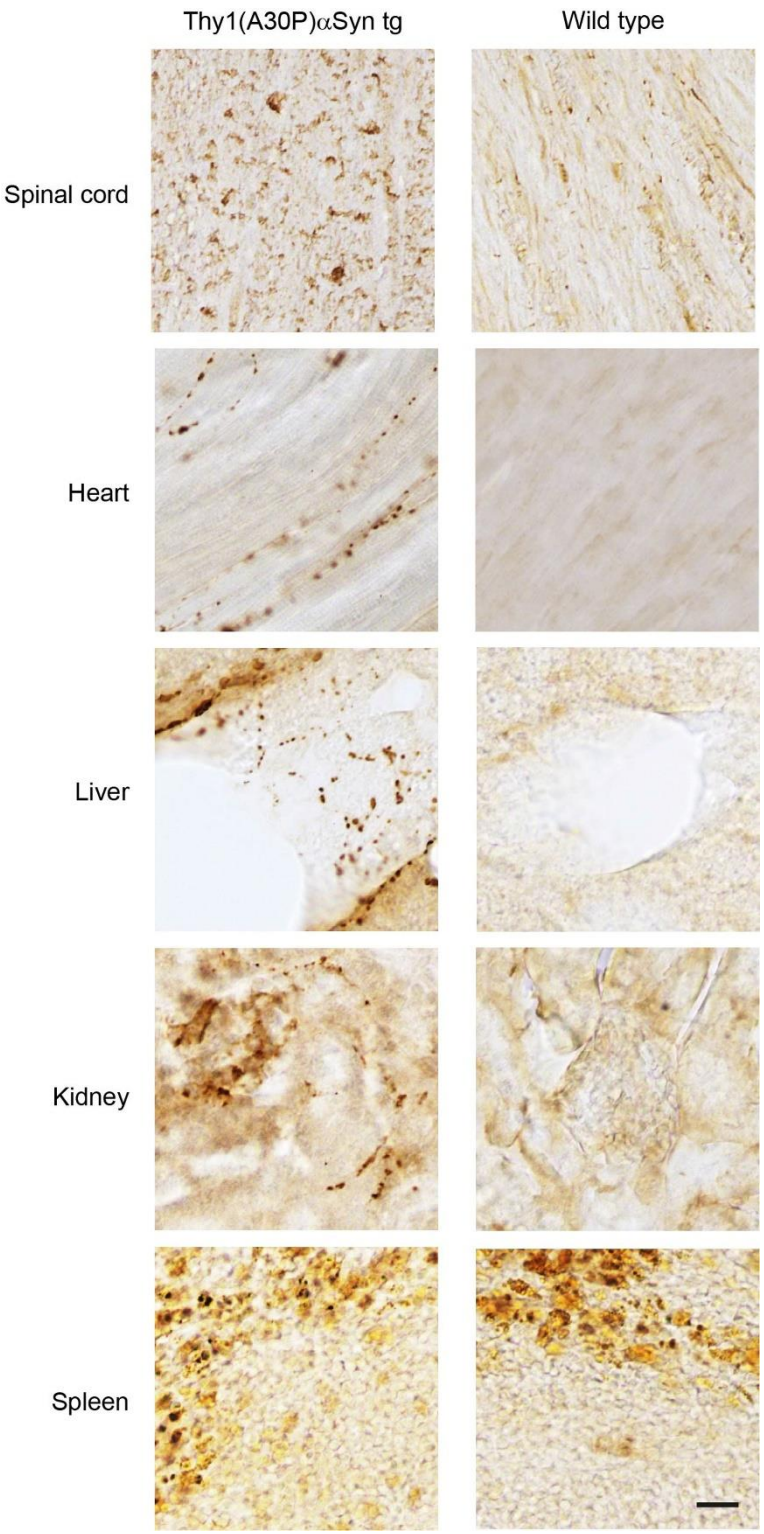
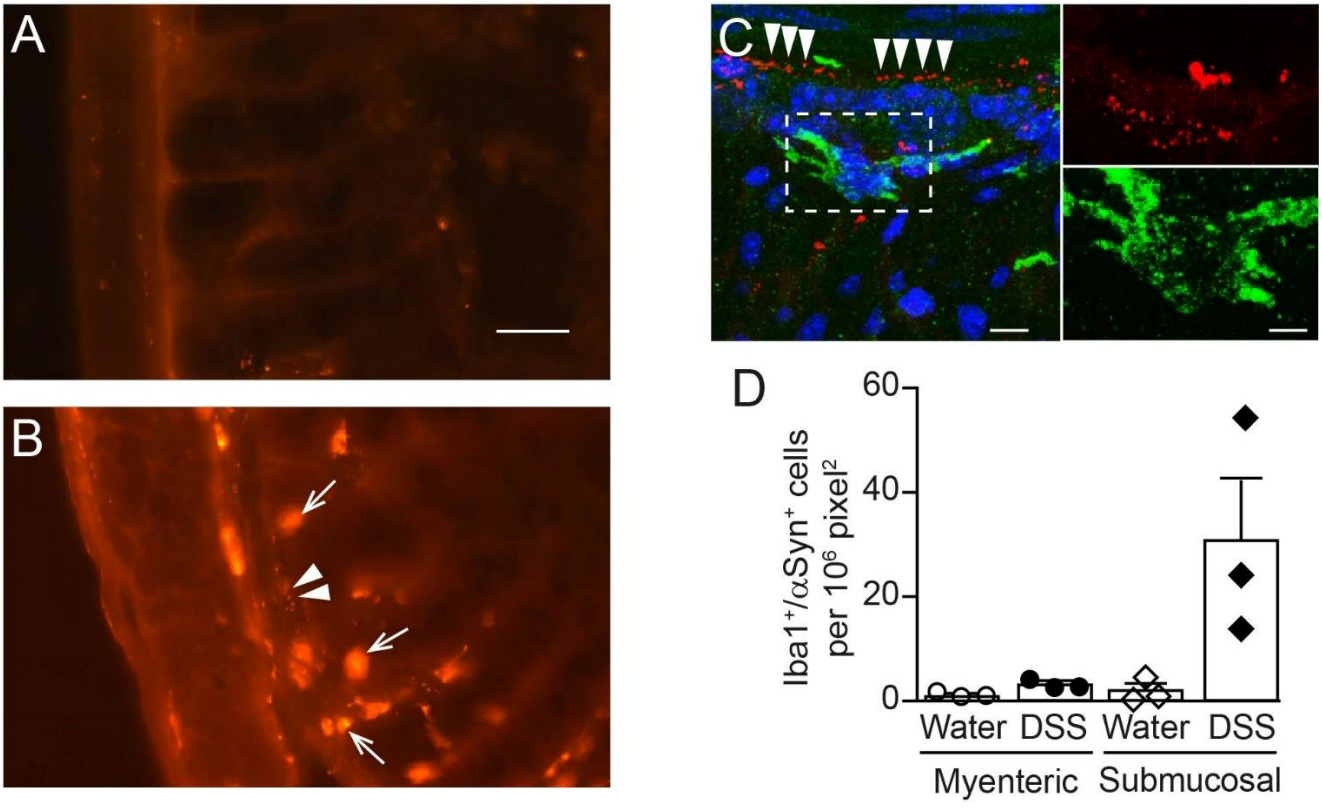
Supplementary material



