Supplementary Material

Mild Microglial Responses in the Cortex and Perivascular Macrophage Infiltration in Subcortical White Matter in Dogs with Age-Related Dementia Modelling Prodromal Alzheimer's Disease

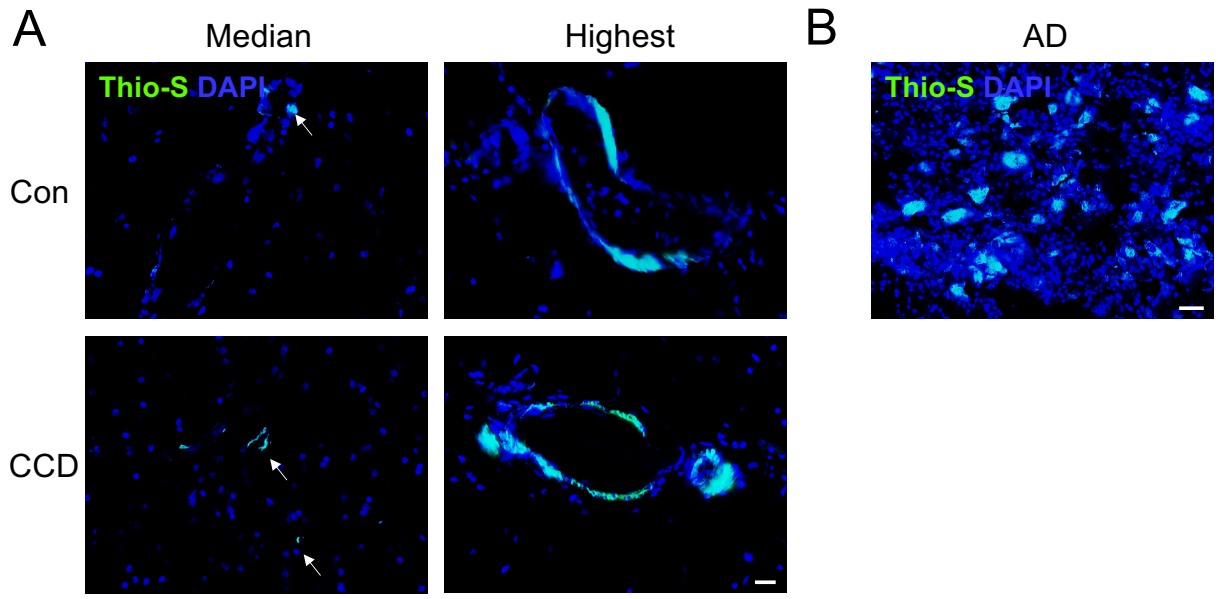
Supplementary Table 1. Detailed data on all dogs included in the study

Dog ID	Breed	Sex	Age at euthanasia (Months)	CCDR score
Controls				
TRSC3	Bearded collie	Male	185	34
TRSC14	Labrador retriever	Male	164	35
TRSC18	Mongrel	Male	119	39
TRSC21[1]	Mongrel	Female	140	34
TRSC22[1]	Golden retriever	Female	182	35
CCD				
TRSC2	Mongrel	Male	188	59
TRSC5	Mongrel	Female	195	52
TRSC6	Mongrel	Female	161	58
TRSC7	Poodle	Female	182	56
TRSC16	Cocker Spaniel	Female	140	50
TRSC19	Cairn Terrier	Male	177	63
TRSC20[1]	Danish/Swedish Farmdog	Male	185	64
17-011[1]	Danish/Swedish Farmdog	Female[2]	189	52
18-004[1]	Jack Russell Terrier	Male	188	58
19-033[1]	Mongrel	Male[2]	169	64

