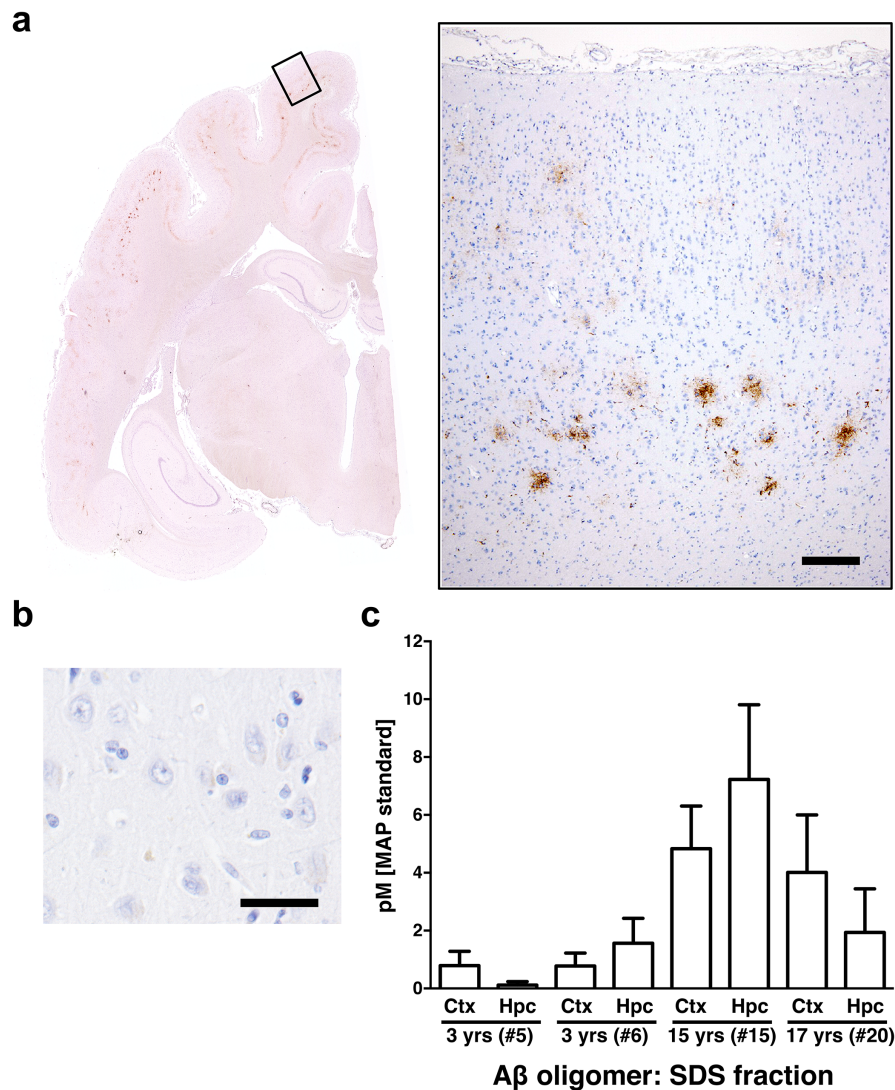


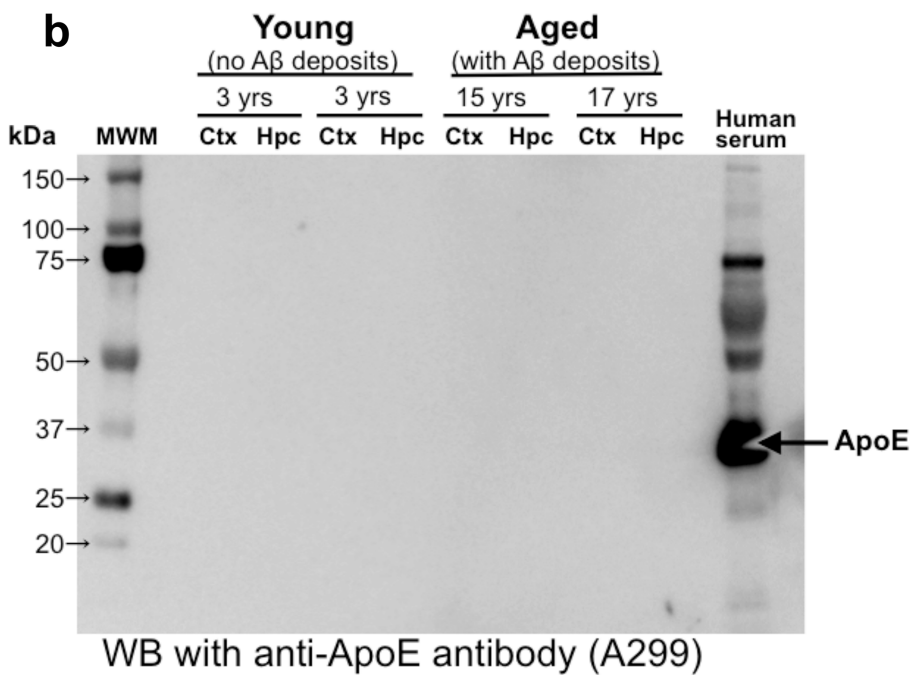