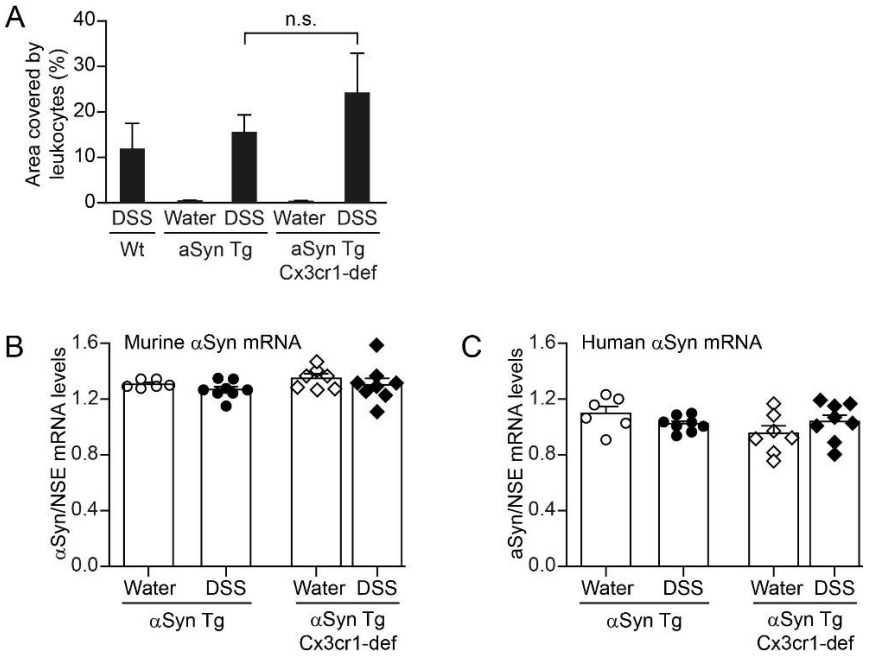
Suppl. Fig. 1 Alpha-synuclein is expressed in the majority of organs in wild type and (Thy1)-h[A30P]αSyn transgenic mice.