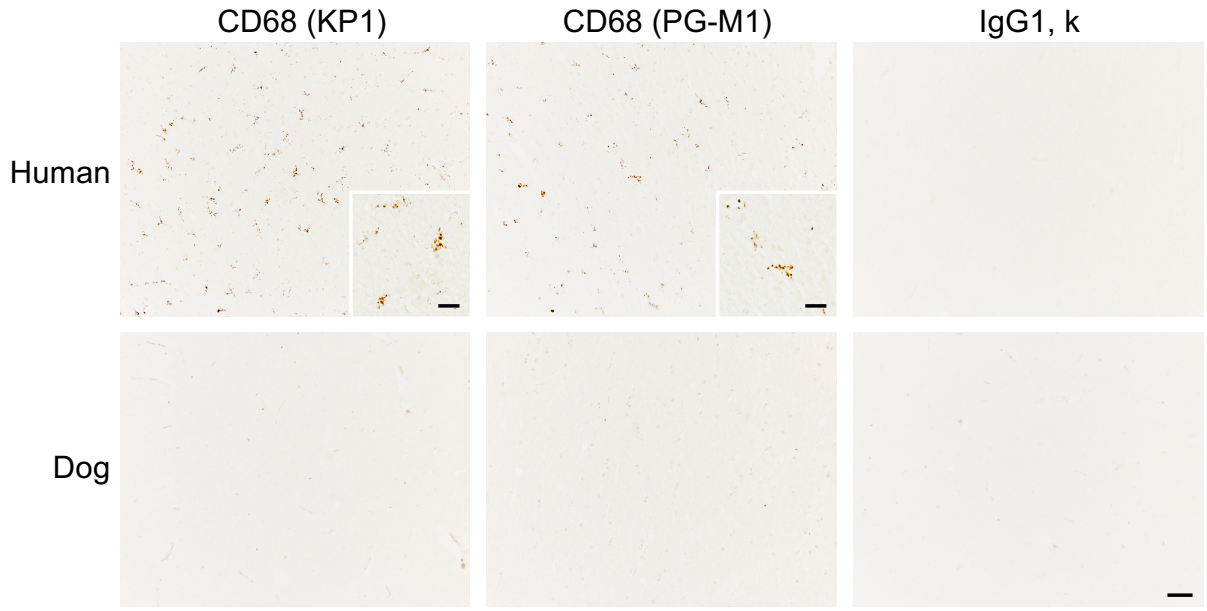
[1] Dogs not included in Schutt et al. 2016 [15].

[2] Neutered dogs.