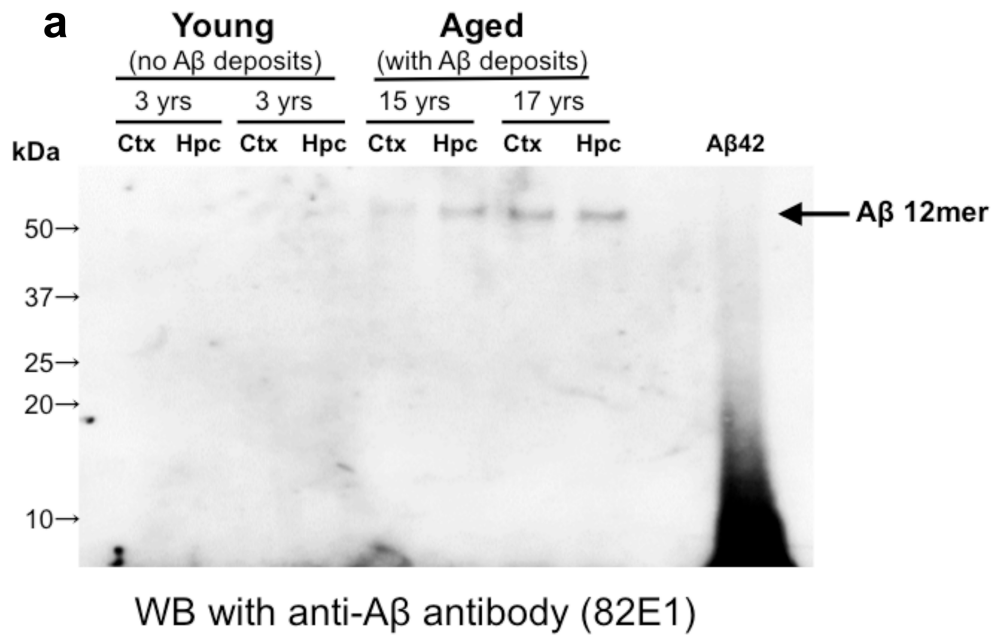
Supplementary material

