Suppl. Table 1

Sampling parameters for neuronal density estimates

Subjects	Left					Right				
	VENs/Fork cells No. Sampling Sites	VENs CE (SH1)	Fork cells CE (SH1)	L5NNs No. Sampling Sites	L5NNs CE (SH1)	VENs/Fork cells No. Sampling Sites	VENs CE (SH1)	Fork cells CE (SH1)	L5NNs No. Sampling Sites	L5NNs CE (SH1)
N1	747	0.075	0.084	45	0.072	791	0.064	0.086	54	0.083
N2	686	0.059	0.082	50	0.099	756	0.066	0.088	49	0.083
N3	726	0.067	0.101	35	0.069	763	0.059	0.115	56	0.085
N4	695	0.056	0.055	69	0.079	700	0.059	0.062	70	0.065
N5	709	0.054	0.055	68	0.067	700	0.051	0.067	69	0.063
N6	699	0.048	0.049	69	0.073	717	0.046	0.063	62	0.071
N7	697	0.090	0.083	71	0.067	698	0.064	0.060	70	0.063
N8	693	0.055	0.057	70	0.088	714	0.064	0.064	77	0.070
N9	697	0.055	0.085	68	0.074	658	0.062	0.063	65	0.066
N10	663	0.061	0.074	65	0.075	692	0.056	0.057	68	0.062
S1	695	0.092	0.085	68	0.067	675	0.113	0.094	65	0.072
S2	645	0.066	0.064	64	0.092	700	0.059	0.059	71	0.061
S3	677	0.058	0.065	68	0.063	657	0.065	0.069	76	0.062
S4	704	0.092	0.089	70	0.078	708	0.091	0.096	69	0.071
S5	679	0.079	0.152	45	0.080	702	0.096	0.225	32	0.102
S6	741	0.098	0.116	48	0.075	727	0.086	0.128	47	0.069
S7	685	0.064	0.103	78	0.068	na	na	na	na	na
S8	688	0.074	0.068	76	0.077	720	0.071	0.074	87	0.058
S9	na	na	na	na	na	697	0.049	0.049	69	0.066
S10	712	0.069	0.063	70	0.073	672	0.062	0.048	66	0.073
S11	na	na	na	na	Na	696	0.085	0.062	69	0.070
S12	662	0.093	0.171	55	0.087	789	0.102	0.205	58	0.080
S13	855	0.076	0.109	63	0.067	699	0.065	0.106	49	0.063
S14	na	na	na	na	na	701	0.080	0.091	70	0.080
S15	na	na	na	na	na	725	0.066	0.068	49	0.083
S16	na	na	na	na	na	757	0.047	0.052	75	0.081
S17	na	na	na	na	na	677	0.059	0.074	64	0.056
C1	788	0.100	0.090	81	0.060	707	0.099	0.116	71	0.062
C2	na	na	na	na	na	664	0.106	0.123	66	0.073
C3	744	0.072	0.075	65	0.066	na	na	na	na	na
C4	689	0.079	0.124	69	0.067	706	0.090	0.098	71	0.069
C5	na	na	na	na	na	na	na	na	na	na
C6	na	na	na	na	na	702	0.068	0.104	71	0.058
C7	na	na	na	na	na	713	0.059	0.064	73	0.082
C8	na	na	na	na	na	695	0.066	0.065	68	0.075
C9	na	na	na	na	na	689	0.117	0.101	66	0.054
C10	698	0.086	0.088	76	0.076	708	0.043	0.046	70	0.066

Key: CE: coefficient of error; SH1: Schmitz-Hof equation, 1st estimated; L5NNs: Neighboring layer 5 neighboring neurons; na: not assessed