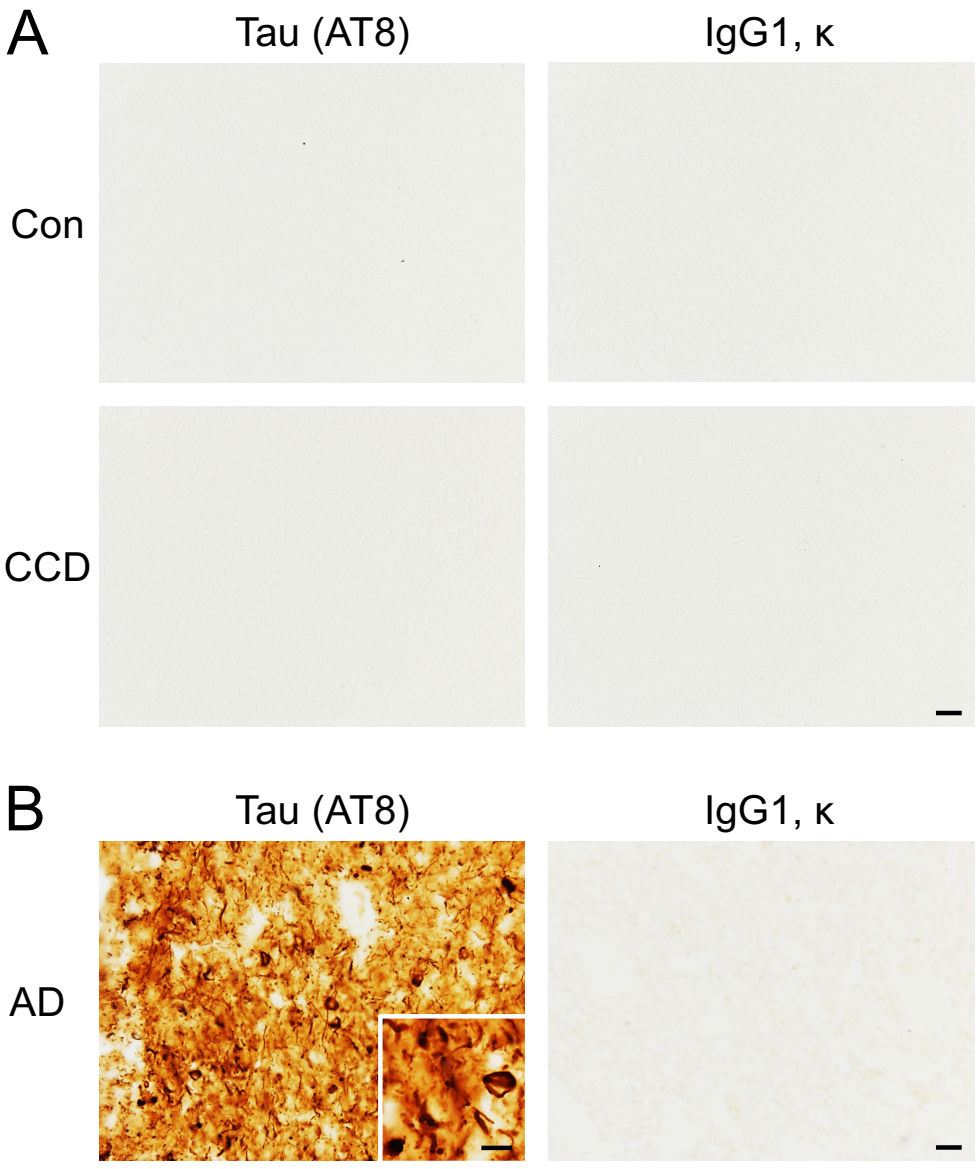
Supplementary Figure 1. Thioflavin S stained Aβ beta-sheet conformation in the vascular wall in frontal cortex of CCD and control dogs. Fluorescence thioflavin S staining of sections of frontal cortex from the two CCD dogs shown in Fig. 1 (A) and AD patient (B). DAPI (dark blue) was used for nuclear staining. A) The thioflavin S signal (green) was observed in the vascular wall in CCD and control dogs also co-localizing with DAPI-stained material in the vascular wall indicated by the light blue signal. Sections without thioflavin S staining were blank (not shown). B) Fluorescence thioflavin S staining in AD grey matter was abundant. AD, Alzheimer's disease; CCD, Canine cognitive dysfunction; Con, control. Scale bars: 20 µm (A), 50 µm (B).



Supplementary Figure 2. Microglial CD68 staining in human, but not in canine tissue. The expression of the microglial lysosomal marker CD68 was examined by immunohistochemistry using two anti-human CD68 antibodies (clones: KP1 and PG-M1). CD68 was visualized in sections of human brain tissue, not in canine brain sections, showing dot-like staining according to the lysosomal localization of CD68. Substitution with IgG1,κ isotype control was blank. Scale bars: 50 µm (inserts: 20 µm).



Supplementary Figure 3. Absence of tau phosphorylation using the AT8 antibody in frontal cortex of CCD and control dogs. Sections from CCD and control dogs (A) and AD patient (B) immunohistochemically stained for phosphorylated tau using the AT8 antibody. A) Tau phosphorylation was not observed in the frontal cortex of CCD and control dogs. IgG1,κ isotype control was blank. B) Tau hyperphosphorylation in AD grey matter showing typical neuropil threads and NFTs (insert). AD, Alzheimer's disease; CCD, Canine cognitive dysfunction; Con, control. Scale bars: 20 µm (inserts: 10 µm).



Supplementary Figure 4. Isotype controls for S396 tau immunohistochemical stainings in frontal cortex of CCD and control dogs. Substitution controls with rabbit IgG were blank indicating a high specificity of the tau S396 antibody in both CCD and control dogs. CCD, Canine cognitive dysfunction; Con, control. Scale bar: 20 µm.