Supplementary material

Methods

Isolation of human brain microvessels

The procedure used for isolation of human brain microvessels has been described in our previous work [2]. Inferior parietal cortex samples (~ 400 mg) were thawed on ice in 500 µl of a microvessel isolation buffer (MIB; 15 mM HEPES, 147 mM NaCl, 4 mM KCl, 3 mM $CaCl_2$ and 12 mM $MgCl_2$) containing a cocktail of protease and phosphatase inhibitors (Bimake, Houston, TX). Meninges and white matter were removed, and the samples were homogenized in a total of 1.5 ml of MIB and centrifuged at 1000 *g* for 10 min at 4°C. The supernatant was discarded, the pellet was resuspended in 5 ml of MIB containing 18% dextran (from leuconostoc mesenteroides, M.W. 60,000–90,000; Sigma-Aldrich, St. Louis, MO) and centrifuged at 4000 *g* for 20 min at 4°C. The resulting supernatant was discarded and the tube was cleaned with absorbent paper to avoid contamination of the pellet by the myelin layer. The pellet was then resuspended in 1 ml of MIB and the resulting homogenate was filtered through a 20 µm nylon filter (Millipore, Temecula, CA). The material that was retained on the filter consists of cerebral microvessels, whereas the filtrate consists of microvessel-depleted parenchymal cell populations. The collected vascular tissue was washed off the filter with 500 µl of lysis buffer (150 mM NaCl, 10 mM NaH_2PO_4, 1% Triton X-100, 0.5% SDS and 0.5% sodium deoxycholate) containing protease and phosphatase inhibitors and 1 mM EDTA. The microvessels were homogenized, disrupted by sonication (3 × 45 s) in a Sonic Dismembrator apparatus (Thermo Fisher Scientific, Waltham, MA) and centrifuged at 100,000*g* for 20 min at 4 °C. The supernatant was concentrated by a centrifugation at 16,000*g* for 60 min at 4 °C in a Vivaspin device (MWCO, 3 kDa; Sartorius Stedim Biotech, Aubagne, France)

and kept for Western immunoblotting analyses as the vascular fraction. In parallel, the filtrate was centrifuged at 16,000 *g* for 20 min at 4 °C. The supernatant was discarded and the pellet was homogenized in 100 µl of lysis buffer, sonicated and spun at 100,000 *g* for 20 min at 4 °C. The supernatant was kept for Western immunoblotting analyses as the microvessel-depleted parenchymal fraction. Protein concentrations in all fractions were determined using the bicinchoninic acid assay (Thermo Fisher Scientific).

Isolation of murine brain microvessels

The procedure used for isolation of murine brain microvessels has been reported in our previous work [59]. Nontransgenic and 3xTg-AD mice aged 12 and 18 months were sacrificed with an intracardiac perfusion of ice-cold PBS containing 0.32 M sucrose and protease (SIGMA*FAST* Protease Inhibitor tablets, Sigma-Aldrich) and phosphatase (1 mM sodium pyrophosphate and 50 mM sodium fluoride) inhibitors, under deep anesthesia with ketamine/xylazine. The brains were immediately collected and brainstem, cerebellum and meninges were removed. Murine brain samples were then chopped and frozen in 0.5 mL of MIB containing 0.32 M sucrose and protease and phosphatase inhibitors (Bimake). The microvessel enrichment procedure was then conducted as described above for human samples. To validate the enrichment of mural cell markers, the microvessel-enriched and the microvessel-depleted fractions were compared to a total brain homogenate obtained from the homogenization of a whole hemisphere of a control mouse in the lysis buffer. Protein concentrations in all fractions were determined using the bicinchoninic acid assay (Thermo Fisher Scientific).