The domestic cat as a natural animal model of Alzheimer's disease

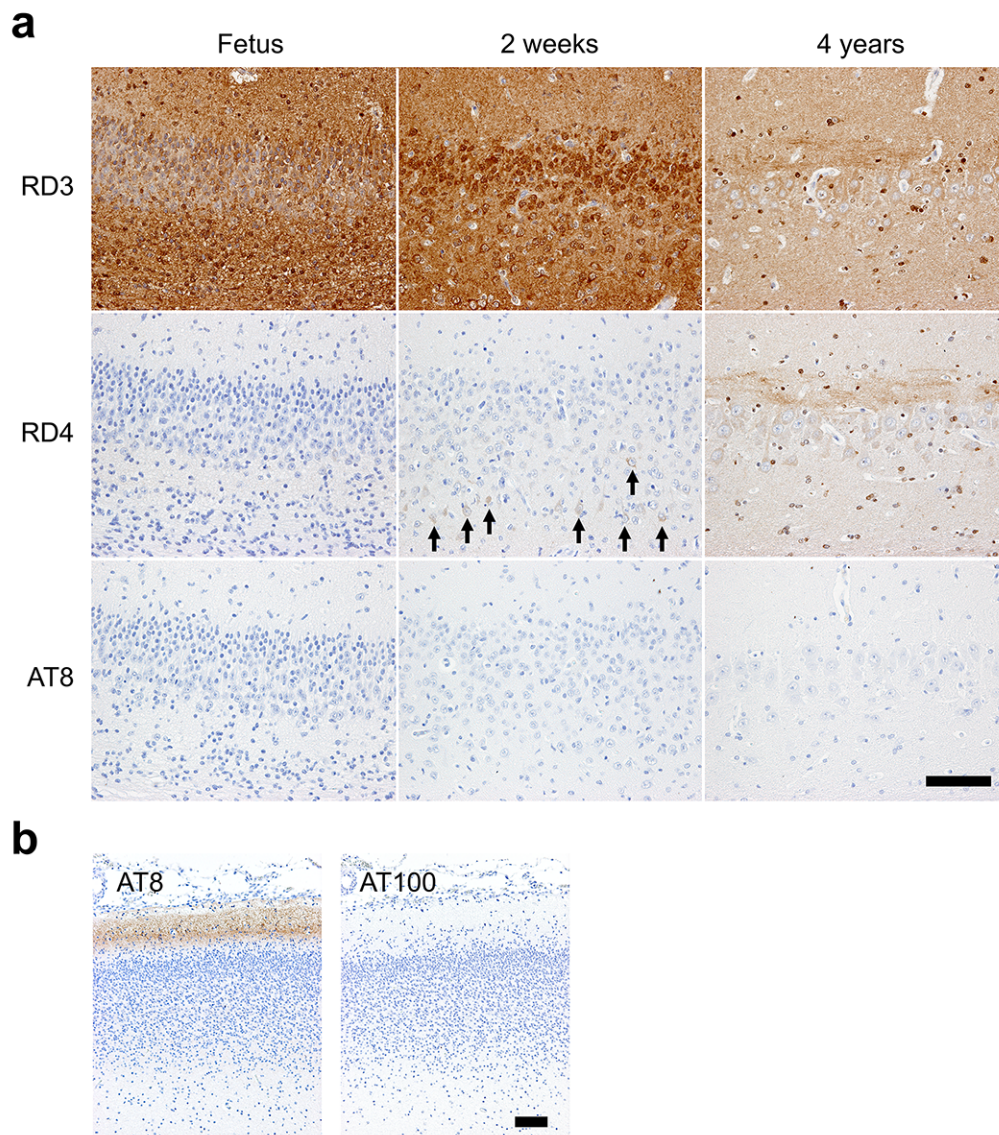
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**Supplementary Fig. 1** A $\beta$  deposition in cat brains. (a) Immunohistochemistry of the cerebrum of a 17-year-old cat (case No. 20) for A $\beta$ 42 with FA pretreatment. A $\beta$ 42 aggregates are observed in the cerebral cortex but not in the hippocampus by immunohistochemistry with FA pretreatment. Higher magnification of the parietal lobe (right). Bar = 100  $\mu$ m. (b) Negative control (without primary antibody) of Fig. 1c. No staining is detected in the cytoplasm. (c) ELISA for high molecular weight A $\beta$  oligomers. Higher amounts of A $\beta$  oligomers were seen in the brains of aged cats (15-year-old, case No. 15; 17-year-old, case No. 20) compared to the brains of young cats (3-year-old, case No. 5; 3-year-old, case No. 6). The ratio of MAP level per unit of A $\beta$  concentration varies depending on the sizes of the A $\beta$  oligomers. One pM of the MAP can be estimated to yield the same signal as 1.56 pM (for 20-mer) to 5.0 pM (for 100-mer) of A $\beta$ 42 oligomers [33]. Ctx, cortex; Hpc, hippocampus.