Suppl. Table 2

Sampling parameters for TDP-43 inclusion fraction estimates

Subjects	Left							Right						
	VENs/Fork cells No. Sampling Sites	VENs CE (SH1)	Fork cells CE (SH1)	L5NNs No. Sampling Sites	L5NNs CE (SH1)	L2-3 Neurons No. Sampling Sites	L2-3 Neurons CE (SH1)	VENs/Fork cells No. Sampling Sites	VENs CE (SH1)	Fork cells CE (SH1)	L5NNs No. Sampling Sites	L5NNs CE (SH1)	L2-3 Neurons No. Sampling Sites	L2-3 Neurons CE (SH1)
S1	697	0.083	0.107	70	0.078	73	0.063	696	0.091	0.121	57	0.074	79	0.063
S2	660	0.067	0.080	68	0.083	91	0.055	717	0.060	0.071	70	0.078	72	0.058
S3	725	0.058	0.074	78	0.058	66	0.058	706	0.076	0.090	69	0.074	88	0.053
S4	702	0.090	0.097	67	0.078	74	0.053	636	0.088	0.110	59	0.086	73	0.049
S5	707	0.092	0.196	46	0.095	76	0.076	956	0.082	0.202	50	0.099	66	0.071
S6	884	0.070	0.113	45	0.092	68	0.067	877	0.084	0.115	54	0.064	78	0.063
S7	691	0.073	0.091	65	0.073	60	0.065	na	na	na	na	na	na	na
S8	622	0.087	0.090	68	0.073	64	0.082	648	0.093	0.101	71	0.082	71	0.065
S9	na	na	na	na	na	na	na	886	0.046	0.070	62	0.064	68	0.056
S10	700	0.083	0.106	63	0.084	87	0.065	702	0.079	0.106	71	0.083	n/a	n/a
S11	na	na	na	na	na	na	na	746	0.100	0.118	71	0.075	90	0.079
S12	1128	0.061	0.110	47	0.073	78	0.073	812	0.080	0.134	43	0.105	70	0.091
S13	735	0.079	0.146	49	0.077	62	0.097	699	0.071	0.097	48	0.097	48	0.077
S14	na	na	na	na	na	na	na	719	0.064	0.083	74	0.067	69	0.071
S15	na	na	na	na	na	na	na	709	0.074	0.088	41	0.096	74	0.061
S16	na	na	na	na	na	na	na	623	0.061	0.082	61	0.054	73	0.064
S17	na	na	na	na	na	na	na	661	0.087	0.095	69	0.065	77	0.068
C1	710	0.098	0.121	75	0.066	na	na	698	0.081	0.123	70	0.060	na	na
C2	na	na	na	na	na	na	na	682	0.085	0.111	69	0.075	na	na
C3	682	0.066	0.087	70	0.067	na	na	na	na	na	na	na	na	na
C4	661	0.077	0.150	40	0.100	na	na	973	0.063	0.141	47	0.110	na	na
C5	na	na	na	na	na	na	na	na	na	na	na	na	na	na
C6	na	na	na	na	na	na	na	690	0.057	0.077	69	0.056	na	na
C7	na	na	na	na	na	na	na	538	0.065	0.083	53	0.097	na	na
C8	na	na	na	na	na	na	na	769	0.064	0.088	71	0.066	na	na
C9	na	na	na	na	na	na	na	685	0.090	0.110	69	0.073	na	na
C10	682	0.069	0.067	86	0.071	na	na	na	na	na	na	na	na	na

Key: CE: coefficient of error; SH1: Schmitz-Hof equation, 1st estimated; L5NNs: Neighboring layer 5 neighboring neurons; L2-3 Neurons: Layer 2-3 neurons; na: not assessed

Suppl. Table 3

Subject demographics, morphometry.

	Controls	Sporadic bvFTD/MND	*C9orf72*-bvFTD/MND
N	4	7	7
Clinical diagnoses	N.A.	4 bvFTD-MND, 3 bvFTD	1 ALS, 5 bvFTD-MND, 1 bvFTD
Neuropathological diagnoses	N.A.	4 FTLD-TDP-B, MND; 2 FTLD-TDP-B, ALS; 1 FTLD-TDP-B	2 ALS-TDP; 4 FTLD-TDP-B, MND; 1 FTLD-TDP-U, MND
Gender (M:F)	2:2	5:2	5:2
Age at death (years), mean (SD)	59.8 (3.8)	61.3 (5.7)	60.1 (3.5)

Suppl. Table 4

Distribution of VENs across clusters by TDP-43 status

	Cluster		
	1	2	3
Normal TDP, control VENs	9	22	8
Normal TDP, patient VENs	61	57	11
TDP-43 inclusion VENs	4	35	43
TDP-43-depleted VENs	0	30	63
Chi-square statistic	135.48		
p	< 0.0001		