Tables

	Insoluble Aβ40	Insoluble Aβ42	Neuritic plaque count	Diffuse plaque count	Tangle count	Insoluble phosphorylated tau
	r^2	r^2	r^2	r^2	r^2	r^2
α-SMA	-0.035	-0.084¶	-0.132¶¶	< -0.001	-0.028	-0.044
PDGFRβ	-0.027	-0.049	-0.112¶	-0.027	-0.019	-0.003
CD13	-0.003	-0.006	-0.054	0.001	-0.008	-0.021

Table S1: Linear regressions between brain mural cell markers and cortical Aβ and tau neuropathologies. Alpha (α)-SMA, PDGFRβ and CD13 were measured by Western immunoblotting in isolated human brain microvessels. Insoluble Aβ peptide concentrations were determined by ELISA in formic acid extracts from the parietal cortex. Insoluble phosphorylated tau content was assessed by Western immunoblotting using the AD2 antibody in formic acid extracts from the parietal cortex. Relative optical density values obtained for brain mural cell markers and insoluble cortical Aβ concentrations were log transformed for statistical analyses. Linear regressions were performed to obtain coefficients of determination (r^2). ¶ $p < 0.05$ and ¶¶ $p < 0.01$. Abbreviations: α-SMA, smooth muscle alpha actin; CD13, aminopeptidase N; PDGFRβ, platelet-derived growth factor receptor β.

	Molecular weight (kDa)	NCI	MCI	AD	One-way ANOVA
phosphorylated soluble TDP-43	43	9.4 ± 0.6	9.8 ± 0.5	9.0 ± 0.5	p = 0.5432
Ser403/404	35	94.8 ± 5.5	82.0 ± 5.2	73.0 ± 3.9*	p = 0.0102
total soluble TDP-43	43	64.7 ± 9.4	54.1 ± 6.4	45.2 ± 5.0	p = 0.1744
(2E2-D3)	35	6.4 ± 0.8	8.2 ± 1.0	7.0 ± 1.0	p = 0.4103
phosphorylated insoluble TDP-43	43	6.6 ± 1.0	10.0 ± 1.3	9.7 ± 1.1	p = 0.0762
Ser409/410	25	3.8 ± 0.9	6.6 ± 1.3	8.3 ± 1.0*	p = 0.0157
total insoluble TDP-43	43	34.2 ± 4.3	48.2 ± 5.7	51.0 ± 5.0	p = 0.0467
(2E2-D3)	35	10.2 ± 1.8	16.6 ± 3.0	17.3 ± 2.4	p = 0.0806

Table S2: Western immunoblotting analyses of phosphorylated and total TDP-43 in TBS- and formic acid-soluble protein homogenates from the parietal cortex. Uncropped gels of all these immunoblotting experiments are shown in Figure S4. Data are expressed as mean ± S.E.M. Statistical analysis: one-way ANOVA followed by a Tukey's post hoc test. * $p < 0.05$ vs NCI.