**Supplementary Fig. 2** Western blotting analysis of the SDS fraction of the cortex (Ctx) and hippocampus (Hpc) of young cats and aged cats. (a) The band corresponding to A $\beta$  dodecamer is detected by anti-A $\beta$  antibody 82E1. (b) A $\beta$  oligomers that are demonstrated in Fig. 1f are not detected by anti-ApoE antibody A299.



**Supplementary Fig. 3** Expression of tau isoforms in the developing cat brain. (a) Immunohistochemistry of the hippocampus CA1 region of a fetus (case No. 1), a 2-week-old cat (case No. 3), and a 4-year-old cat (case No. 7) for 3-repeat tau (RD3), 4-repeat tau (RD4), and hyperphosphorylated tau (AT8). Only the 3-repeat tau isoform is expressed in the fetal hippocampus. The hippocampal pyramidal cells begin to express 4-repeat tau in the 2-week-old cat (arrows). Both 3-repeat and 4-repeat tau isoforms are expressed in the hippocampus of adult cat brain. Bar = 50  $\mu$ m. (b) Immunohistochemistry of the cerebral cortex of a fetal cat for hyperphosphorylated tau (AT8 and AT100). The surface layer of the fetal cerebral cortex is positive for AT8 and negative for AT100. Bar = 100  $\mu$ m.



Supplementary Table1 A $\beta$  protein amino acid sequences of different animal species.

	Species [species, sequence ID]	Amino acid sequence	
Human-type A $\beta$	Human [ <i>Homo sapiens</i> , NP_000475.1]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Chimpanzee [ <i>Pan troglodytes</i> , NP_001013036.1]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Cynomolgus monkey [ <i>Macaca fascicularis</i> , XP_005548940.1]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Dog [ <i>Canis familiaris</i> , NP_001006601.2]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Polar bear [ <i>Ursus maritimus</i> , XP_008699989.1]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Camel [ <i>Camelus bactrianus</i> , XP_010954929.1]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Horse [ <i>Equus caballus</i> , XP_003364220.1]	1 DAEFRHDSGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42	
	Felid-type A $\beta$	Cat [ <i>Felis catus</i> , XP_006936005.1]	1 DAEFRHESGVEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42
	Rodent-type A $\beta$	Mouse [ <i>Mus musculus</i> , NP_001185752.1]	1 DAEFGHDSGFVRRHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42
		Rat [ <i>Rattus norvegicus</i> , NP_062161.1]	1 DAEFGHDSGFVRRHQKLVFFAEDVGSNKGAIIGLMVGGVVIA 42

Supplementary Table 2 Tau protein amino acid sequences of different animal species.

