α-Synuclein-induced myelination deficit defines a novel interventional target for multiple system atrophy

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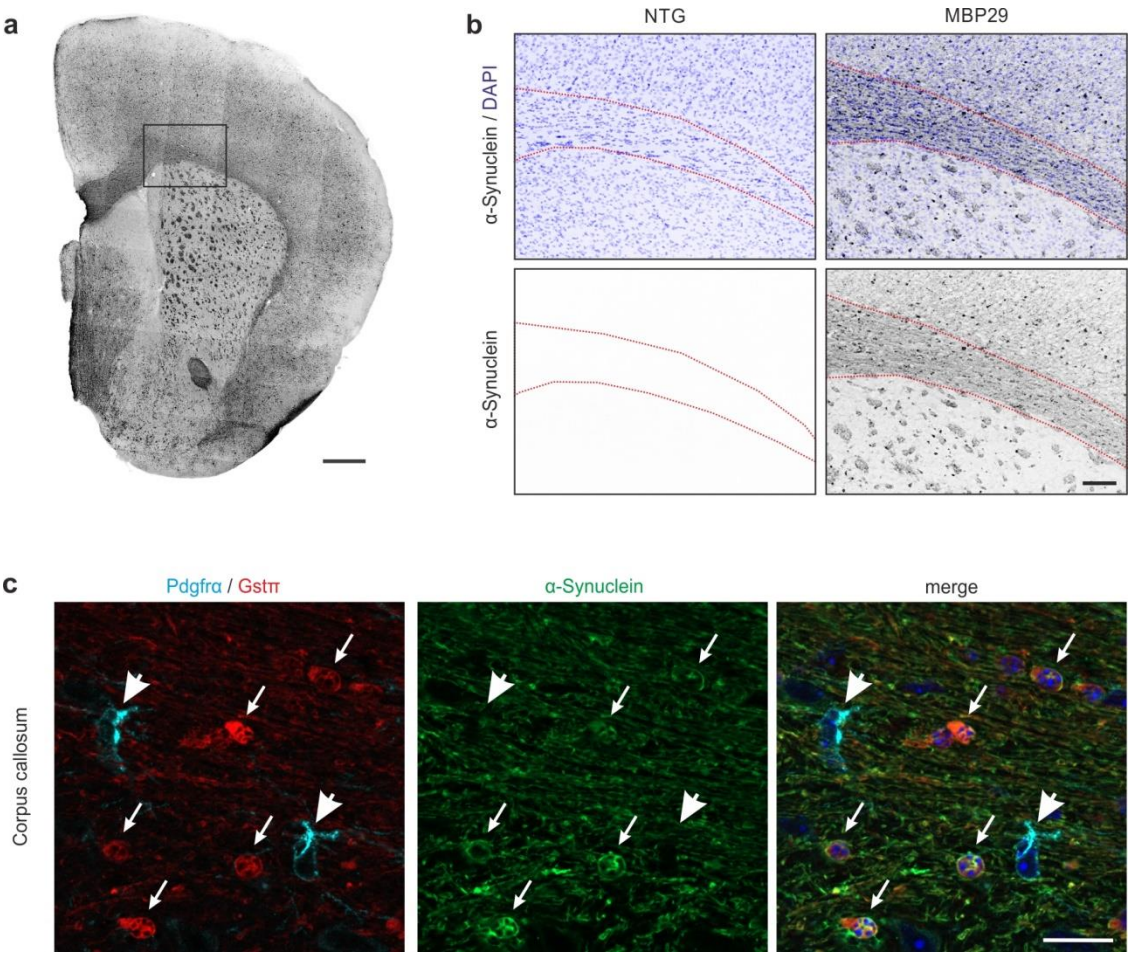
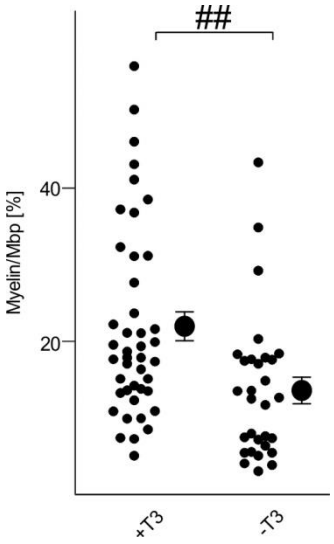
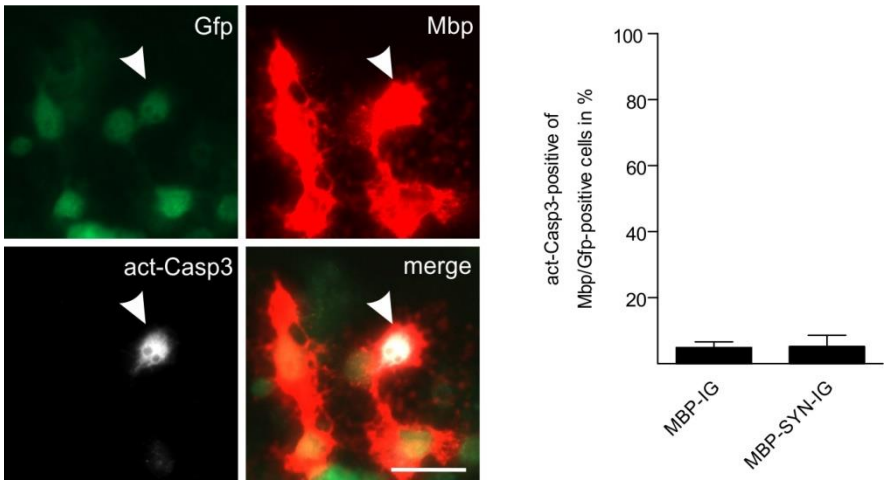


