SUPPORTING INFORMATION

Nature-inspired design and evolution of anti-amyloid antibodies

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Table S1. Comparison of the number of each type of amino acid and net charge (pH 7.4) of heavy chain CDR3 for AF1 relative to previously reported antibodies specific for Aβ. Antibodies with reported conformational specificity ("conf. Ab") are noted in the table. The average values do not include AF1. The antibody C706 recognizes a coiled conformation of the Aβ peptide.

			# of amino acids in HCDR3																				
Antibody	**Source (patent or PDB)**	**Conf. Ab?**	**A**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**K**	**L**	**M**	**N**	**P**	**Q**	**R**	**S**	**T**	**V**	**W**	**Y**	**H3 net charge (pH 7.4)**
AF1	This work	Y	0	0	5	0	2	3	0	0	0	0	0	1	0	0	0	1	0	1	0	6	-5
3B3	WO2013009703A2	Y	1	0	1	0	0	1	0	1	0	1	0	0	0	0	0	0	2	0	0	1	-1
Hu20C2A3	US20120164158A1	Y	1	0	2	0	0	2	0	0	0	1	1	0	0	1	2	0	2	0	0	1	0
13C3	EP3257865A1	Y	0	0	2	0	0	2	0	0	0	0	0	0	0	0	0	1	0	0	0	1	-2
aducanumab	PDB: 6CO3	Y	1	0	2	0	0	3	0	1	0	0	1	0	1	0	3	0	0	1	0	2	1
bapineuzumab	PDB: 4HIX	Y	0	0	2	0	0	1	1	0	0	0	0	0	0	0	0	3	0	0	0	3	-1.9
gantenerumab	PDB: 5CSZ	Y	0	0	1	0	1	3	1	0	2	0	0	1	1	0	1	0	1	2	0	3	2.1
BAN2401	US8025878B2	Y	0	0	1	1	0	3	0	0	0	0	1	0	0	0	1	1	1	0	0	6	-1
AMG 864 (mAb 2.1)	US2008292639A1	N	1	0	2	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	5	-1
C706	PDB: 5W3P	N	2	0	0	1	0	1	0	0	0	1	0	3	0	0	0	1	0	0	0	1	-1
82E1	WO2008030251	N	1	0	3	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	3	-4
ponezumab	PDB: 3U0T	N	0	0	0	0	0	0	0	0	0	2	0	0	1	0	0	1	0	1	0	2	0
solanezumab	PDB: 4XXD	N	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	-1
Average values	**Conf. Abs**		0.4	0.0	1.6	0.1	0.1	2.1	0.3	0.3	0.3	0.3	0.4	0.1	0.3	0.1	1.0	0.7	0.9	0.4	0.0	2.4	-0.4
	Non-conf. Abs		0.8	0.0	1.2	0.4	0.2	0.6	0.0	0.0	0.0	0.6	0.0	0.8	0.2	0.0	0.2	0.4	0.2	0.2	0.0	2.4	-1.4
	All Abs		0.6	0.0	1.4	0.3	0.2	1.5	0.2	0.2	0.2	0.4	0.3	0.4	0.3	0.1	0.7	0.6	0.6	0.3	0.0	2.4	-0.8