Species [species, sequence ID]	Amino acid sequence
Human [ <i>Homo sapiens</i> , NP_005901.2]	1 MAERROEFVEMEDHAGTYGIGDGRK---QGGYTMHDDQREPTDAGIKESPLQPTREDGSEEPSETSDAKSTPTAEDVTAFLVDEGARPKQAAPHTTEI 97
Chimpanzee [ <i>Pan troglodytes</i> , XP_009430187.1]	1 MAERROEFVEMEDHAGTYGIGDGRK---QGGYTMHDDQREPTDAGIKESPLQPTREDGSEEPSETSDAKSTPTAEDVTAFLVDEGARPKQAAPHTTEI 97
Cat [ <i>Felis catus</i> , XP_003997079.1]	1 MAERRODFVMDHAGTYGTERKRDPSQGGYTMDDHEDVYQGGIKESPLQPTPADGSEEPSETSDAKSTPTAEDVTAFLVDEGARPKQAAPHTTEI 100
Dog [ <i>Canis lupus familiaris</i> , XP_005624235.1]	1 MAERROEFVEMEDHAGTYGIGDGRK---QEGYTMDDQREPTDAGIKESPLQPTPADGSEEPSETSDAKSTPTAEDVTAFLVDEGARPKQAAPHTTEI 97
Mouse [ <i>Mus musculus</i> , NP_001033698.1]	1 MADRROEFVEMEDHAG-----DYTLDDQREPTDAGIKESPLQPTPADGSEEPSETSDAKSTPTAEDVTAFLVDEGARPKQAAPHTTEI 86
	98 PEGTTAEAGIGDTPPSLEDEAAGHVTAQARMVSKSKDGTGSDDKKAKGADGK--TKIATPRGAAPRGQKQANATRIIPAKTTPPAKTPPSSGEPKSGDRS 195
	99 PEGTTAEAGIGDTPPSLEDEAAGHVTAQARMVSKSKDGTGSDDKKAKGADGK--TKIATPRGAAPRGQKQANATRIIPAKTTPPAKTPPSSGEPKSGDRS 195
	101 PEGTTAEAGIGDTPPSLEDEAAGHVTAQARMVSKSKDGTGSDDKKAKGADGK--TKIATPRGAAPRGQKQANATRIIPAKTTPPAKTPPSSGEPKSGDRS 200
	98 PEGTTAEAGIGDTPPSLEDEAAGHVTAQARMVSKSKDGTGSDDKKAKGADGK--TKIATPRGAAPRGQKQANATRIIPAKTTPPAKTPPSSGEPKSGDRS 195
	87 PEGTTAEAGIGDTPPSLEDEAAGHVTAQARMVSKSKDGTGSDDKKAKGADGK--RTGNDDEKAKGADGKQAKIATPRGAAPRGQKQANATRIIPAKTTPPAKTPPSSGEPKSGDRS 184
	196 GYSSPSPGTPGSRSRRTPSLPTPTREPKKVAVVRTPPKSPSSAKSRLQTAFLVPMFDLKNVSKIGSTENLKHQREGGVQIINKKLDLSNVQSKSGSKD 295
	197 GYSSPSPGTPGSRSRRTPSLPTPTREPKKVAVVRTPPKSPSSAKSRLQTAFLVPMFDLKNVSKIGSTENLKHQREGGVQIINKKLDLSNVQSKSGSKD 295
	201 GYSSPSPGTPGSRSRRTPSLPTPTREPKKVAVVRTPPKSPSSAKSRLQTAFLVPMFDLKNVSKIGSTENLKHQREGGVQIINKKLDLSNVQSKSGSKD 300
	196 GYSSPSPGTPGSRSRRTPSLPTPTREPKKVAVVRTPPKSPSSAKSRLQTAFLVPMFDLKNVSKIGSTENLKHQREGGVQIINKKLDLSNVQSKSGSKD 295
	185 GYSSPSPGTPGSRSRRTPSLPTPTREPKKVAVVRTPPKSPSSAKSRLQTAFLVPMFDLKNVSKIGSTENLKHQREGGVQIINKKLDLSNVQSKSGSKD 284
	296 NIKHVPGGGSVQIYVKFVDSLKVTSKCGSLGNTHHNRGGQVVEKSEKIDFKDRVQSKIGSLDNI THVPGGNKKIETHKLTFRRNAKAKTDHGAELIYK 395
	297 NIKHVPGGGSVQIYVKFVDSLKVTSKCGSLGNTHHNRGGQVVEKSEKIDFKDRVQSKIGSLDNI THVPGGNKKIETHKLTFRRNAKAKTDHGAELIYK 395
	301 NIKHVPGGGSVQIYVKFVDSLKVTSKCGSLGNTHHNRGGQVVEKSEKIDFKDRVQSKIGSLDNI THVPGGNKKIETHKLTFRRNAKAKTDHGAELIYK 400
	296 NIKHVPGGGSVQIYVKFVDSLKVTSKCGSLGNTHHNRGGQVVEKSEKIDFKDRVQSKIGSLDNI THVPGGNKKIETHKLTFRRNAKAKTDHGAELIYK 395
	285 NIKHVPGGGSVQIYVKFVDSLKVTSKCGSLGNTHHNRGGQVVEKSEKIDFKDRVQSKIGSLDNI THVPGGNKKIETHKLTFRRNAKAKTDHGAELIYK 385
	396 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
	397 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
	396 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
	401 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 446
	396 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
	385 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 430
	396 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
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	396 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
	401 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 446
	396 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 441
	385 SPVYSGDTPSRHLNNSVSTGSDIMVDSPOLATLADDEVASLAKQGL 430

MBD: microtubule binding domain.