NSERPSKRYLHQAAGGGERKE RQLCS	Nuclear factor NF-kappa-B p105 subunit	P19838	NP_001158884.1	11	66.2
1M4	NSVFYHWAVNVHNISPVEFL	Dyslexia-associated protein KIAA0319	A0A087X 0U9	NP_001239257.1	4	
1L17	NSRQKRKHNYQIL	Nuclear fragile X mental retardation-interacting protein 1	Q9UHK0	NP_036477.2	3.9	40.3
2O12	NSPVTASNPT	DNA repair protein XRCC1	P18887	NP_006288.2	2.1	24.4
1E12	NSSN	No significant similarity found				
1H11	NSSKMLGHHYVQHC	Protocadherin Fat 3 precursor	Q8TDW7	NP_001008781.2	19	49
2G19	NSVSLNQFRNDLCISK	Arachidonate 12-lipoxygenase, 12S-type	P18054	NP_000688.2	2.3	25.7
5C14	NSS	No significant similarity found	-	-	-	-
2P3	NSSK	No significant similarity found	-	-	-	-
2P22	NSVSAEWCSTYALIGKEQMGR GSEGSI	Constitutive coactivator of PPAR-gamma-like protein 2 isoform 1	Q9NX05	NP_060318.4	9	39.9
5M6	NSST	No significant similarity found	-	-	-	-
2P1	NSCSNISMTFHCCKXD	Leptin receptor isoform 3 precursor	P48357	NP_001003679.1	3.2	25.2
5G12	NSSRKHPASAQSMCLLLENPVP VIAWTENVRVLGRGGRE	Immunoglobulin superfamily DCC subclass member 3 precursor	Q8IVU1	NP_004875.2	3	25.8
5P19	NSSK	No significant similarity found	-	-	-	-
5P8	NSSGGGVGGRGGGGGGGRG AGGGRGAGAGGGRPEAA	No significant similarity found	-	-	-	-
3A13	NSPVTASNPT	DNA repair protein XRCC1	P18887	NP_006288.2	2.1	24.4

5N13	NSSDESGQQSKTPVSKNNKKKLAQHGGMHLXSQLLGMGG	No similarity found	-	-	-	-
3L4	NSSCGYNAKLR	Transmembrane channel-like protein 7 isoform c	H3BNW8	NP_001287661.1	22	21.8
3A10	NSKKKKK	No significant similarity found	-	-	-	-
5K79	NSKKKKKKKKKKKKKKKK	No significant similarity found	-	-	-	-
10-3AM (Random 2)	NSSCINKIDFHNKMVYPQIKIKNN	Lethal(3)malignant brain tumor-like protein 2	Q969R5	NP_113676.2	6.6	25.7
44AM (Random 3)	NSSISYTSILNHFYCHN	Olfactory receptor 8J1	Q93034	NP_003469.2	0.007	32.9
554AS (Random 5)	NSSPPCSEPSAFWALRL	E3 ubiquitin-protein ligase TRIM38	O00635	NP_006346.1	2.6	25.7
55CS (Random 7)	NSSRSLIVLGQ	Solute carrier family 23 member 2	Q9UGH3	NP_005107.4	0.91	25.7
803AS (Random 8)	NSKEVVWKEKFTDTR	Kinesin-like protein (KIF14), isoform 2	Q15058	NP_001292721.1	3.9	37.3
52CS (Random 9)	NSSLKTHLVPESCPQLLNITALKL	Cullin-5	Q93034	NP_003469.2	2.4	26.9

