

Soluble and insoluble lysates from the human A53T mutant α -synuclein transgenic mouse model induces α -synucleinopathy independent of injection site.

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Affiliations

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Supplementary Material

Table S1

Figures and Legends (Figures S1-S9)

Loading Controls	Company	Reference	RRID	Use
GAPDH (D16H11)	Cell Signaling	5174	AB_10622025	WB
α -tubulin	Abcam	4074	AB_2288001	WB
Actin	Millipore-Sigma	A2066	AB_476693	DB
α-Synuclein Species	Company	Reference		Use
α -Synuclein (total)	BD Biosciences	610787	AB_398108	WB
pS129 α S	Abcam	Ab51253	AB_869973	IHC, WB
pS129 α S	Wako	015-25191	AB_2537218	IF
FILA-1	Poul H. Jensen (gift)	Ref a	N/A	DB
Glial and Neuronal Markers	Company	Reference		Use
Iba1	Wako Chemical	019-19741	AB_839504	IHC, WB, IF
GFAP	Dako Cytomation	Z0334	AB_10013382	IHC, WB, IF
Misc	Company	Reference		Use
Anti-KDEL (Grp78/94)	Abcam	Ab176333	AB_2819147	WB
Grp78/Bip	Novus	NB300-520	AB_10000968	WB
CytoC	BD Biosciences	556432	AB_396416	WB
Transketolase	Invitrogen	PA5-56166	AB_2648498	WB
Calnexin	Novus	NB100-1965	AB_10002123	WB
Anti-OC (Amyloid Fibril)	Millipore-Sigma	AB2286	AB_1977024	DB

Supplementary Table S1. Antibodies used in this report. RRID-Research Resource Identifier, WB-Western Blot, DB-Dot Blot, IHC-Immunohistochemistry, IF-Immunofluorescence, N/A-not applicable.

- a. Lindersson, E. *et al.* Proteasomal inhibition by alpha-synuclein filaments and oligomers. *J Biol Chem* **279**, 12924-12934 (2004).

Supplementary Figures:

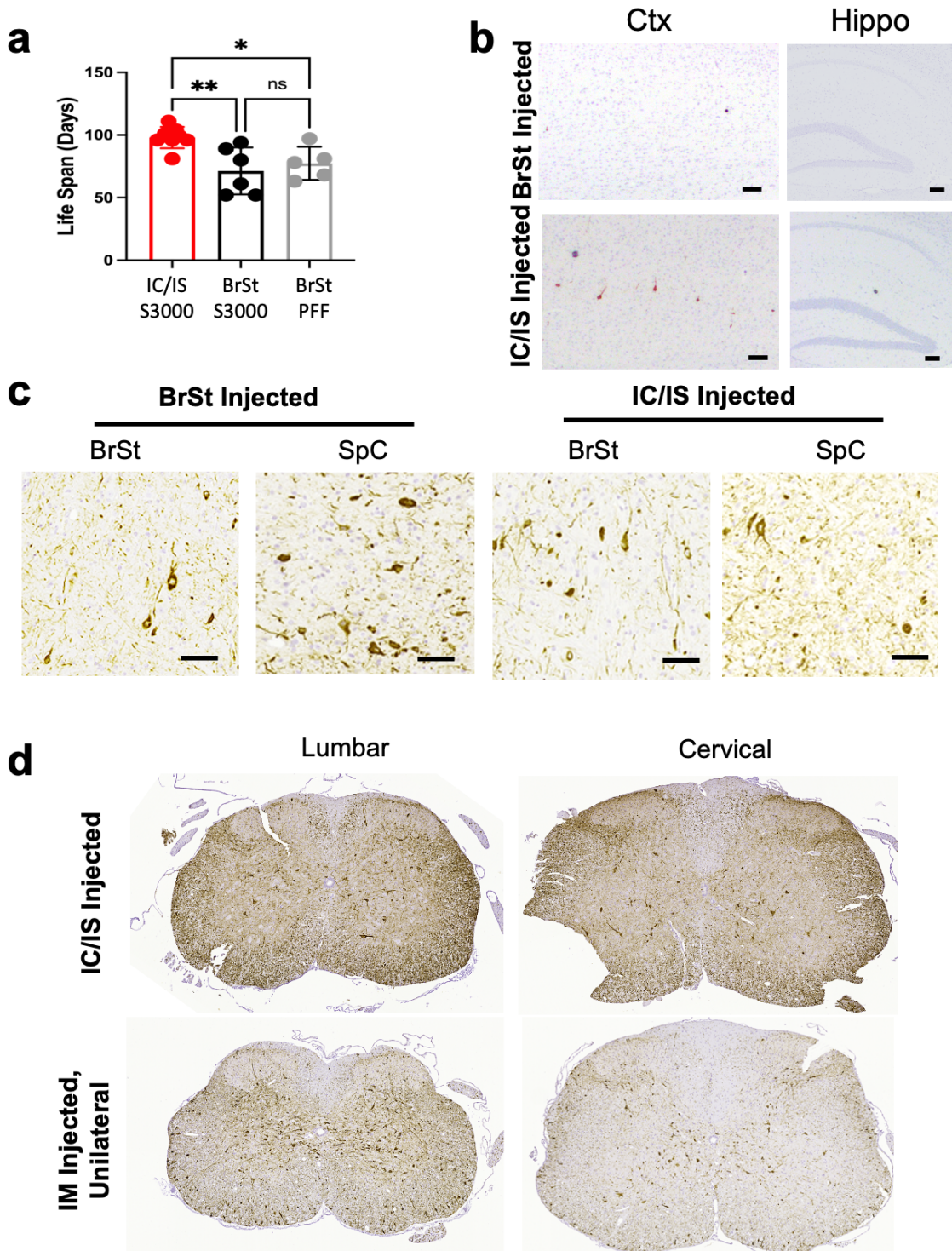


Figure S1. a) Injection of ESL or α S PFF into BrSt leads to shorter lifespan than IC/IS injections of ESL in TgA53T mice. $*p < 0.05$, $**p < 0.01$, One-way ANOVA, $n = 5-6$. **b, c)** α S pathology following BrSt or IC/IS injections are most prominent in BrSt and SpC at ES.

b) IC/IS injections lead to more pS129 α S⁺ neurons in cortex but both models lack pS129 α S in hippocampus (Hippo). **c)** Abundant pS129 α S immunoreactivity is seen with both BrST and IC/IS injection model. **d)** Bilateral SpC pS129 α S pathology following unilateral IC/IS injection of ESL or unilateral IM injection of α S PFF. In addition to bilateral pathology in the grey matter, pS129 α S is bilaterally present in the descending and ascending axon tracts. Abbreviations: end-stage lysate, ESL; α -synuclein, α S; preformed fibril, PFF; brainstem, BrSt; intracortical/intrastriatal, IC/IS; intramuscular, IM; spinal cord, SpC; end-stage, ES. Scale Bars: 100 μ m.