Figures

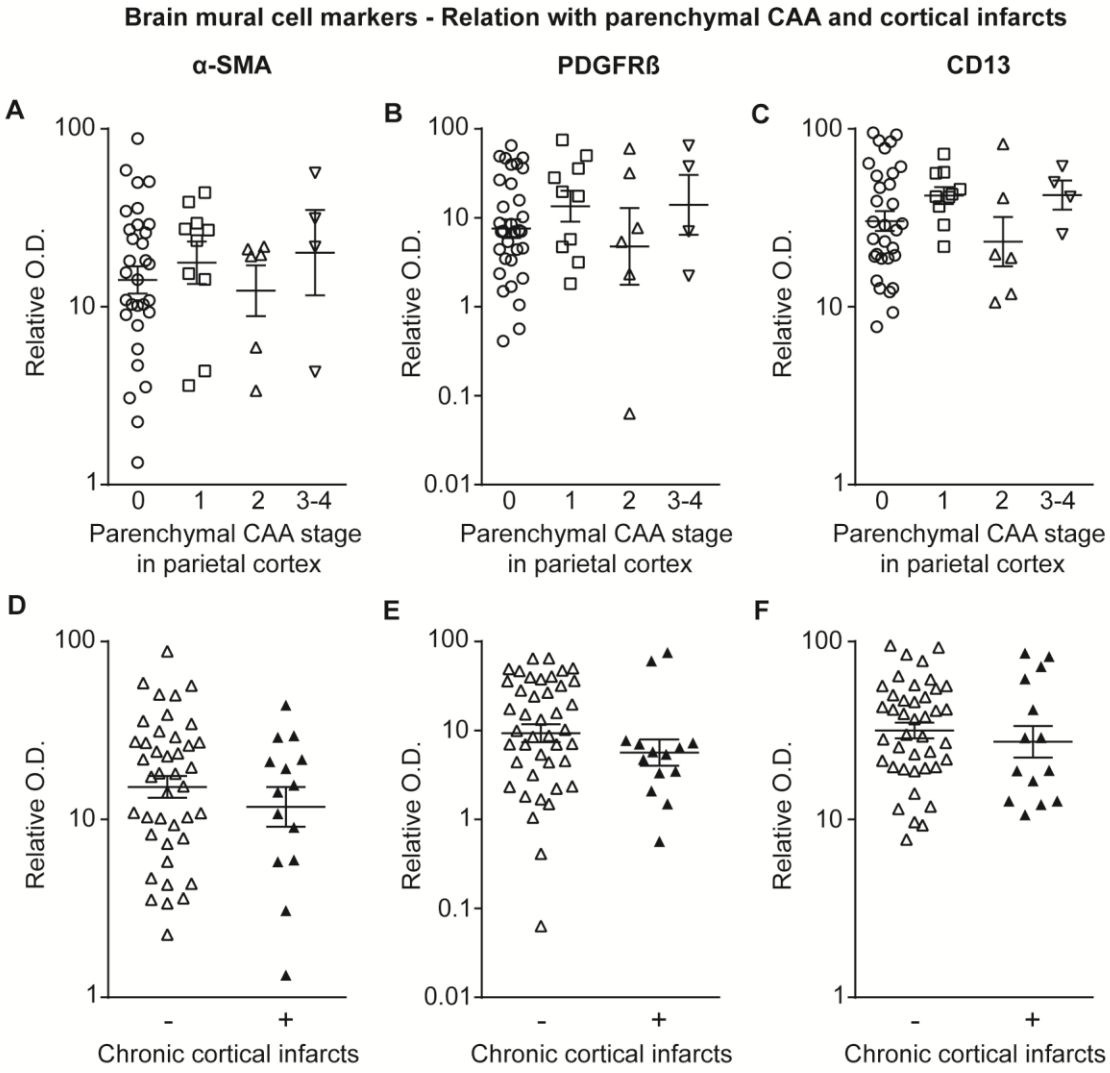

Figure S1: Brain mural cell marker levels are not altered by parenchymal cerebral amyloid angiopathy and cortical infarcts. Levels of mural cell markers in human microvessel extracts were determined by Western immunoblotting. Data are represented as scatterplots, with relative optical density values indicated using a logarithmic scale. A-C) Participants were grouped based on their parenchymal cerebral amyloid stage in the parietal cortex (see Methods section). Individuals with stages 3 or 4 were pooled in the same group for statistical reasons. No difference was noted for all mural cell markers investigated. Statistical analysis: one-way analysis of variance followed by a Tukey's post hoc test, $p > 0.05$. D-F) Volunteers were divided based on the presence of one more chronic cortical macro- and/or

microinfarcts. Levels remained similar between groups for all mural cell markers. Statistical analysis: unpaired Student's t-test, $p > 0.05$. Abbreviations: -, no chronic cortical infarcts; +, presence of one or more chronic cortical infarcts; α-SMA, smooth muscle alpha actin; CAA, cerebral amyloid angiopathy; CD13, aminopeptidase N; O.D., optical density; PDGFRβ, platelet-derived growth factor receptor β.

