Supplement Wirths et al.

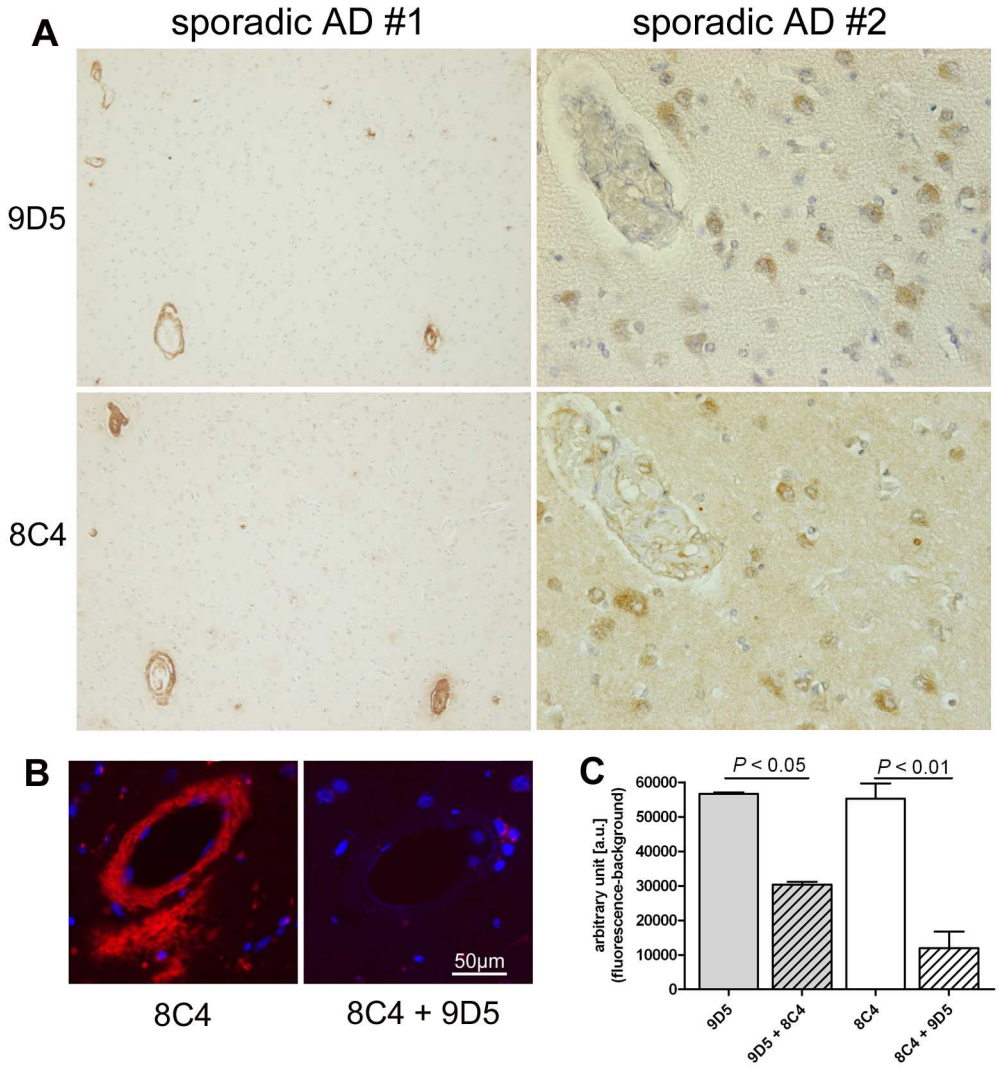
Dot blot competition assay

PVDF membrane (Millipore) was activated in methanol for 3 seconds, washed with ddH2O and equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol). AβpE3-38 (500 ng in H2O) was spotted on the wet PVDF membrane and left to dry for 10 minutes. After blocking of the membrane by 5% non-fat dry milk TBST solution the competitor antibodies 9D5 (IgG2b subclass) and 8C4 (IgG1 subclass) were diluted in 5% non-fat dry milk TBST solution (10 µg/ml) and incubated with the membrane over night at 4°C while gently shaking. Then the corresponding detector antibody (8C4 after pre-incubation with 9D5 and vice versa) was added (at 10 µg/ml) to each competitor antibody solution and incubated by gently shaking for two hours. After washing the membrane three times in 1xTBST for 15 minutes, IgG2b and IgG1 subclass-specific secondary antibodies conjugated to Cy3 (Jackson Immunoresearch) were diluted 1:200 in 5% non-fat dry milk TBST and used to