Suppl. Table 5

Association between atrophy severity rank and neuronal compartment

VENs, TDP-43 inclusions	Rank				VENs, nuclear TDP-43 depleted	Rank			
	1	2	3	4		1	2	3	4
Apical dendrite	53	31	14	7	Apical dendrite	61	27	9	8
Basal dendrite	30	51	16	8	Basal dendrite	20	55	23	7
Nucleus	13	10	30	52	Nucleus	21	19	27	38
Soma	9	13	45	38	Soma	3	4	46	52
Chi-square statistic	167.73				Chi-square statistic	231.69			
p	< 0.0001				p	< 0.0001			
Fork cells, TDP-43 inclusions	**Rank**				**Fork cells, nuclear TDP-43 depleted**	**Rank**			
	1	2	3	4		1	2	3	4
Apical dendrite	51	39	9	2	Apical dendrite	38	18	5	1
Basal dendrite	39	38	13	11	Basal dendrite	21	31	8	2
Nucleus	9	16	45	31	Nucleus	3	12	31	16
Soma	2	8	34	57	Soma	0	1	18	43
Chi-square statistic	200.28				Chi-square statistic	191.23			
p	< 0.0001				p	< 0.0001			
Pyramidals, TDP-43 inclusions	**Rank**				**Pyramidals, nuclear TDP-43 depleted**	**Rank**			
	1	2	3	4		1	2	3	4
Apical dendrite	43	35	20	12	Apical dendrite	25	19	5	38
Basal dendrite	21	28	37	24	Basal dendrite	24	24	18	21
Nucleus	14	23	31	42	Nucleus	23	23	26	15
Soma	32	24	22	32	Soma	15	21	38	13
Chi-square statistic	45.31				Chi-square statistic	47.86			
p	< 0.0001				p	< 0.0001			

Suppl. Table 6

Association between atrophy severity rank and neuronal compartment – pairwise comparisons

VENs, TDP-43 inclusions		
Comparison	Chi-squared statistic	p (FDR corrected)
apical dendrite: basal dendrite	11.45	0.0114
apical dendrite: nucleus	75.14	1.01×10^{-15}
apical dendrite: soma	76.23	1.01×10^{-15}
basal dendrite: nucleus	70.81	5.74×10^{-15}
basal dendrite: soma	67.22	2.52×10^{-14}
nucleus: soma	6.30	0.098
VENs, nuclear TDP-43-depletion		
Comparison	Chi-squared statistic	p (FDR corrected)
apical dendrite: basal dendrite	36.51	7.02×10^{-08}
apical dendrite: nucleus	84.88	3.62×10^{-15}
apical dendrite: soma	126.78	1.60×10^{-26}
basal dendrite: nucleus	72.72	2.22×10^{-15}
basal dendrite: soma	98.64	9.15×10^{-21}
nucleus: soma	20.92	0.000109
Fork cells, TDP-43 inclusions		
Comparison	Chi-squared statistic	p (FDR corrected)
apical dendrite: basal dendrite	8.57	0.0356
apical dendrite: nucleus	88.50	9.18×10^{-19}
apical dendrite: soma	131.55	1.50×10^{-27}
basal dendrite: nucleus	54.89	1.09×10^{-11}
basal dendrite: soma	93.46	1.19×10^{-19}
nucleus: soma	16.34	0.00116
Fork cells, nuclear TDP-43-depletion		
Comparison	Chi-squared statistic	p (FDR corrected)
apical dendrite: basal dendrite	9.37	0.0247
apical dendrite: nucleus	63.09	2.56×10^{-13}
apical dendrite: soma	100.65	6.78×10^{-21}
basal dendrite: nucleus	46.35	7.17×10^{-10}
basal dendrite: soma	90.33	5.58×10^{-19}
nucleus: soma	28.11	4.13×10^{-6}
Pyramidal neurons, TDP-43 inclusions		
Comparison	Chi-squared statistic	p (FDR corrected)
apical dendrite: basal dendrite	17.41	0.00175
apical dendrite: nucleus	36.28	3.93×10^{-7}
apical dendrite: soma	12.85	0.00994
basal dendrite: nucleus	7.33	0.0621
basal dendrite: soma	7.55	0.0621
nucleus: soma	9.94	0.0285
Pyramidal neurons, nuclear TDP-43-depletion		
Comparison	Chi-squared statistic	p (FDR corrected)
apical dendrite: basal dendrite	1.82	0.611
apical dendrite: nucleus	24.36	6.33×10^{-5}
apical dendrite: soma	4.71	0.233
basal dendrite: nucleus	14.11	0.00552
basal dendrite: soma	10.10	0.0266
nucleus: soma	40.76	4.40×10^{-8}