HCDR3

Position	95	96	97	98	99	100	100a	100b	100c	100d	100e	100f	100g	100h	100i	100j	100k
Wild-type	**W**	**G**	**G**	**D**	**G**	**G**	**L**	**M**	**V**	**G**	**G**	**V**	**V**	**I**	**A**	**X**	**X**
	D 50, 59%	**G** 50, 48%	**S** 50, 48%			**G** 50, 44%	**Y** 25, 26%	**F** 25, 22%	**G** 17, 4%	**G** 33, 44%	**Y** 25, 30%	**Y** 25, 33%	**Y** 25, 30%	**Y** 25, 37%	**Y** 17, 11%	**Y** 33, 37%	**Y** 33, 52%
	G 50, 41%	**R** 50, 52%	**Y** 50, 52%			**S** 50, 56%	**F** 25, 7%	**L** 25, 7%	**Y** 17, 30%	**W** 33, 22%	**G** 25, 19%	**F** 25, 22%	**F** 25, 37%	**F** 25, 11%	**F** 17, 15%	**F** 33, 41%	**F** 33, 19%
							L 25, 22%	**I** 25, 41%	**F** 17, 22%	**R** 33, 33%	**D** 25, 26%	**D** 25, 26%	**D** 25, 33%	**N** 25, 44%	**D** 17, 30%	**S** 33, 22%	**S** 33, 30%
							H 25, 44%	**M** 25, 30%	**D** 17, 26%		**C** 25, 26%	**V** 25, 19%	**V** 25, 0%	**I** 25, 7%	**A** 17, 19%		
									V 17, 11%						**S** 17, 19%		
									C 17, 7%						**V** 17, 7%		

Figure S1. Summary of the expected and observed amino acid frequency in the initial antibody library. The expected (first value) and observed (second value) frequencies of each amino acid at each CDR site in heavy chain CDR3. The observed frequencies are based on sequencing results for 27 antibody variants in the initial library.

A

HCDR3

B

V_L

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQ
QKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLT
ISSLQPEDFATYYCQQHYTTPPTFGQGTKVEIK

Linker

RTSPNSASHSGSAPQTSSAPGSQ

V_H

EVQLVESGGGLVQPGGSLRLSCAASGFNIKDTYIHWV
RQAPGKGLEWVARIYPTNGYTRYADSVKGRFTISADT
SKNTAYLQMNSLRAEDTAVYYCARDGYDGSYFVGYDY
NDFYDYWGQGTLVTVSS

Figure S2. Sequence of the AF1 antibody. The amino acid sequences of (A) HCDR3 and (B) V_L-linker-V_H for the AF1 scFv.