cover the membrane probed with the 9D5 and 8C4 respectively with gentle shaking for one hour. Then the membrane was washed three times by 1xTBST for 15 minutes. Fluorescent dots were scanned using a Fuji CCD camera LAS-4000 mini and a fluorescence filter.

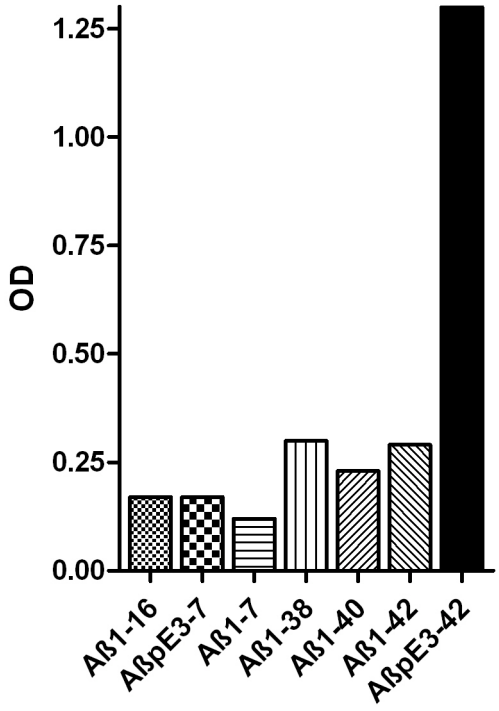
Immunostaining competition

Immunostaining on paraffin embedded sections was performed on 4 µm sagital paraffin sections, as described above. In order to study competition between 8C4 and 9D5, the specific binding sites of 8C4 (IgG1 subclass; 10 µg/ml) were blocked by application of the competitor antibody 9D5 (IgG2b subclass) (0.04 µg/ml) together with the non-specific treatment with skim milk and fetal calf serum in PBS, prior to the addition of the primary antibodies. Primary antibody 8C4 (10 µg/ml) was incubated overnight in a humid chamber at room temperature, followed by incubation with a IgG1 subclass specific secondary antibodies conjugated to Cy3 (Jackson Immunoresearch) diluted 1:200 in 5% non-fat dry milk TBST.