Primary clinical diagnosis

78 bvFTD ± MND/ALS or ALS,
UCSF NDBB 2004-2014

Neuropathological diagnosis

Excluded: n = 31
(6 FTLD-FUS, 13 FTLD-Tau, 2 FTDP-17, 4 FTLD atypical or
multiple pathology, 1 ALS-FUS, 1 ALS-NOS, 1 ALS-U, 3 AD)

41 FTLD-TDP, 6 ALS-TDP

Excluded: n = 9
(7 FTLD-TDP-A, 2 FTLD-TDP-C)

C9orf72-negative: n = 21
(17 FTLD-TDP-B ± MND, 4
ALS-TDP)

n = 38
(24 FTLD-TDP-B ± MND,
8 FTLD-TDP-U ± MND, 6
ALS-TDP)

C9orf72-positive: n = 17
(7 FTLD-TDP-B ± MND, 8
FTLD-TDP-U ± MND, 2 ALS-
TDP)

Excluded: n = 4
(1 cormorbid tau pathology, 1 AD Braak
stage > 4, 1 poor tissue quality, 1 PMI >
48h)

Excluded: n = 2
(1 Transitional limbic Lewy body disease,
1 poor tissue quality)

Final number of cases available: n = 17
(13 FTLD-TDP-B ± MND, 4 ALS-TDP)

Final number of cases available: n = 15
(6 FTLD-TDP-B ± MND,
7 FTLD-TDP-U ± MND, 2 ALS-TDP)

Cell counting study: n = 17
(13 FTLD-TDP-B ± MND, 4 ALS-TDP)

**Cell counting study: n = 10
included for blinding**
(5 FTLD-TDP-B ± MND, 4 FTLD-
TDP-U ± MND, 1 ALS-TDP)

Morphometry study: n = 7
with long-fixed right FI
(7 FTLD-TDP-B ± MND)

Morphometry study: n = 7
with long-fixed right FI
(4 FTLD-TDP-B ± MND, 1 FTLD-TDP-U
with MND, 2 ALS-TDP)

**Suppl. Figure 1
Subject selection process**

Flowchart showing patients selected for sporadic and C9orf72-bvFTD/MND groups.
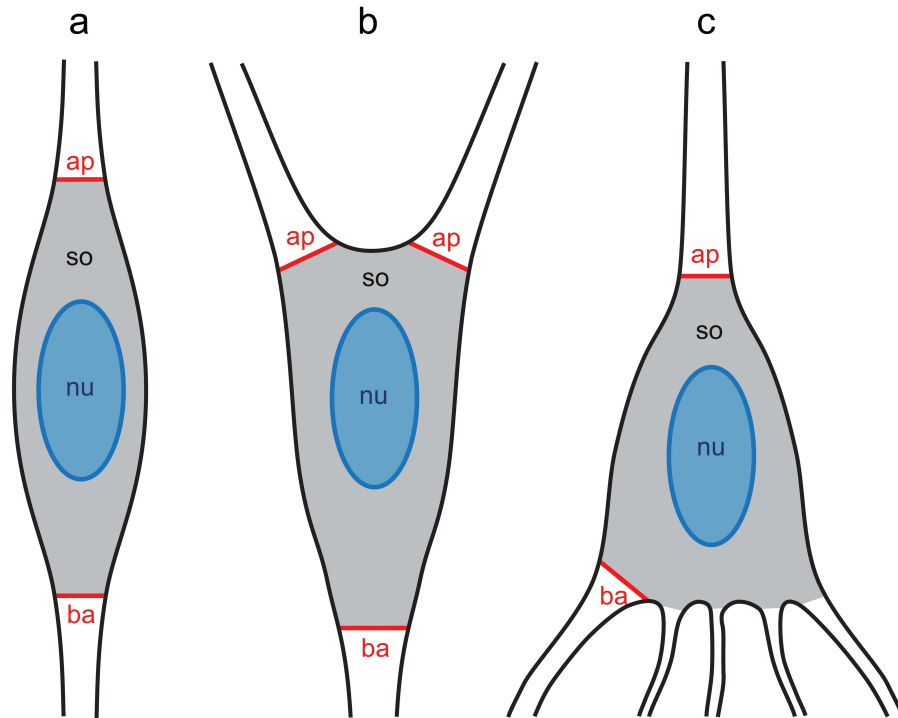
Suppl. Figure 2
Morphometric measurements in VENs fork cells, and neighboring Layer 5 pyramidal cells.