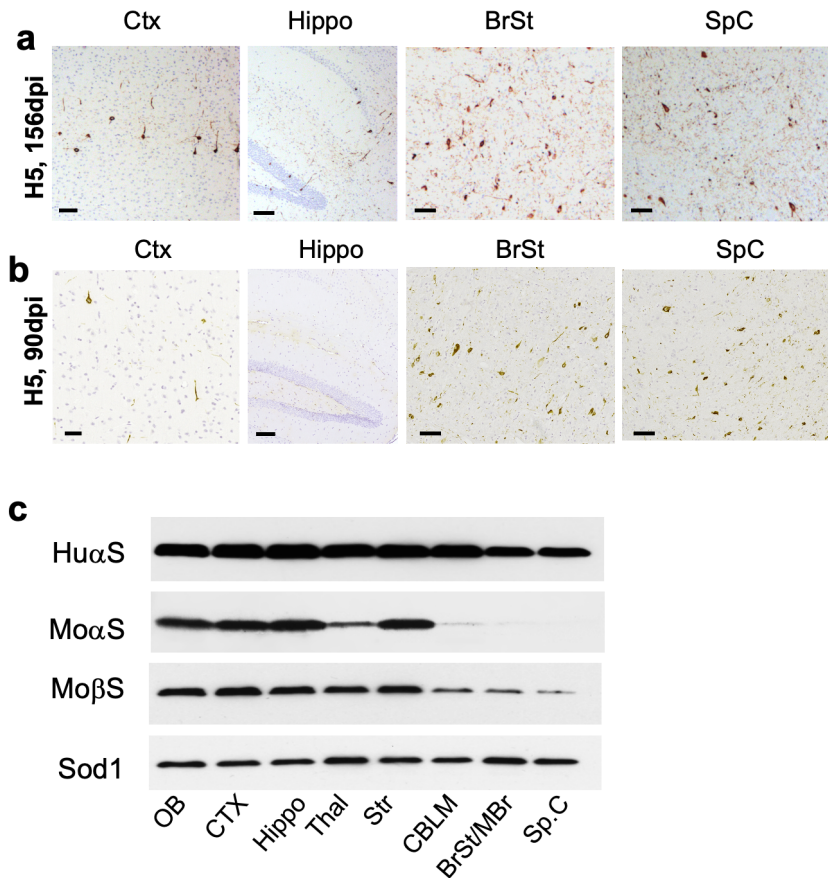
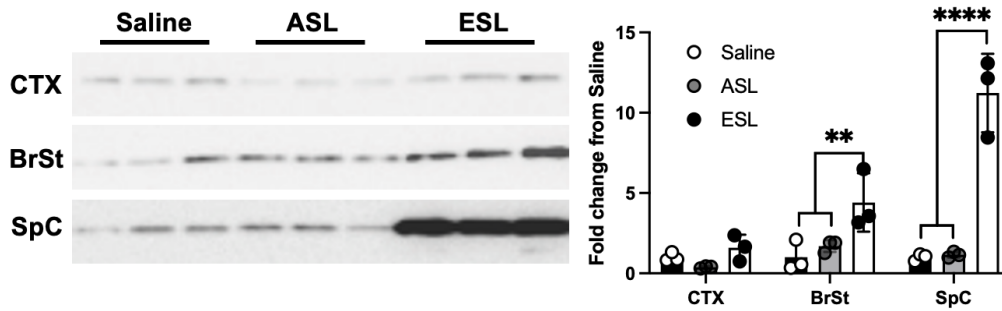


Figure S2. a, b) α S pathology following IC/IS injections of ESL into mice from *TgA53T(H5)* line. **a)** ES *TgA53T(H5)* mice (156 dpi) following IC/IS injection of ESL (S3000) were analyzed for accumulation of pS129 α S. While the *TgA53T(H5)* mice show more pS129 α S pathology in the cortex (CTX) and hippocampus (Hippo) than the IC/IS injected *TgA53T(G2-3)* mice (see Fig. S1B), most prominent pS129 α S pathology is localized to BrSt and SpC. **b)** Intermediate stage *TgA53T(H5)* (90 dpi) mice were analyzed for accumulation of pS129 α S. These mice did not show any overt motor abnormalities. There is a low level of pS129 α S staining in CTX with more abundant pS129 α S staining in the BrSt and SpC. Abbreviations: α -synuclein, α S; intracortical/intrastratial, IC/IS; end-stage lysate, ESL; end-stage, ES. Scale Bars: 100 μ m. **c)** Analysis of α -synuclein and β -synuclein expression in *TgA53T* mice. Total SDS-soluble lysates from mouse brain regions from *TgA53T* mice were immunoblotted using antibodies selective for human α -synuclein (HuSyn, Hu α S)²⁸, mouse α -synuclein (mo α S)³⁴, and β S (Mo β S, Abcam). Sod1 was used to confirm equal loading. The results show that transgenic Hu α S is widely expressed all brain regions. While high levels of Mo α S or Mo β S is expressed in forebrain areas [Olfactory Bulb (OB), Cortex (CTX), Hippocampus (Hipp), Thalamus (Thal), and Striatum (Str)] very low levels are expressed in Cerebellum (CBLM), Brain Stem/Mid-brain (BrSt/MidBr), and Spinal Cord (SpC). Mo α S expression is also low in Thal.