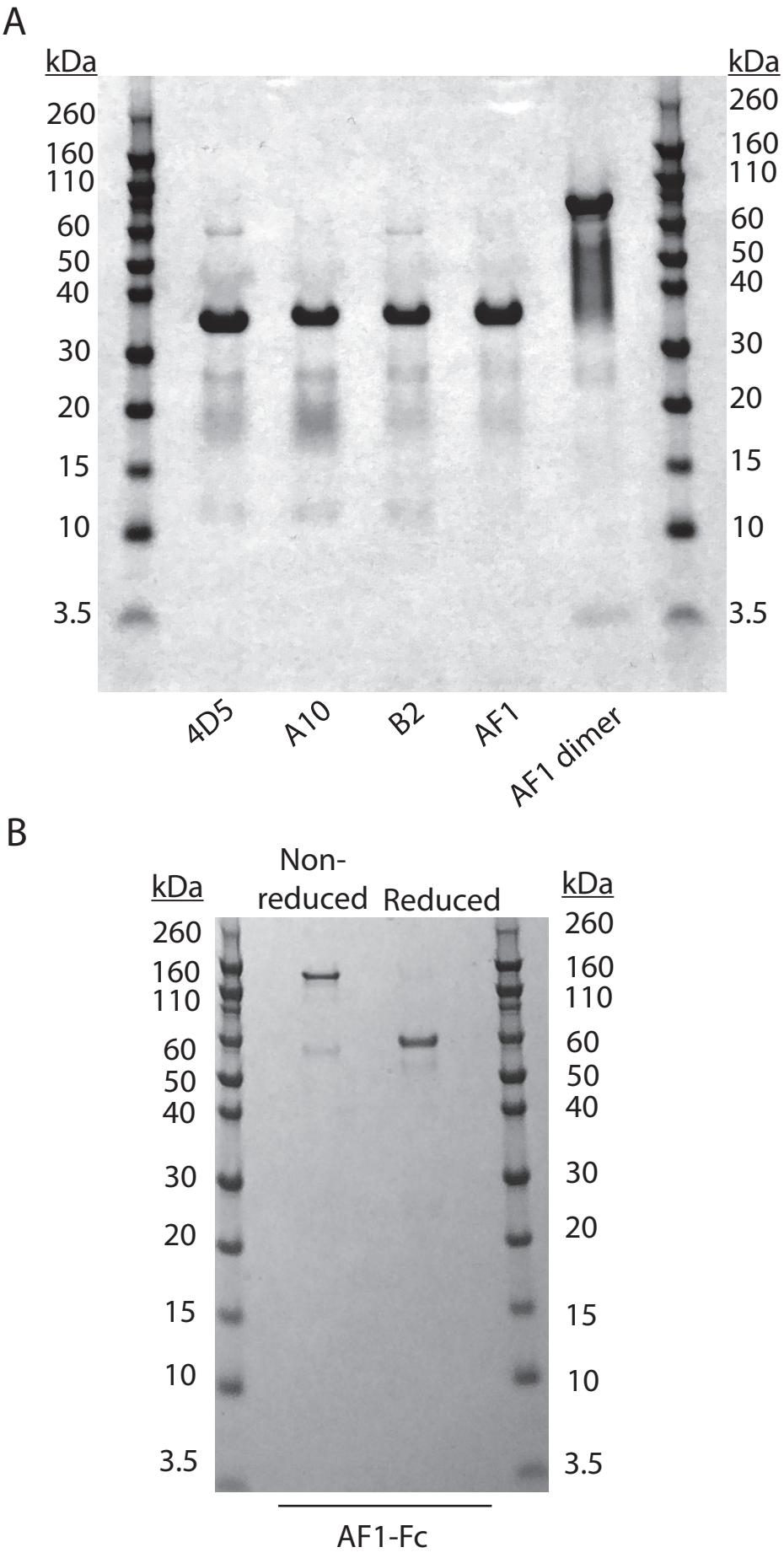

Figure S3. Analysis of the purity of the monovalent and bivalent AF1 and control antibodies. (A) SDS-PAGE analysis of AF1 scFv and AF1 scFv dimer (peptide linker) along with the wild-type scFv (4D5) and two anti-Aβ scFvs (A10 and B2). (B) SDS-PAGE analysis of AF1-Fc (scFv-Fc) for non-reducing and reducing conditions.