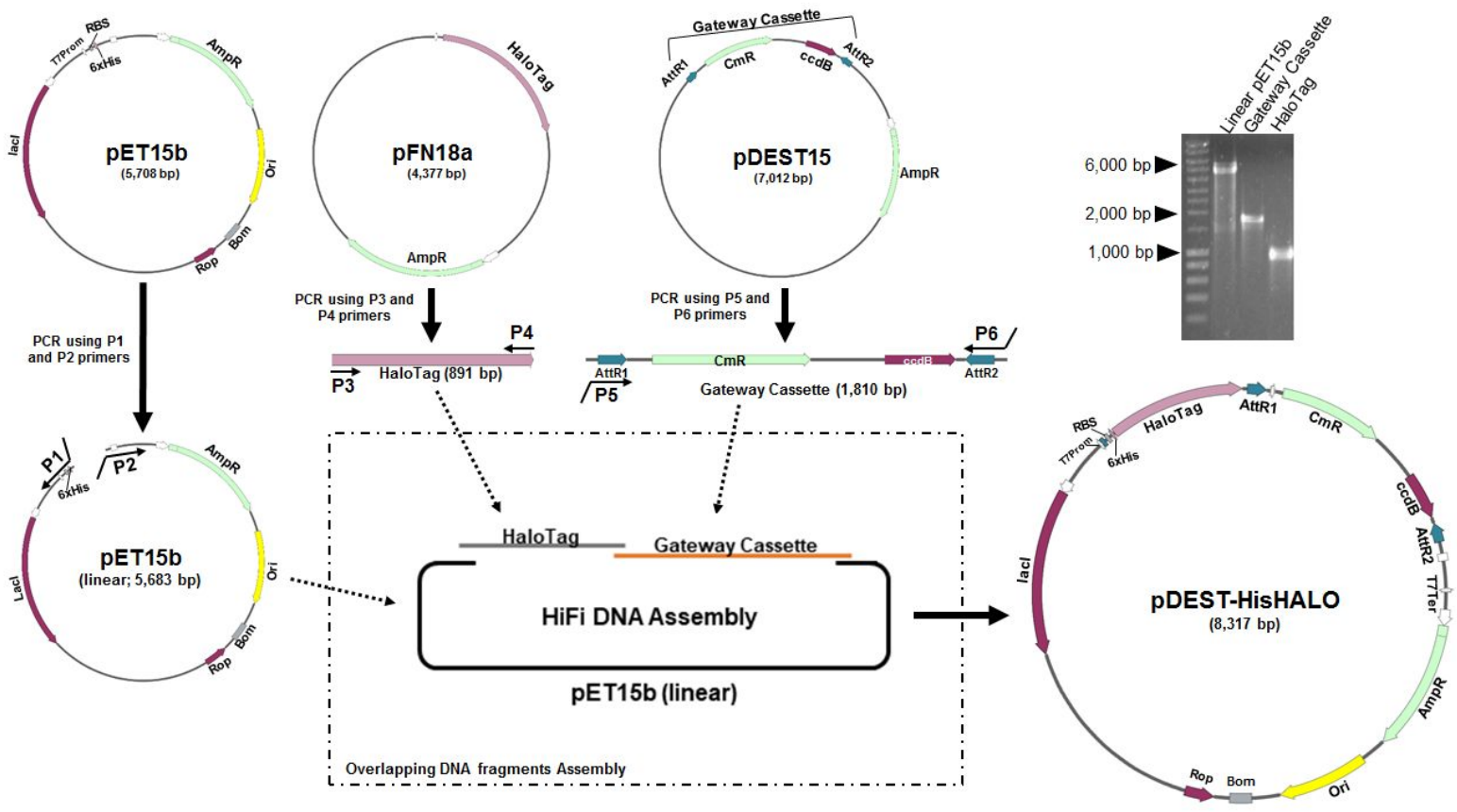

Figure S1. Assembly of the pDEST-HisHALO vector. For further validation of the microarray results, peptides displayed on the most immunoreactive phages after statistical analysis were cloned into a self-designed pDEST-HisHALO vector. (A) The vector was assembled using overlapping oligonucleotides to amplify the pET15b plasmid as template, and join the coding sequence of the HaloTag protein in frame with a Gateway cassette obtained from the pFN18a and pDEST15 plasmids, respectively. (B) 1% agarose gel electrophoresis of 100 ng of the purified PCR-amplified DNA fragments before vector assembly.