a. Iba1



b. GFAP

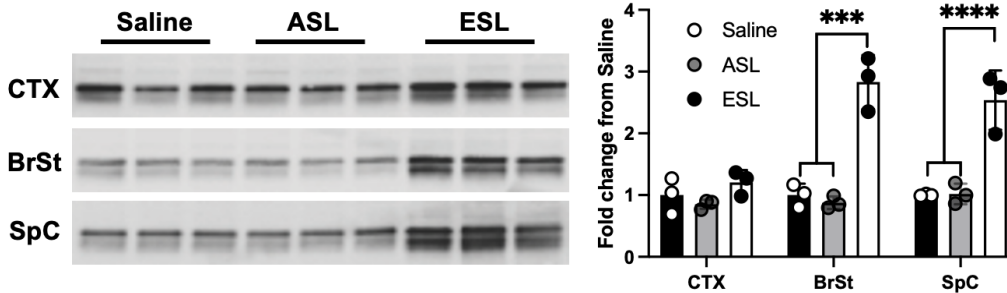


Figure S3. Quantitative analysis of increased microglia and astrocytes in ES *TgA53T* mice following ESL injection. **a)** Immunoblot analysis of whole-tissue lysates from CTX, BrSt, and SpC of saline-, ASL-, and ESL-injected mice for Iba-1 and corresponding quantitative analysis. Quantification of the blots confirms no change in the Iba1 signal in CTX but significant increases in BrSt and SpC (bottom). Values are mean ± SD ($n=3$ each). Two-way ANOVA, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, Tukey's *post hoc* test for multiple comparisons. $n=3$. **b)** Immunoblot analysis of lysates in **a** for GFAP confirms no change in GFAP levels in CTX but significant increases in BrSt and SpC (bottom). Values are mean ± SD ($n=3$ each). One-way ANOVA, * $p < 0.05$. Tukey *post hoc* test for multiple comparisons. Abbreviations: end-stage, ES; end-stage lysate, ESL; cortex, CTX; brainstem, BrSt; spinal cord, SpC; asymptomatic lysate, ASL.

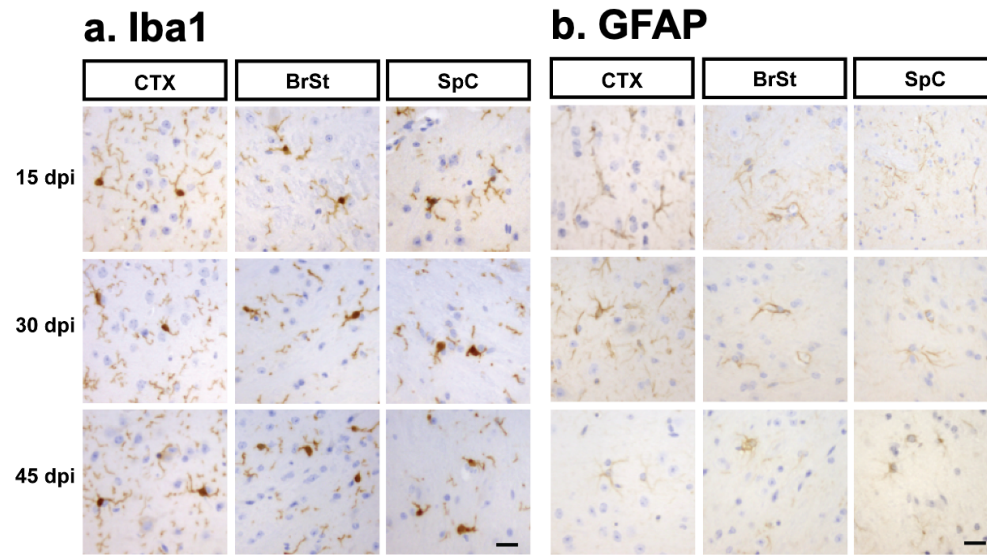


Figure S4. Tissues from end-stage lysate-BrSt injected mice were collected at 15-, 30-, and 45-days post injection and immunostained for Iba1 (**a**) and GFAP (**b**). There is no increase in neuroinflammation within 45 dpi. Increased astrogliosis and microgliosis occur only at end-stage disease when severe α S pathology is present. Abbreviations: brainstem, BrSt; cortex, CTX; spinal cord, SpC; days post-inoculation, dpi. Scale bars: 25 μ m.