Immunohistochemical detection of human αSyn (clone LB509 monoclonal antibody) in (Thy1)-h[A30P]αSyn transgenic mice or of endogenous murine αSyn in wild type mice (syn1 monoclonal antibody) in various organs. Note the typical dot-like structures of the human αSyn in the transgenic mice reminiscent of neuritic inclusions and the very low expression of endogenous murine αSyn in the wild type mice. The pronounced brownish staining in the spleen is due to the abundant iron which is exposed by the chromogenic staining method. Note, thymocytes in the spleen do not stain for human αSyn supporting the selectivity of the expression of the transgenic human αSyn under the modified Thy1.2 cassette, e.g., not expressed in thymocytes [41], n = 3 per group. Scale bar: 20 µm.



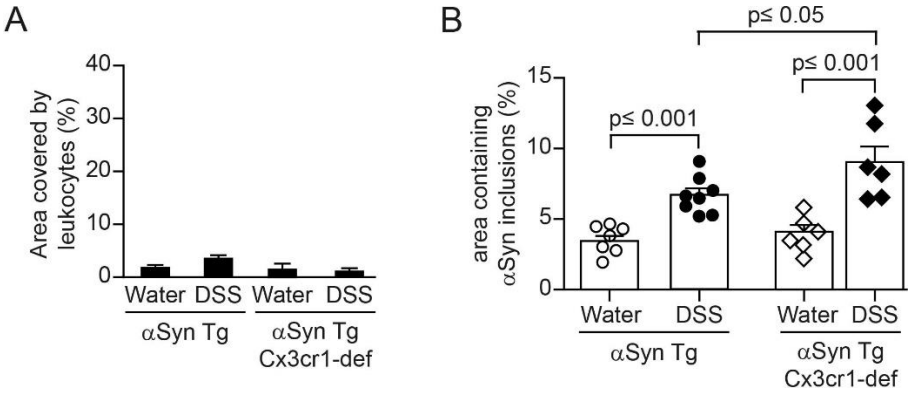
Suppl. Fig. 2 Alpha-synuclein accumulation co-localizes with ENS and Iba-1 positive macrophages upon DSS colitis in αSyn transgenic mice.

A, B Immunofluorescence image of αSyn staining (clone 211; human αSyn specific, detecting both normal αSyn and pathological/abnormal αSyn inclusions containing the respective epitope) in colonic region of (Thy1)-h[A30P]αSyn transgenic mice on water (**A**) or after acute DSS colitis (2.5%) (**B**). Note the small, dotted structures of the typical αSyn inclusions in the submucosal plexus (arrow heads) and the large features of immunoreactivity which localize to infiltrating leukocytes (arrows; identified by their typical cellular morphology). Scale bar: 100 µm. **C** 2D stacks and close-up of confocal images co-localizing αSyn (red) with the macrophage marker Iba-1 (green) in the colon of a (Thy1)-h[A30P]αSyn transgenic mouse after DSS colitis. Note the dotted structures of the typical αSyn inclusions in the submucosal plexus (arrow heads). Scale bar: 40 µm and 13 µm for the close-up. **D** Quantification of numbers of Iba-1/αSyn-double positive macrophages (n = 3 per group; mean and S.E.M.).