(a-c) Apical (ap) and basal (ba) dendritic diameter and nuclear (nu) and somatic (so) cross sectional area were measured for VENs (a), fork cells (b), and neighboring Layer 5 pyramidal cells (c). In fork cells, the diameter was measured for both apical dendrites. In pyramidal neurons the diameter of the largest basal dendrite was measured.
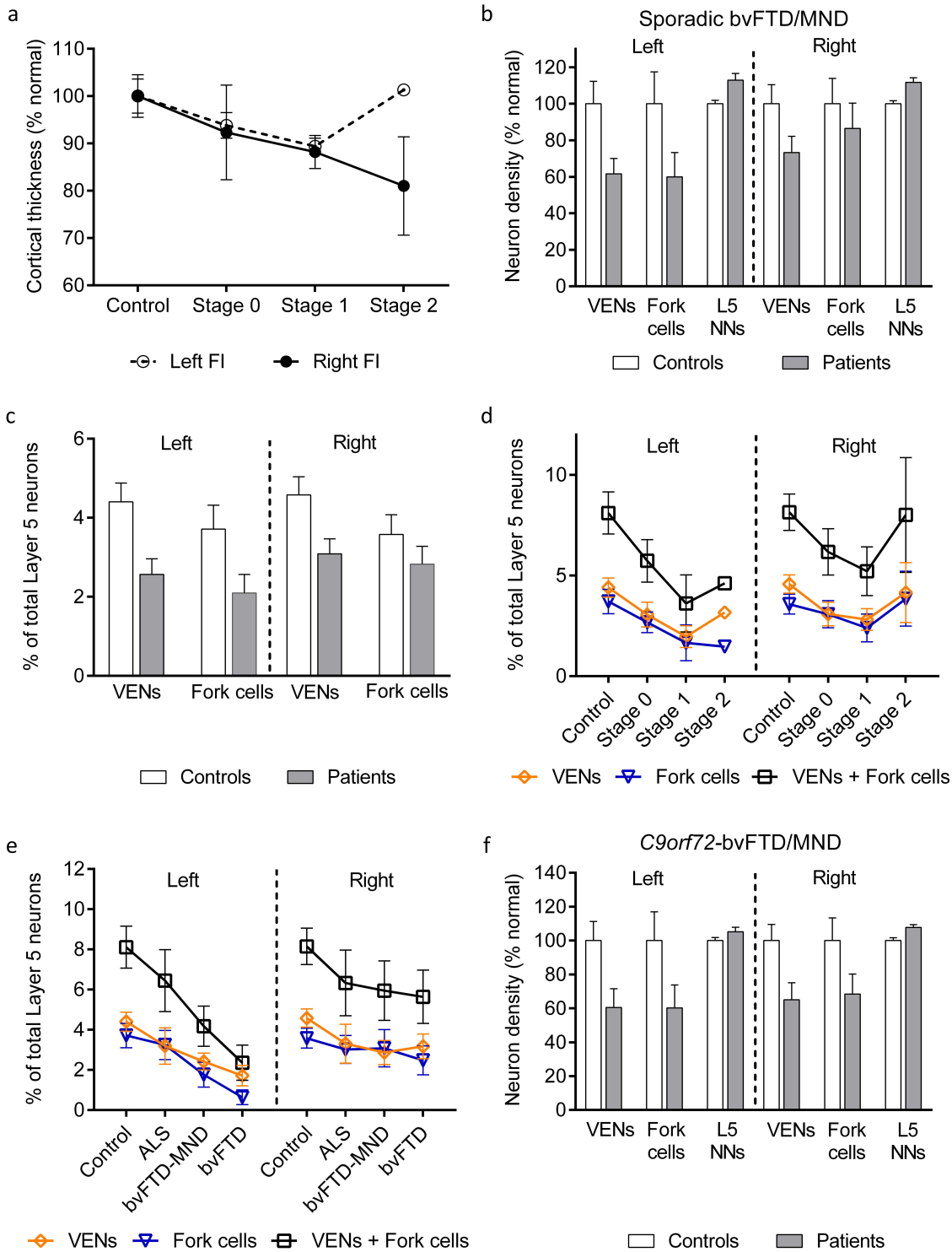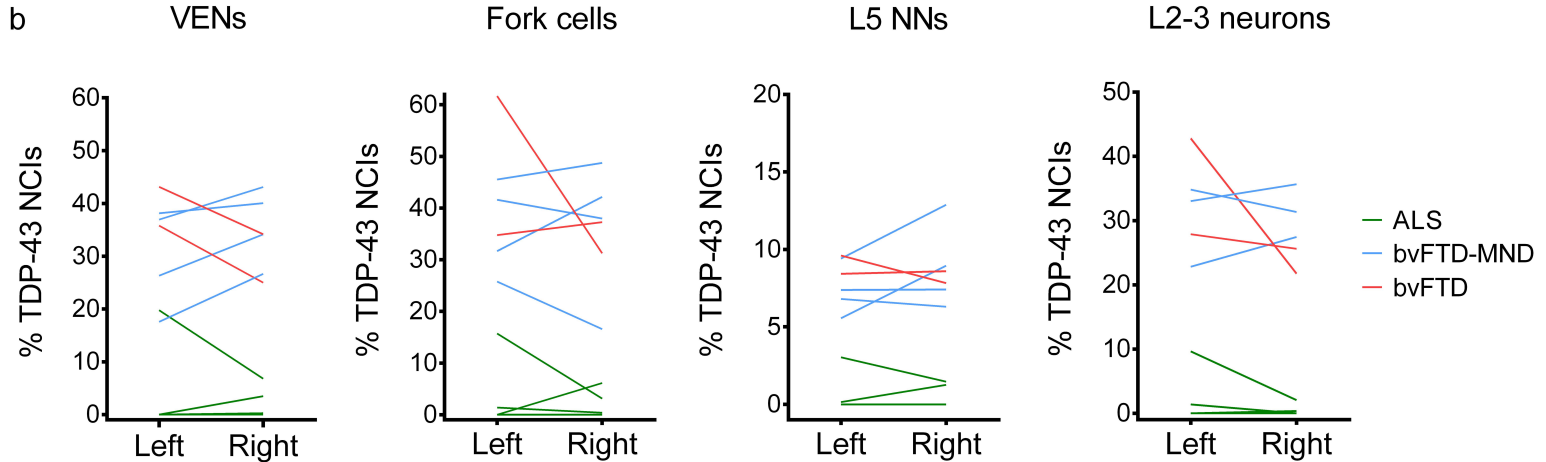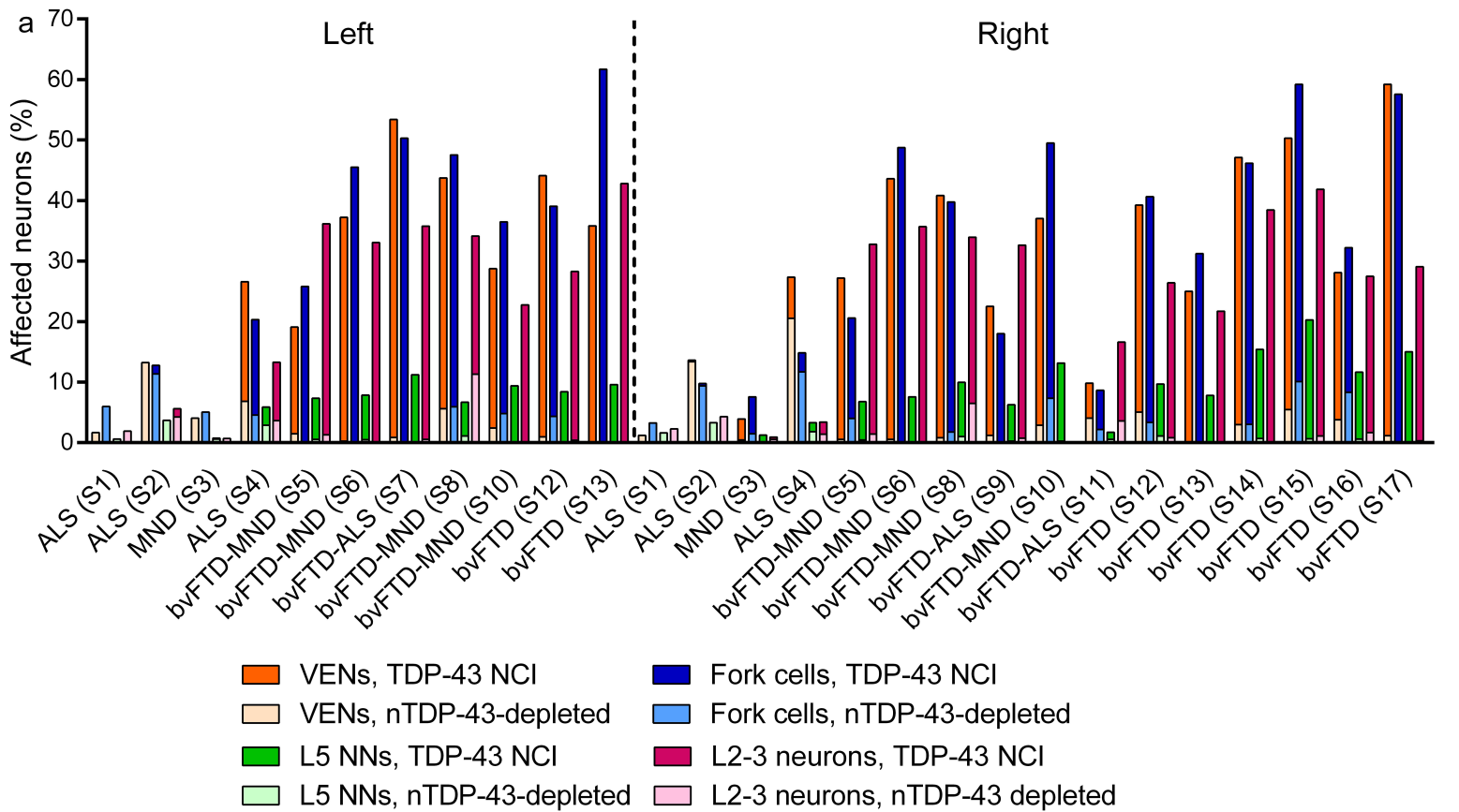
Suppl. Figure 3
Mild FI VEN and fork cell loss in early stage cases.