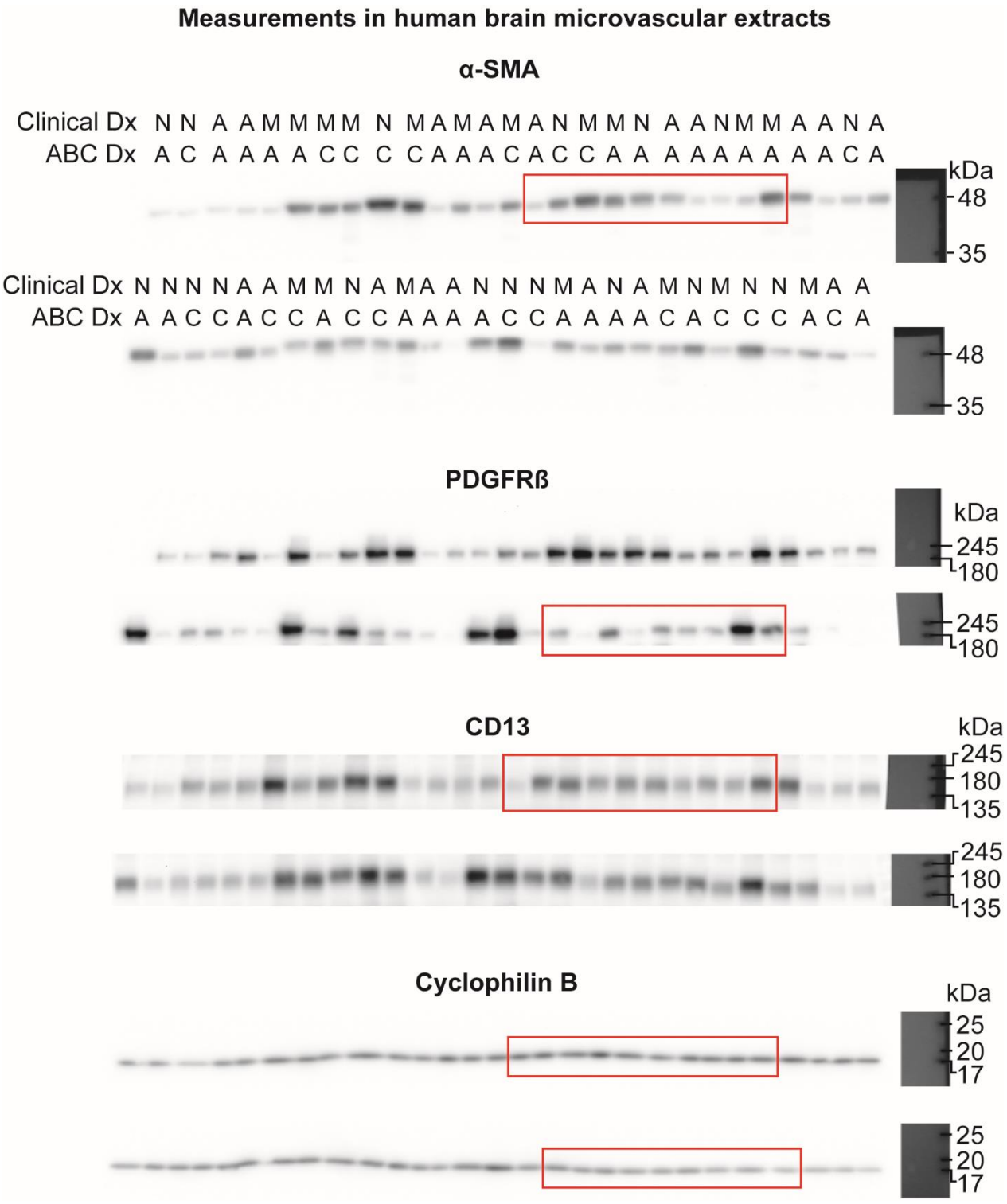

Figure S2: Uncropped gels of immunoblotting experiments in human brain microvessels. The clinical and neuropathological diagnosis are given above each sample. Gels for PDGFRβ, CD13 and Cyclophilin B were loaded in the same order than α-SMA. An equal amount (8 µg) of proteins per sample

was loaded. Red rectangles indicate the bands that were taken as representative photo examples in Figure 2 and Figure 3. Abbreviations: A, Alzheimer's Disease; ABC Dx, ABC neuropathological diagnosis; C, control; Clinical Dx, clinical diagnosis; M, mild cognitive impairment; N, healthy control with no cognitive impairment.