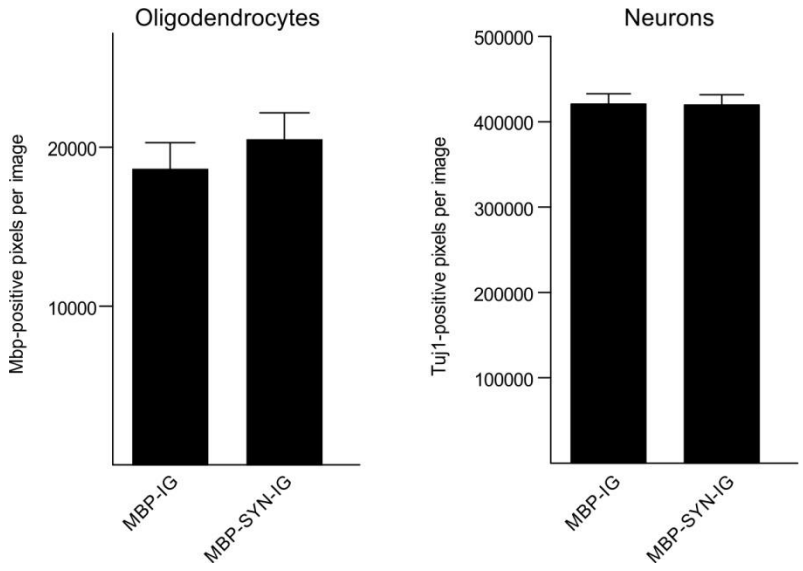
Suppl. Fig. S1 Expression pattern of human α-synuclein in MBP29 mice. **a** Human α-synuclein-stained (black) coronal brain section shows regional expression pattern of α-synuclein in MBP29 mice. The corpus callosum (boxed area is magnified in b) exhibits most abundant α-synuclein expression. Scale bar: 500µm. **b** The corpus callosum is highlighted with dotted red lines and shows a consistent and strong α-synuclein expression pattern. Nuclei were visualized using DAPI (blue). Scale bar: 100µm. **c** α-Synuclein expression was frequently detected in Gstπ-positive mature oligodendrocytes (arrows), whereas it was absent from Pdgfrα-positive OPCs (arrowheads). Scale bar: 20µm.



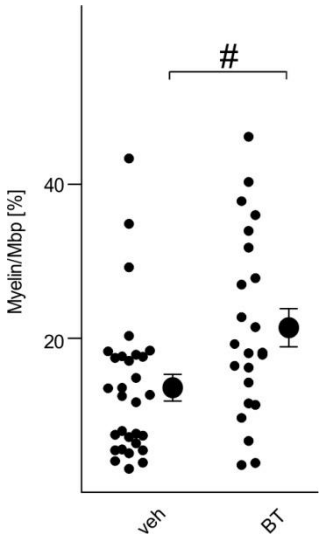
Suppl. Fig. S2 Reduced myelin formation by stem cell-derived oligodendrocytes upon withdrawal of T3. Upon withdrawal of the myelinogenic thyroid hormone T3, myelination of stem cell-derived oligodendrocytes was reduced by 38%. At least 30 individual cells per condition were analyzed and were derived from at least two independent experiments. T-Test: ##$p < 0.01$.