(a) Cortical thickness decreased with increasing Broe stage and was therefore included in the models as a fixed effect. (b-c) VENs and fork cells showed a non-significant trend toward decreased density (b) and percentage of total neurons (c) in both left and right FI. (d) FI VENs and fork cells were lost in both left and right FI in subjects with mild gross atrophy (Broe stage 0-1) but VEN and fork cell loss was less evident in subjects with more severe global degeneration. (e) There was no clear association between clinical diagnosis and VEN or fork cell loss in the right FI, but more VEN and fork cell loss in subjects with bvFTD±MND than ALS alone in the left FI. (f) A trend toward mild VEN and fork cell loss was seen in C9orf72-bvFTD/MND. Predicted values from linear mixed effect models are shown for a-f. Data for each group is presented as percent of the normal mean ± SEM in a, b and f, and mean ± SEM in c-e. The error bar for Broe stage 2 in d represents the range. L5 NNs: Neighboring Layer 5 neurons.
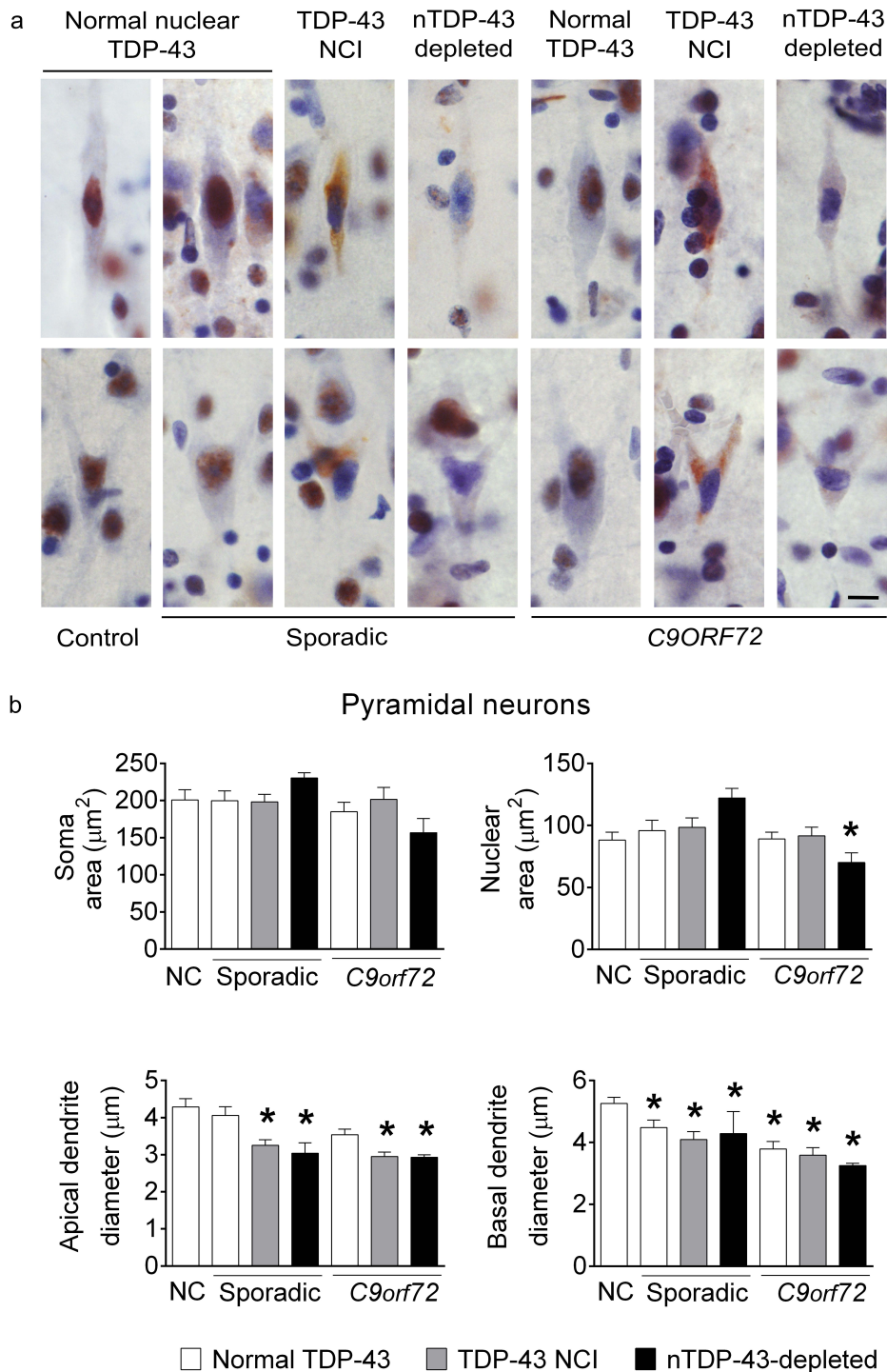
Suppl. Figure 4