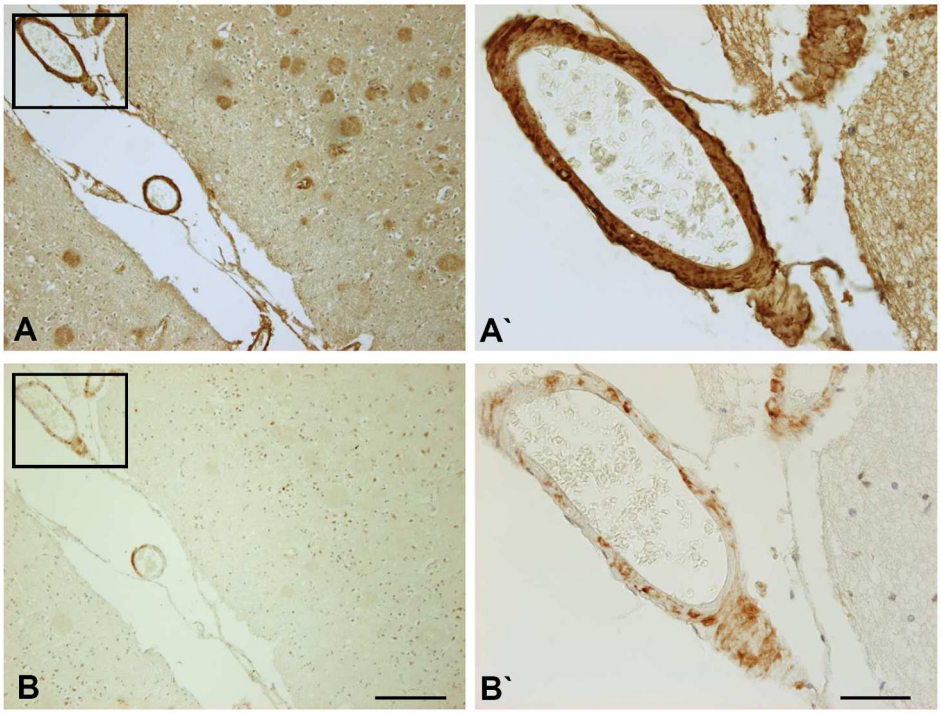
Supplementary Figure 1: 9D5 and 8C4 monoclonal antibodies were competing for the same epitope. (**A**) Parallel sections stained with 9D5 and 8C4 revealed indistinguishable pattern in two cases of sporadic AD showing either prominent blood vessel staining (sporadic case #1) or intraneuronal immunoreactivity (sporadic case #2). (**B**) 9D5 pre-incubation completely blocked 8C4 blood vessel staining in an AD case. (**C**) Dot blot competition assay. Significant lower 9D5 signal was detected after competition with 8C4 ($P<0.05$). The same was observed for the 8C4 signal after competition with 9D5 ($P<0.01$) by t-testing.



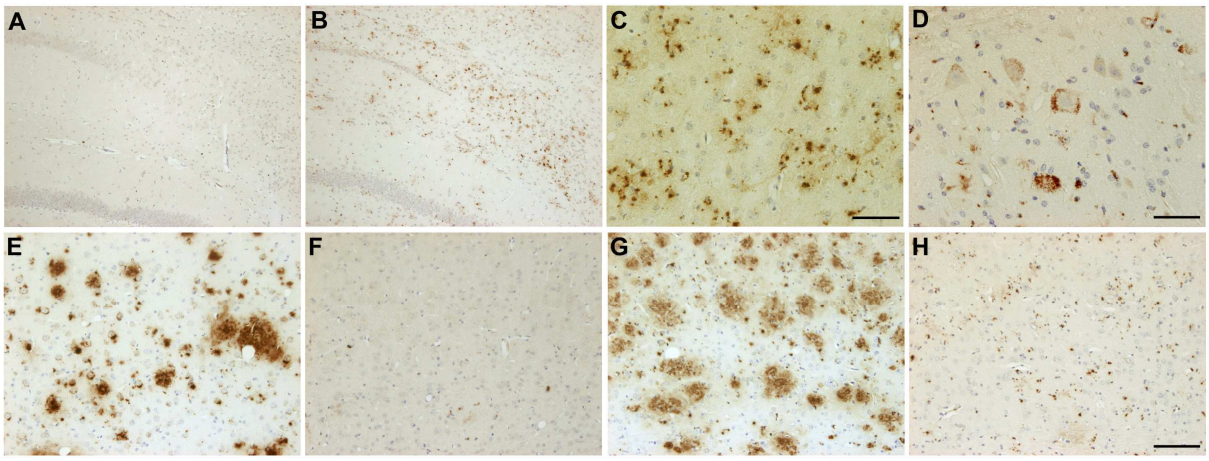
Supplementary Figure 2: 9D5 specifically recognizes AβpE3-42 by direct ELISA. 50 ng of each peptide was used. 9D5 did not recognize Aβ 1-7, Aβ1-16, Aβ1-38, Aβ1-40, Aβ1-42 or the N-terminal truncated peptide AβpE3-7.