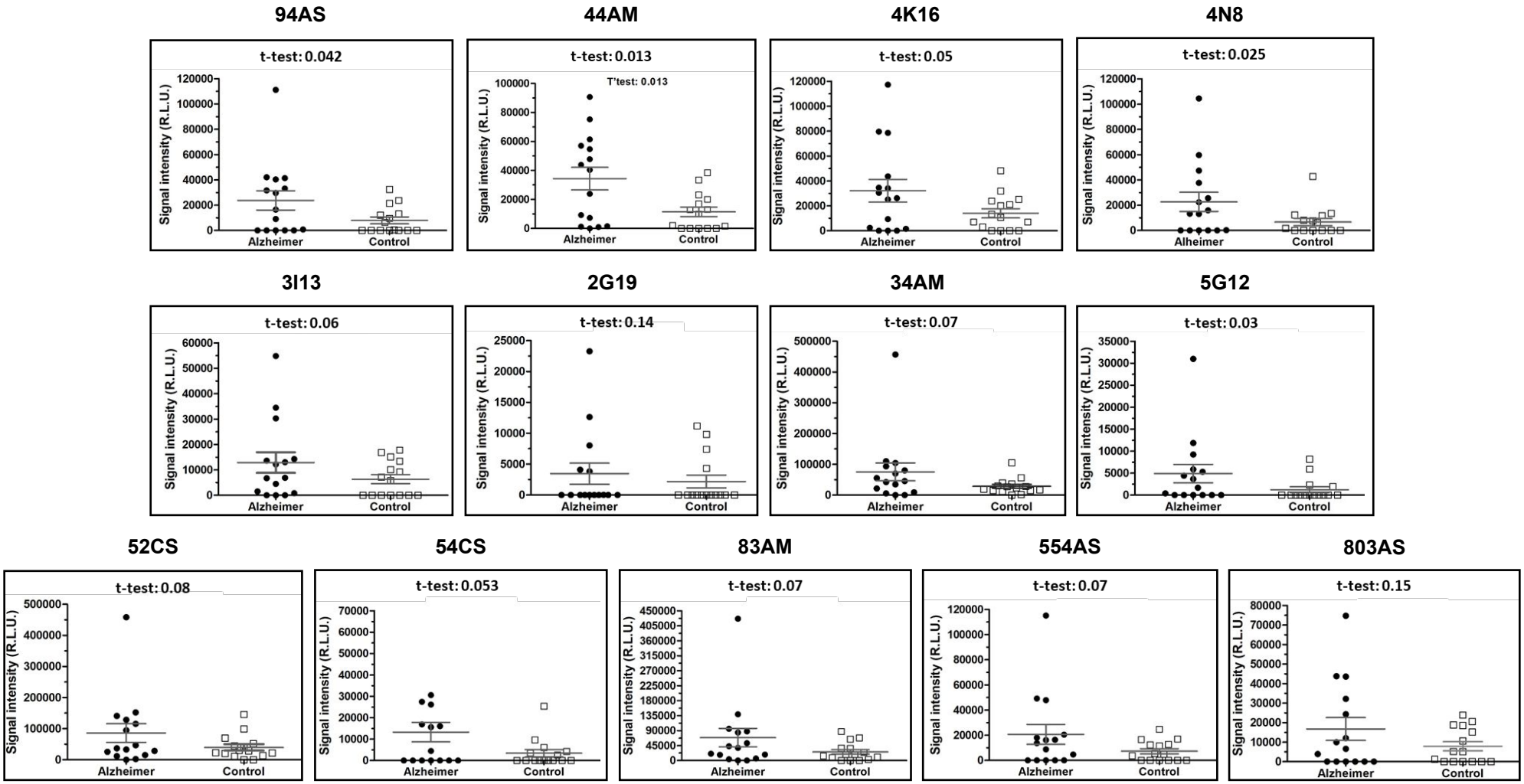

Figure S2. Dot-plots representing the analyzed seroreactivity of all 6xHisHalo-tagged peptides using 15 random individual AD patients' sera (Alzheimer) and 15 random healthy controls (Control) after HaloTag-based luminescence beads immunoassay. T-test p-value is shown in each case. Signal intensity is shown in arbitrary relative luminescence units (R.L.U). Mean and SEM are depicted in the figure for Alzheimer and control groups.