Suppl. Figure 4
Percentage of affected VENs, fork cells, and neighboring Layer 5 neurons in each patient with sporadic bvFTD/MND.

(a) Although TDP-43 aggregation was more prominent than nuclear TDP-43 depletion in patients with sporadic bvFTD/MND, patients with ALS showed a higher proportion of nuclear TDP-43-depleted VENs, fork cells, and neighboring Layer 5 neurons (L5 NNs) and Layer 2-3 (L2-3) neurons. (b) No consistent pattern of right-left asymmetry in TDP-43 neuronal cytoplasmic inclusion (NCI) fraction was evident in patients with sporadic bvFTD/MND with bilateral FI available. Each line in b represents a single subject.

Suppl. Figure 5

TDP-43 aggregation and nuclear TDP-43 depletion are associated with nuclear and somatodendritic atrophy in VENs and fork cells and dendritic atrophy in Layer 5 pyramidal cells.

(a) TDP-43 neuronal cytoplasmic inclusion (NCI)-bearing and nuclear TDP-43-depleted VENS (a, upper panel) and fork cells (a, lower panel) appeared shrunken compared to VENs and fork cells with a normal nuclear TDP-43 staining pattern. Normal VENs and fork cells in patients were larger than the normal VENs and fork cells of control subjects. (b) Pyramidal neurons showed dendritic atrophy patterns that were similar to but less pronounced than VEN and fork cell dendritic atrophy. No significant difference in soma or nuclear area were observed between pyramidal neurons with or without nuclear TDP-43. Data in (b) are shown as mean ± SEM. * p < 0.01 compared with normal controls.