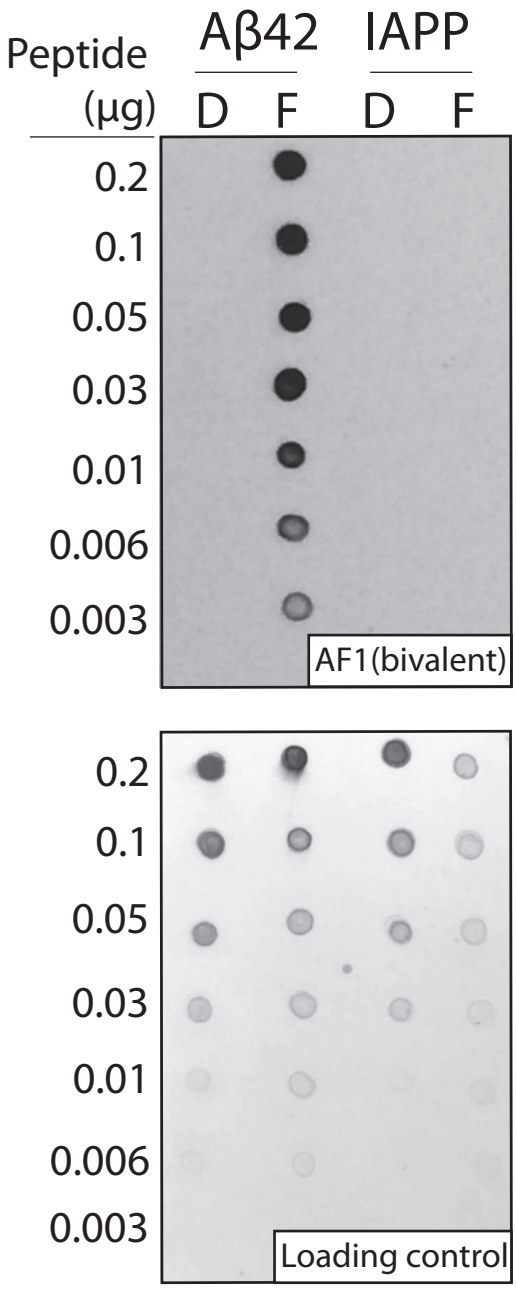

Figure S4. Immunoblot analysis of the conformational and sequence specificity of the AF1 antibody. Dilutions of Aβ and IAPP [disaggregated (D) and fibril (F)] were deposited on nitrocellulose membranes. Binding of the bivalent AF1 (scFv-Fc) antibody was evaluated at 10 nM in 5% milk. The immunoblot was imaged using X-ray film (~10 s of exposure time). The loading control blot was detected using silver colloidal stain.

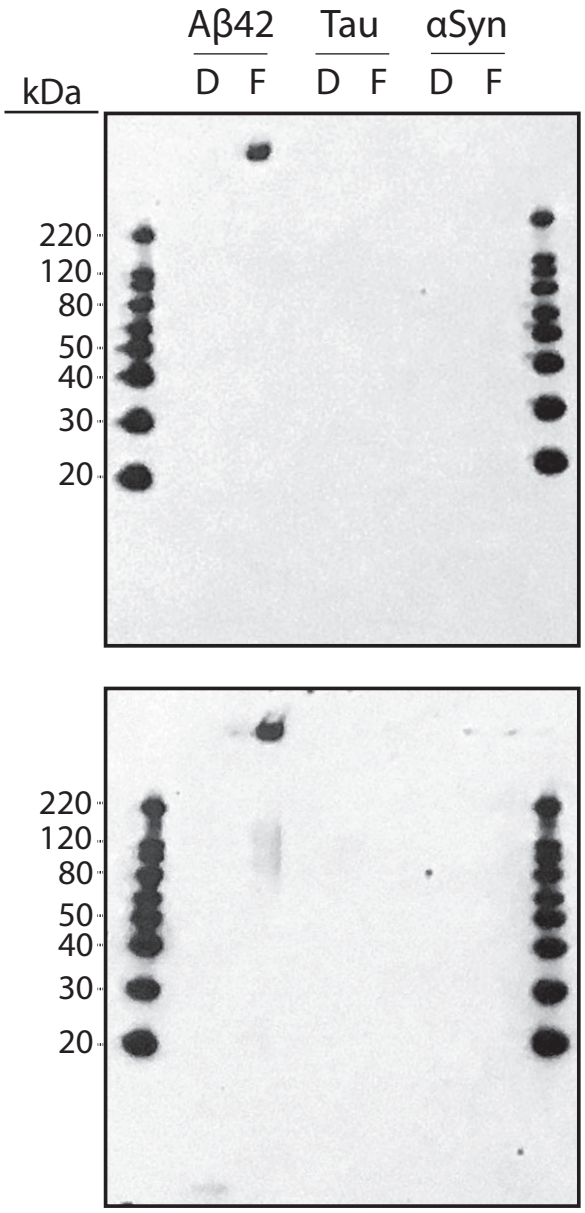

Figure S5. Western blot analysis for the AF1-Fc antibody at different exposure times. Aβ, tau, and α-synuclein [disaggregated (D) and fibril (F); 0.85 µg] were separated via SDS-PAGE, transferred to nitrocellulose membranes, and evaluated as described in Figure 5. The images are for short (top, 15 s) and long (bottom, 90 s) exposure times. At long exposure times, the AF1-Fc antibody displays some reactivity with low and intermediate molecular weight Aβ conformers.