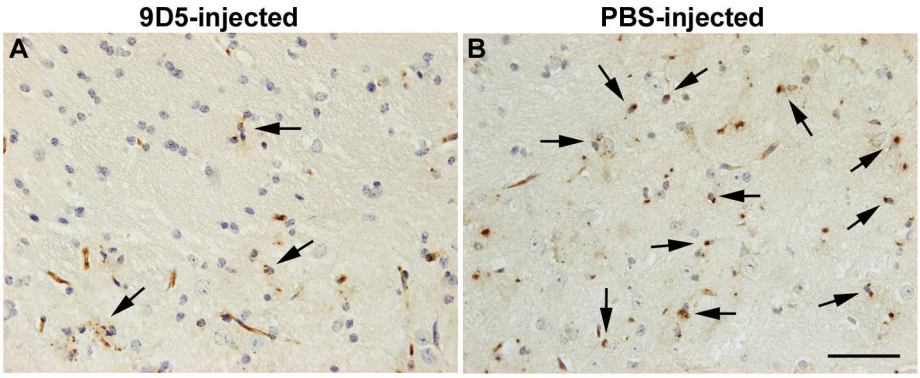
Supplementary Figure 3: 9D5 and 2-48 (against N-terminal AβpE3-x) monoclonal antibodies recognize a differential staining pattern. Adjacent sections of FAD case harboring the PS1 (Δexon9) mutation were stained with 2-48 (**A, A'**) and 9D5 (**B, B'**). While 2-48 recognized abundant plaques and vessel staining, 9D5 did not label plaques. The CAA staining did only partially overlap and was much less abundant compared to 2-48. Scale bars: A, B: 200µm; A`, B`: 50 µm.



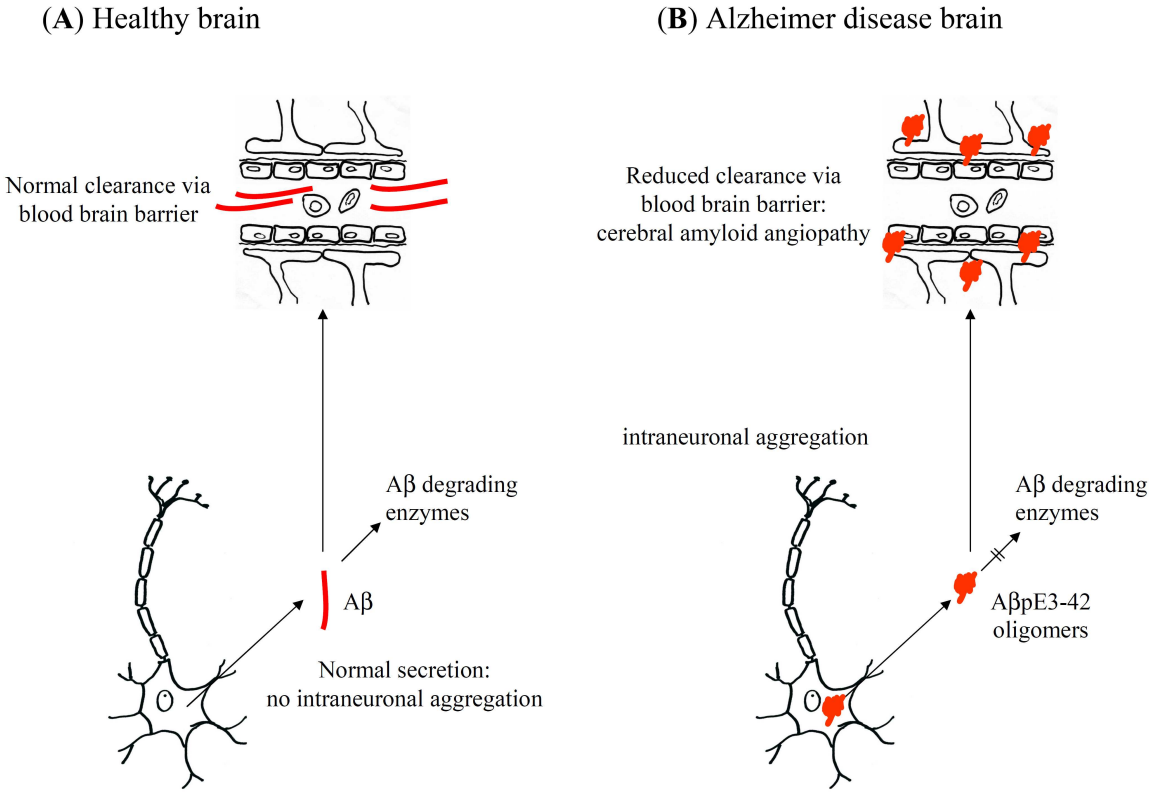
Supplementary Figure 4: 9D5 immunoreactivity in APP/PS1KI and APP single transgenic mice. In 2-month-old APP/PS1KI did not show any 9D5 immunoreactivity in the subiculum (**A**), whereas in 10-month-old APP/PS1KI mice abundant 9D5-staining could be detected (**B**). In cortical regions, abundant 9D5 staining could be detected already at the age of 6 month (**C**). In addition, strong intraneuronal 9D5 staining could be detected in spinal cord motor neurons at 12 months of age (**D**). 10-month-old APP single transgenic mice showed abundant 4G8 staining (**E**) and only minor 9D5-immunoreactivity (**F**). Age-matched APP/PS1KI bigenic mice harbouring mutant PS1 on a homozygous knock-in background showed strong 4G8 staining (**G**), as well as abundant 9D5 immunoreactivity (**H**). Scale bars: A,B: 200 µm; E-H: 100 µm; C,D: 50 µm