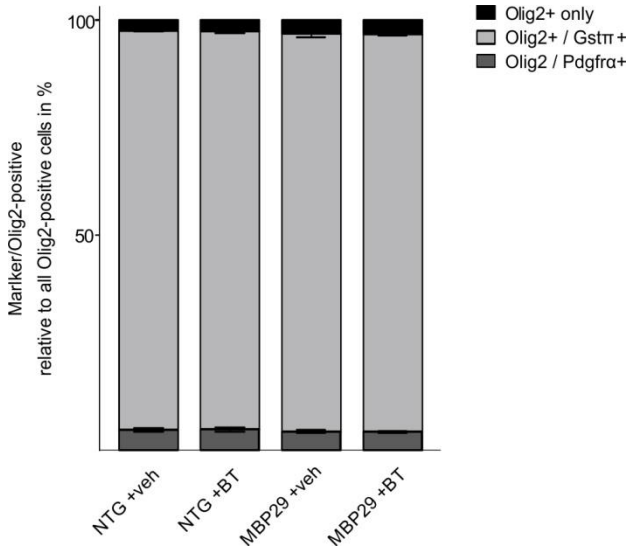
Suppl. Fig. S3 No alteration in numbers of apoptotic pre-myelinating oligodendrocytes upon α-synuclein overexpression. Stem cell-derived oligodendrocytes were lentivirally transduced with control (MBP-IG) and α-synuclein expression vectors (MBP-SYN-IG) and kept for two weeks in culture before staining for the apoptosis marker activated Caspase 3 (act-Casp3). Activated Caspase 3-positive (arrowhead) transduced (Gfp-positive), pre-myelinating (Mbp-positive) oligodendrocytes were only rarely detected. Quantification showed no difference between control (4.6 ± 1.7) and α-synuclein-overexpressing (5.2 ± 3.4) oligodendrocytes (n = 4). T-Test: $p > 0.05$. Scale bar = 50µm.



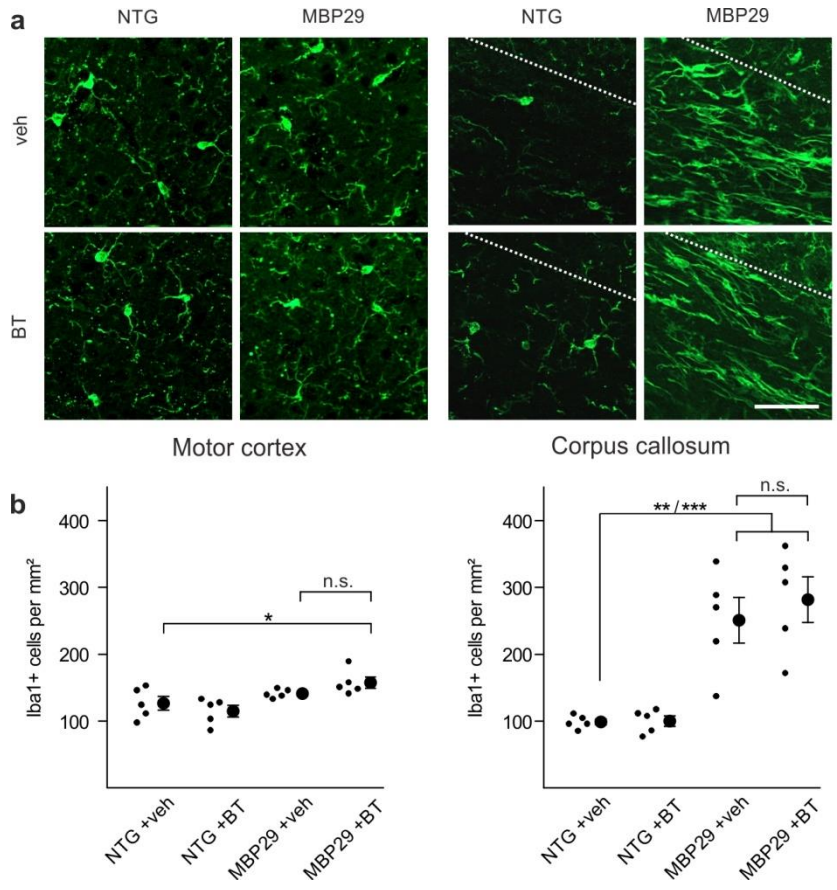
Suppl. Fig. S4 No effect of oligodendrocytic α-synuclein overexpression on oligodendrocytic and neuronal survival in the stem cell-derived oligodendrocyte-neuron co-culture. Stem cell-derived oligodendrocytes were lentivirally transduced with control (MBP-IG) and α-synuclein expression vectors (MBP-SYN-IG) prior to co-culturing for two weeks. Mbp- and Tuj1-positive pixels per analyzed image (altogether: MBP-IG: 235 images, MBP-SYN-IG: 215 images; derived from six independent experiments) were quantified. No significant differences were detected when comparing α-synuclein-overexpressing and control cultures (Mbp-positive pixels: MBP-IG 18597 ± 1688, MBP-SYN-IG 20467 ± 1676, t-test: $p > 0.05$; Tuj1-positive pixels: MBP-IG 421211 ± 11897, MBP-SYN-IG 419714 ± 12183, t-test: $p > 0.05$), indicating that α-synuclein overexpression induces neither neuronal nor oligodendroglial degeneration. Data are shown as mean ± standard error of mean.