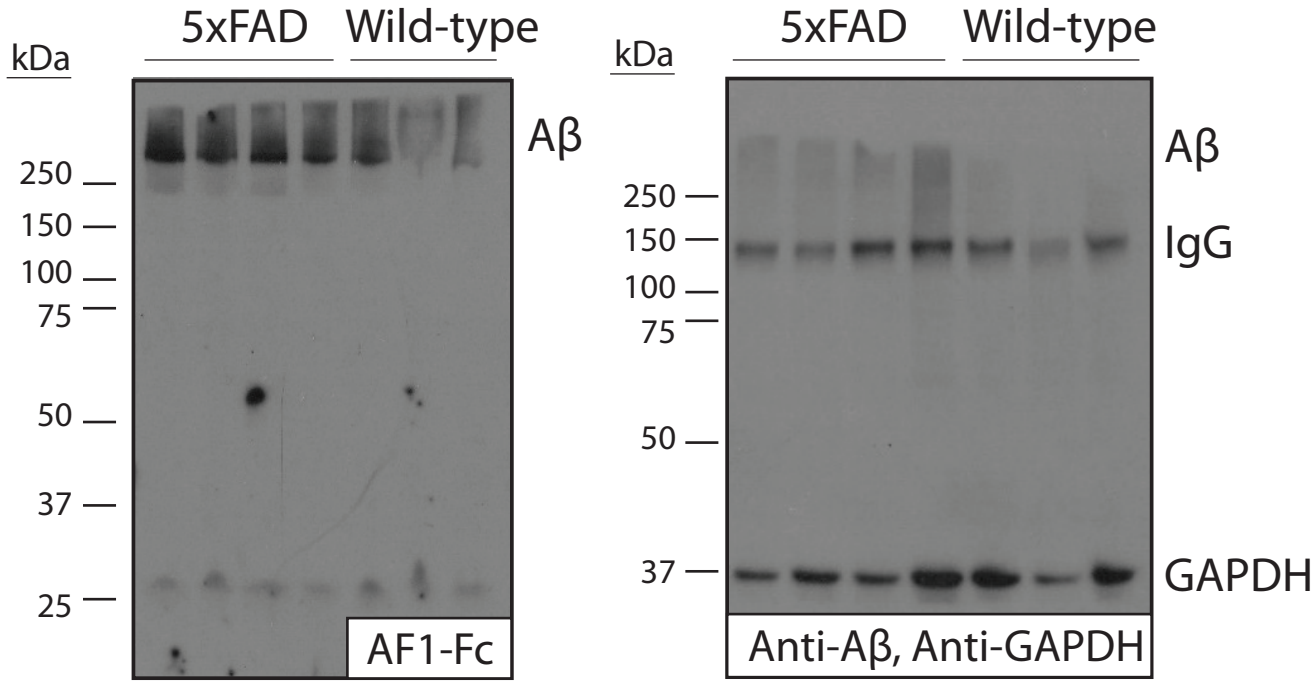

Figure S6. Western blot analysis of brain tissue from transgenic mice overexpressing human amyloid precursor protein relative to wild-type mice. Mouse transgenic (5xFAD) and non-transgenic (wild-type) forebrain samples were homogenized and insoluble fractions (50 µg of total protein) were loaded (without boiling) on precast NuPAGE 4-12% Bis-Tris gels (Invitrogen) for SDS-PAGE analysis. Gels were subsequently transferred onto nitrocellulose membranes and probed overnight at 4 °C with (left blot) AF1-Fc antibody (10 nM) and (right blot) anti-Aβ (2000x dilution; NAB228, Sigma-Aldrich) and anti-GAPDH (10000x dilution; MAB374, Millipore) antibodies. The blots were detected using HRP-conjugates specific for human (AF1-Fc) and mouse (NAB228 and MAB374) primary antibodies. Analyses were completed in triplicate.