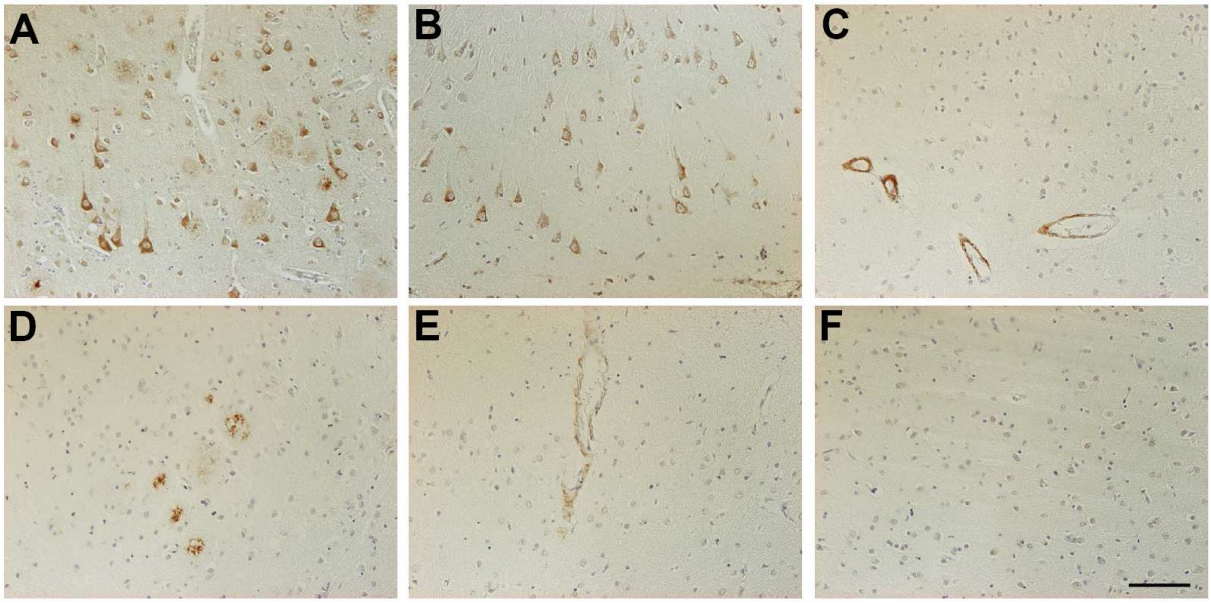
Supplementary Figure 5: Reduction of intracellular signal in the subiculum after passive immunisation of 5XFAD mice with 9D5. (**A**) Reduction of intracellular 9D5 immunoreactivity after passive immunisation with 9D5. (**B**) PBS treated 5XFAD mouse with intracellular 9D5 staining. 9D5-positive cells are indicated by arrows. Scale bar: 50 µm.