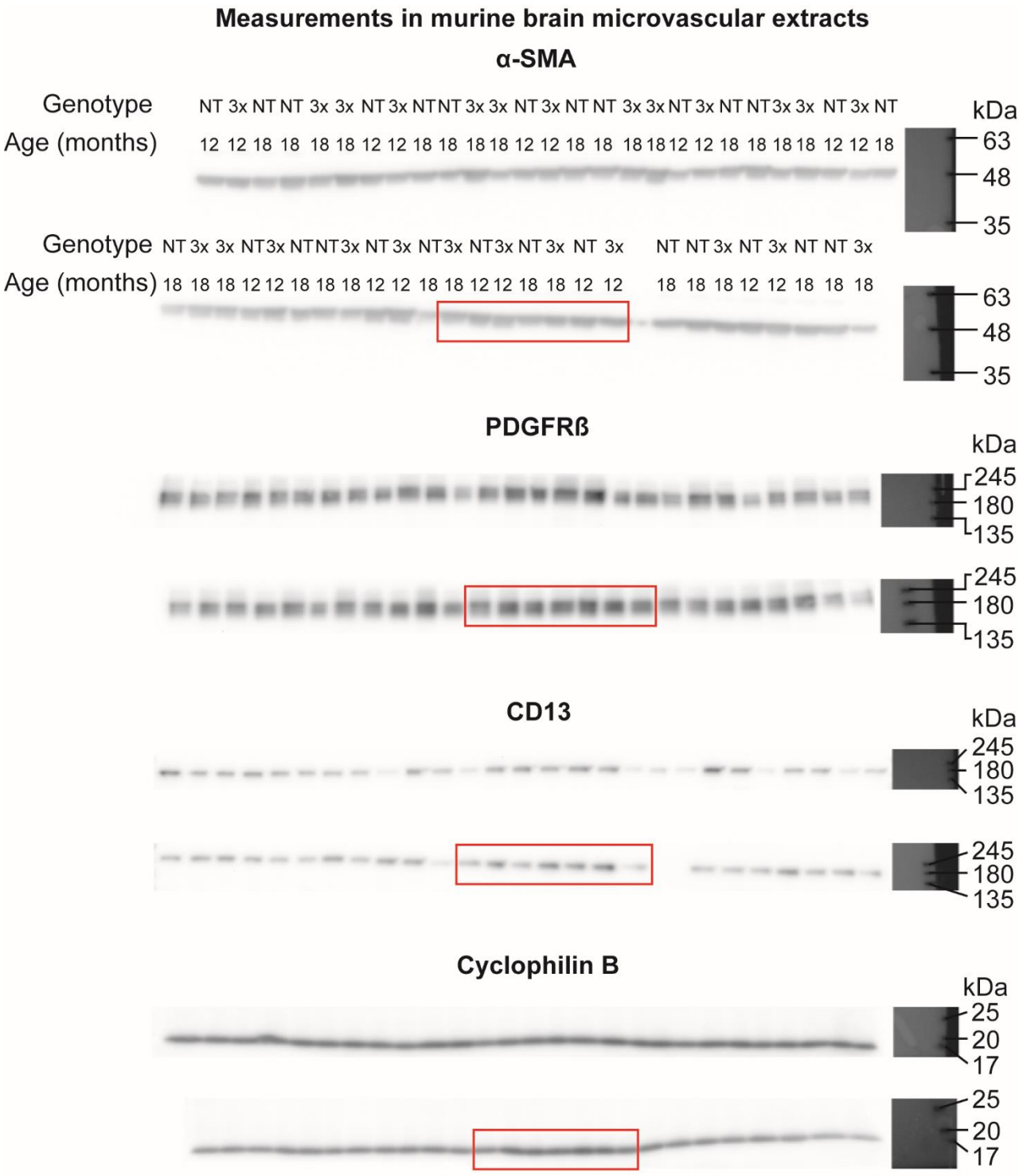

Figure S3: Uncropped gels of immunoblotting experiments in murine brain microvessels. The genotype and age are given above each sample. Gels for PDGFRβ, CD13 and Cyclophilin B were loaded in the same order than α-SMA. An equal amount (8 µg) of proteins per sample was loaded. Red rectangles

indicate the bands that were taken as representative photo examples in Figure 5. Abbreviations: 3x, triple transgenic mouse; NT, non transgenic mouse.