Suppl. Fig. S5 Increased myelin formation of stem cell-derived oligodendrocytes upon benztropine treatment. Stem cell-derived oligodendrocyte-neuron co-cultures were treated with vehicle (veh) or benztropine (BT, 1 µM) for two weeks. Myelination of at least 20 individual oligodendrocytes per condition derived from two independent experiments was quantified, showing a significant increase in myelin formation in the presence of benztropine (Mbp/Tuj1- relative to Mbp-positive pixels in percent: vehicle 13.6 ± 1.7, benztropine 21.4 ± 2.5). Data are shown as mean ± standard error of mean. T-Test: #$p < 0.05$.



Suppl. Fig. S6 Oligodendroglial subpopulations were identified by co-labeling for the pan-oligodendroglial marker Olig2 and stage-specific markers (Pdgfrα for OPCs and Gstπ for mature oligodendrocytes). The percentage of specific subpopulations relative to all Olig2-positive oligodendroglia in vehicle- (veh) or benztropine- (BT) treated mice (*n* = 5) is illustrated (Pdgfrα/Olig2 in dark gray: 4-5%, Gstπ/Olig2 in light gray: 92-93%, Olig2 only in black: 2-3%). There was no statistical difference.



Suppl. Fig. S7 No alteration of microglial cell density in mice overexpressing α-synuclein in oligodendrocytes upon benztropine treatment. **a** Representative images show Iba1-positive microglia within the motor cortex and the corpus callosum of non-transgenic controls (NTG) and α-synuclein transgenic mice (MBP29) treated with vehicle (veh) or benztropine (BT). The callosal-cortical border is depicted with dotted lines. Scale bar: 20µm. **b** Densities of Iba1-positive microglia within the motor cortex and the corpus callosum are blotted (n = 5). In the corpus callosum only, MBP29 mice showed a marked increase in the density of microglia. Neither in the motor cortex nor in the corpus callosum, benztropine changed microglial cell density. Note that motor cortical microglial density was slightly increased in benztropine treated MBP29 mice compared to non-transgenic control mice treated with vehicle. Data are shown as mean ± standard error of mean. ANOVA: *p < 0.05, **p < 0.01, ***p < 0.001. T-test: n.s. (not significant) p > 0.05.

Group	Clinical diagnosis	Sex	Age [yr]	Brain weight [g]	PMD [min]	Disease duration [mo]	Cause of death
MSA	Striatonigral degeneration	M	55	1380	520	39	Pneumonia
MSA	Striatonigral degeneration	M	67	1376	370	58	Pneumonia
MSA	Striatonigral degeneration	F	70	1460	490	65	Pneumonia
MSA	Striatonigral degeneration	F	66	1005	485	67	Septic shock
MSA	Striatonigral degeneration	F	59	1102	400	53	Euthanasia
MSA	Striatonigral degeneration	F	67	1244	435	96	Respiratory failure
		2M/4F	**64±6**	**1261±178**	**450±58**	**63±19**	
Control		M	51	1450	465	-	Suicide
Control		M	55	1393	435	-	Intestinal ischemia
Control		F	55	1363	335	-	Intracerebral bleeding
Control		F	60	1240	450	-	Septic shock
Control		F	64	1221	340	-	Respiratory failure
Control		F	77	1111	340	-	Respiratory failure
		2M/4F	**60±9**	**1296±127**	**394±62**		

Suppl. Table T1 Demographic, clinical, and neuropathological characteristics of the MSA cohort. Patients and controls do not differ (t-test: $p > 0.05$) in regard to age, brain weight, and post-mortem delay (PMD).