Supplementary Figure 6: Hypothesis of oligomeric AβpE3 pathology in Alzheimer disease brain. (**A**) In non-demented controls Aβ is secreted by neurons with no intraneuronal accumulation. Clearance of Aβ occurs by crossing blood brain barrier via the blood stream and/or brain derived Aβ degrading enzymes. (**B**) In AD oligomeric AβpE3 might be increased in brain parenchyma and blood vessels due to reduced blood brain barrier clearance. In addition, reduced secretion, impaired clearance mechanisms and/or transport problems might lead to intraneuronal aggregation of oligomeric AβpE3. AD cases harboring intraneuronal oligomeric AβpE3 might represent a certain state of the disease.



Supplementary Figure 7: Assessment of 9D5-positive neuropathological staining in sporadic (AD) and familial (FAD) Alzheimer disease patients with APP (arc, artic; swe, Swedish) and PS1 mutations ((P264L, L418F, Δexon9) and non-demented controls. ++, most if not all neurons, vessels or plaques were positive (**A**); +, weak to moderate staining of neurons (**B**), vessels (**C**) or plaques (**D**); (+) occasionally staining of neurons, vessels or plaques (**E**); -, no staining was detected (**F**).



Supplementary Table 1. **Demographic data of sporadic (AD) and familial (FAD) Alzheimer disease patients with APP (arc, artic; swe, Swedish) and PS1 mutations ((P264L, L418F, Δexon9) and non-demented controls.** Oligomeric AβpE staining in sporadic and familial AD cases was observed in pyramidal neurons and blood vessels (CAA) of the hippocampus and frontal cortex. Minor plaque staining was only seen in some AD cases. Abbreviations: iAβ, intraneuronal Aβ; CAA, cerebral amyloid angiopathy; m, male; f, female; na, not analyzed; ++, most if not all neurons, vessels or plaques were positive; +, weak to moderate staining of neurons, vessels or plaques; (+) occasionally staining of neurons, vessels or plaques; -, no staining was detected (3 sections per case were analysed in a blinded fashion by the assessor).

			Oligomeric AβpE3				
	sex	age	iAβ	CAA	Plaques	Braak stage	ApoE
control	m	73	-	-	-	0	33
control	f	82	-	-	-	I	33
control	m	78	-	(+)	-	I	43
control	m	84	-	-	-	I	33
control	m	91	-	-	-	I	33
control	m	70	-	-	-	0	43
control	f	78	-	-	-	I	33
control	m	70	-	-	-	0	32
control	f	90	-	(+)	-	I	22
control	f	88	-	-	-	I	33
AD	f	79	+	+	+	IV	43
AD	m	93	-	+	-	IV	33
AD	f	86	-	+	-	IV	43
AD	f	86	-	-	-	IV	33
AD	m	86	-	(+)	+	IV	33
AD	m	92	-	-	-	IV	33
AD	f	92	-	(+)	-	IV	33
AD	f	88	-	-	-	IV	33
AD	f	85	+	-	-	IV	22
AD	f	88	-	(+)	-	IV	43
AD	m	81	-	+	+	IV	
AD	f	84	+	-	+	IV	32
AD	f	84	+	+	-	IV	43
AD	m	91	-	+	-	IV	42
AD	f	88	-	-	+	IV	33
AD	f	91	-	+	-	IV	43
AD	f	87	-	+	-	IV	43
AD	f	92	+	(+)	-	IV	42
AD	f	91	-	(+)	-	IV	43
AD	f	93	-	-	-	IV	33
FAD arc	m	64	(+)	+	+	na	na
FAD swe	f	61	(+)	++	+	na	na
FAD PS1 (P264L)	m	54	+	+	+	na	na
FAD PS1 (L418F)	m	38	++	-	++	na	na
FAD PS1 (Δexon9)	m	61	++	+	-	na	na
FAD PS1 (Δexon9)	m	64	++	+	-	na	33
FAD PS1 (Δexon9)	m	69	++	+	-	na	33