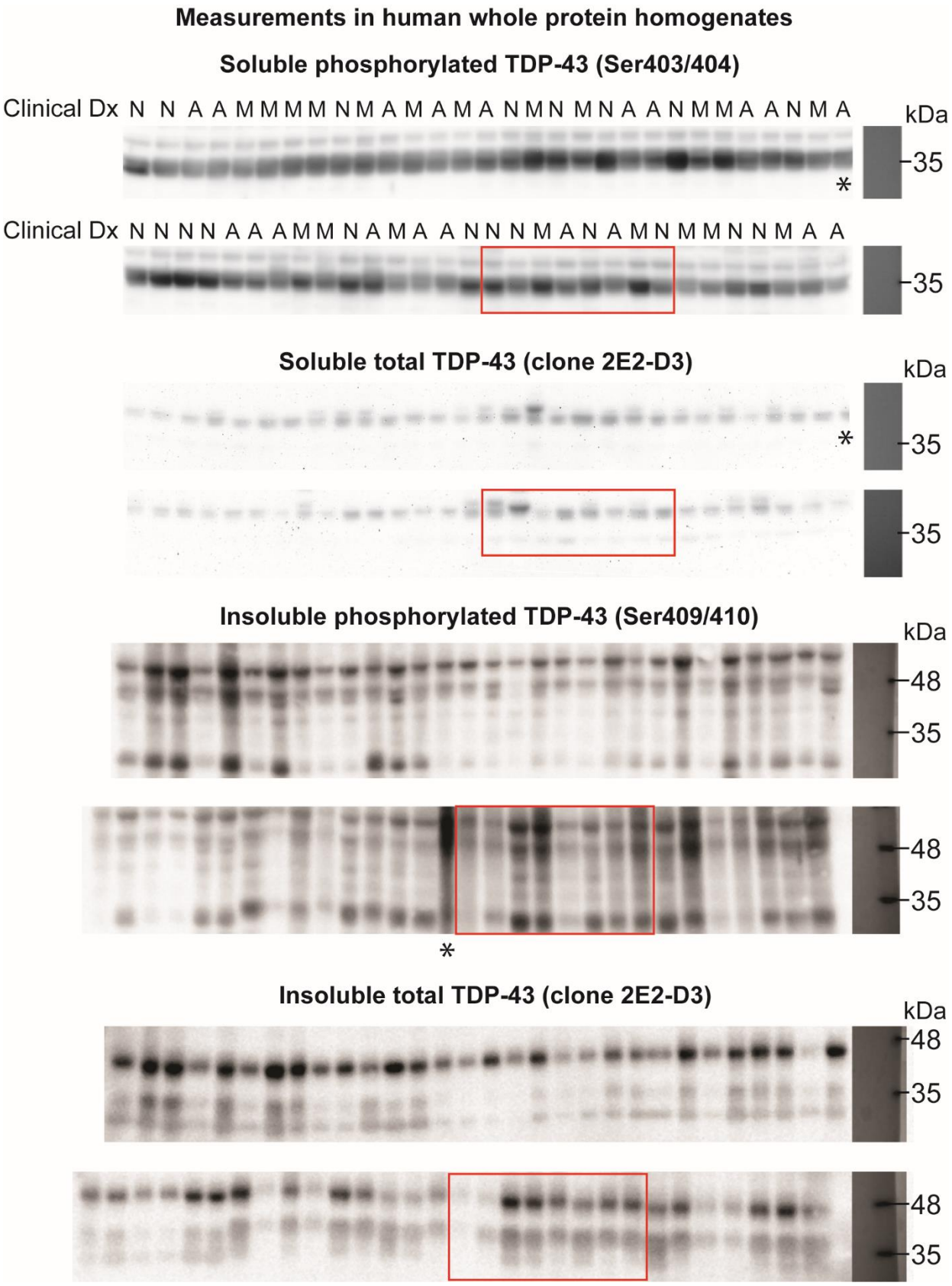
Measurements in human whole protein homogenates
Soluble phosphorylated TDP-43 (Ser403/404)
Clinical Dx N N A A M M M M N M A M A M A N M N M N A A N M M A A N M A
kDa
35
*
Clinical Dx N N N N A A A M M N A M A A N N N M A N A M N M M N N M A A
35
Soluble total TDP-43 (clone 2E2-D3)
kDa
*
35
35
Insoluble phosphorylated TDP-43 (Ser409/410)
kDa
48
35
48
35
*
Insoluble total TDP-43 (clone 2E2-D3)
kDa
48
35
48
35

Figure S4: Uncropped gels of immunoblotting experiments on TDP-43 pathology in whole protein homogenates. The clinical diagnosis is given above each sample. Gels for soluble total TDP-43, insoluble phosphorylated TDP-43 (Ser409/410) and insoluble total TDP-43 (clone 2E2-D3) were loaded in the same order than soluble phosphorylated TDP-43 (Ser403/404). Red rectangles indicate the bands that were taken as representative photo examples in Figure 6. Asterisk symbols indicate samples that were not included in the analyses. Abbreviations: A, Alzheimer's Disease; Clinical Dx, clinical diagnosis; M, mild cognitive impairment; N, healthy control with no cognitive impairment.