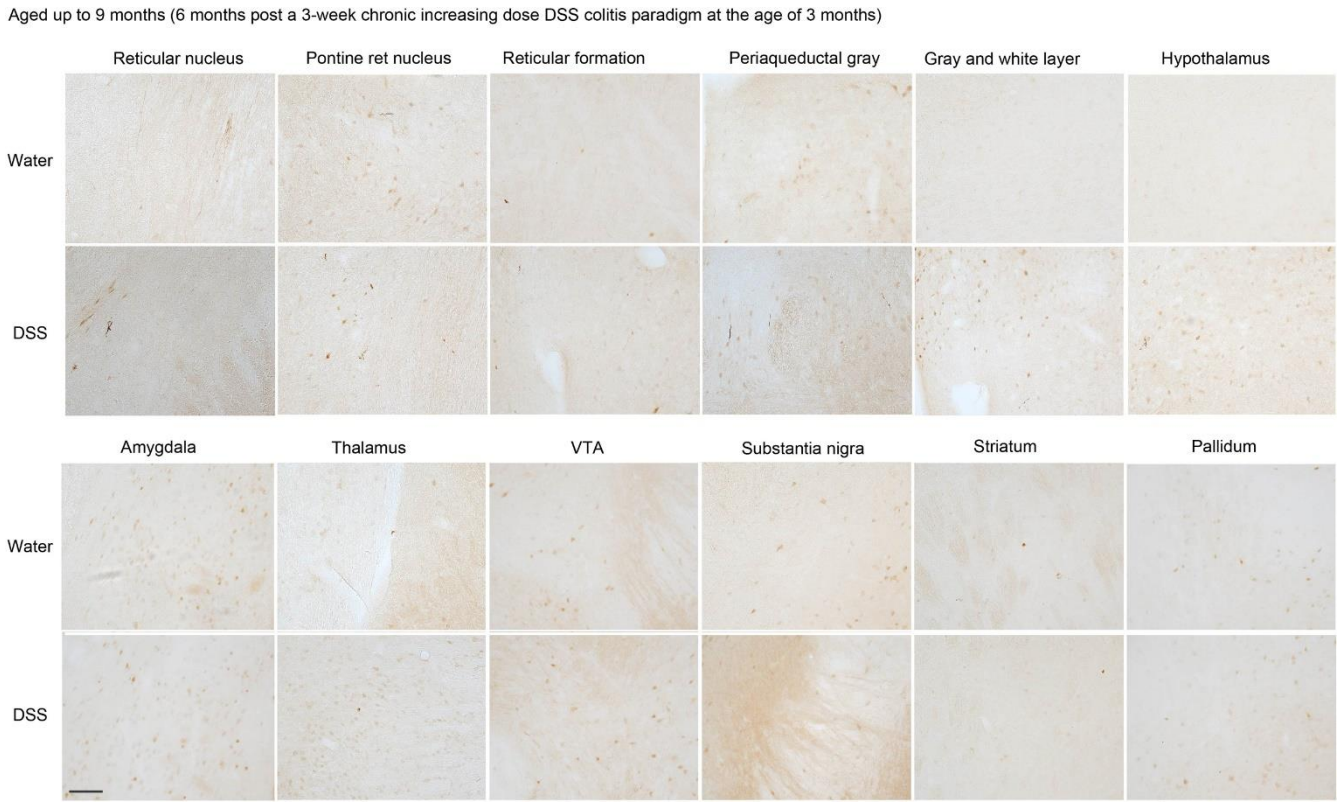
Suppl. Fig. 3 mRNA expression of endogenous murine and transgenic human αSyn in the colon is unchanged after acute DSS colitis.

A Administration of DSS (acute 5%) induces leukocyte infiltration in wild type, (Thy1)-h[A30P]αSyn transgenic (αSyn Tg) and Cx3cr1-deficient (Thy1)-h[A30P]αSyn transgenic mice (αSyn Tg Cx3cr1-def) (Two-way ANOVA with Tukey post hoc test; covariates genotype and treatment paradigm). Expression levels of endogenous murine (**B**) or transgenic human αSyn (**C**) mRNA were normalized to mRNA levels of the neuronal marker neuron specific enolase (NSE) to correct for potential neuronal loss (n = 5-8 per group; mean and S.E.M.).



Suppl. Fig. 4 DSS colitis induced accumulation of αSyn in submucosal plexus of (Thy1)-h[A30P]αSyn transgenic mice remains stable long after recovery.

A 4-week chronic DSS paradigm was performed with (Thy1)-h[A30P]αSyn (αSyn Tg) and (Thy1)-h[A30P]αSyn crossed with Cx3cr1-def mice (αSyn Tg Cx3cr1-def). After recovery for 2 months and thus analysis at the age of 6 months, (**A**) the colon was inspected for signs of inflammation (area covered by leukocytes) and, (**B**) amount of αSyn inclusions (area containing αSyn inclusions), n = 6-8 per group. Statistical analyses were performed using two-way ANOVA with Tukey post hoc testing, covariates genotype and treatment paradigm.



Suppl. Fig. 5 Minor to no detectable αSyn pathology in the brain of a 9-month-old (Thy1)-h[A30P]αSyn transgenic mouse six months after a 23-days chronic DSS colitis insult at young age.

A 23-day chronic DSS paradigm 'increasing dose' (see Fig. 1) was performed with 3-month-old (Thy1)-h[A30P]αSyn transgenic mice. After recovery and further aging under normal conditions, various brain regions were analyzed for proteinase K-resistant pSer129-αSyn immunoreactivity (pathological/abnormal pSer129-positive features; note the only rarely observable neuritic and punctate inclusion-type morphology) Shown are representative images from one 9-month-old mouse. Scale bar: 500 µm.