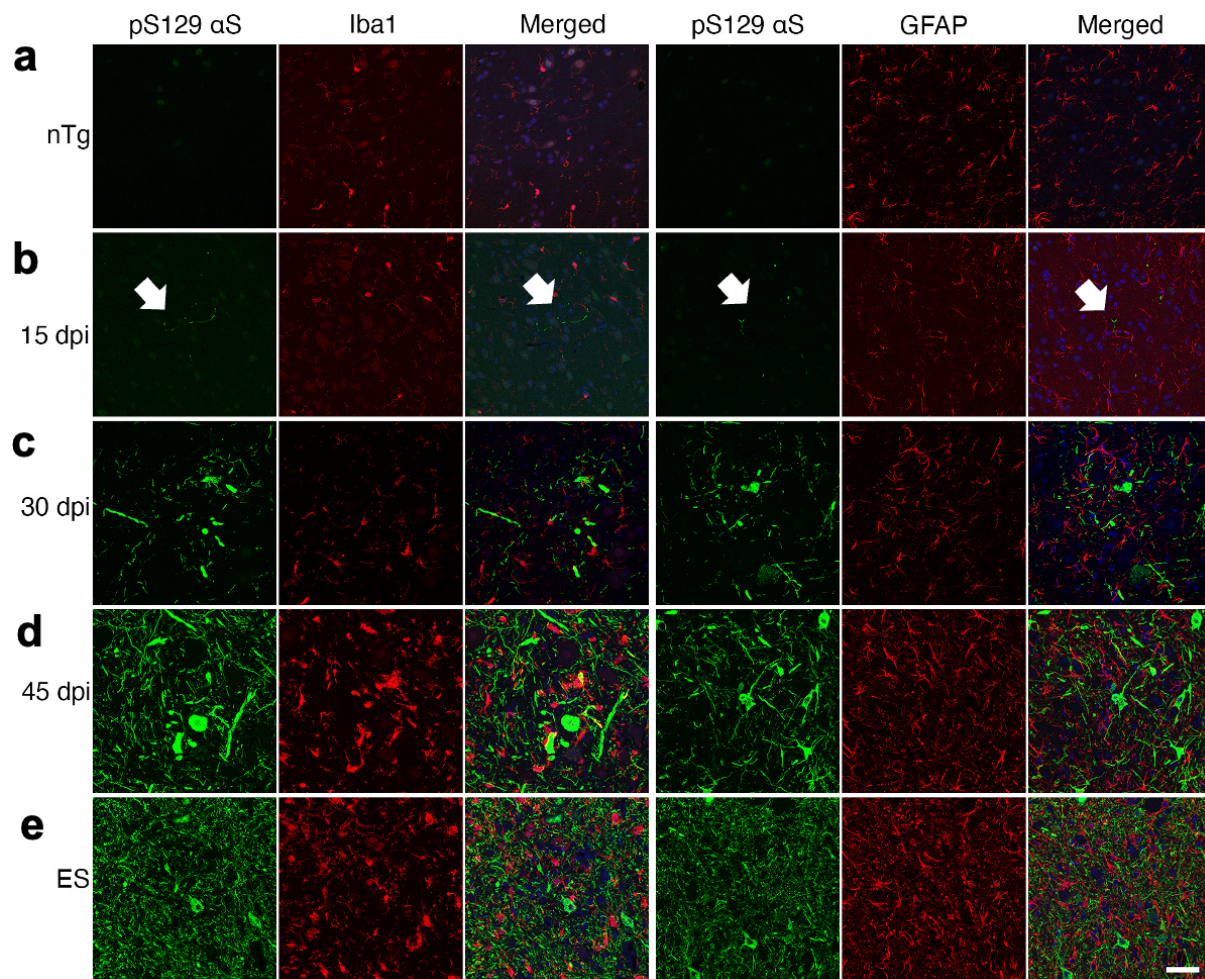


Figure S5. Neuroinflammation occurs after α S pathology in *TgA53T* mice IM injected with α S PFF. SpC sections from Control mice (**a**, **ntg**) and *TgA53T* mice injected with ESL into BrSt were analyzed at 30 dpi (**b**) 45 dpi (**c**), and ES (**d**, **injected**; **e**, **aged**). The sections were stained for pS129 α S and Iba1 or GFAP. Aggregated α S (pS129 α S) is seen as early as 15 dpi (**b**, **arrow**) but the overall morphology and density of Iba-1 and GFAP staining at 15- and 30-dpi (**b,c**) is comparable to nTg mice (**a**). There are clear increases in Iba1 and GFAP staining with more established pS129 α S pathology in 45-dpi and ES animals (**d**, **e**). Abbreviations: alpha synuclein, α S; intramuscular, IM; preformed fibril, PFF; spinal cord, SpC; end-stage lysate, ESL; brainstem, BrSt; days post inoculation, dpi; non transgenic, nTg; end-stage, ES. Scale Bar: 50 μ m.

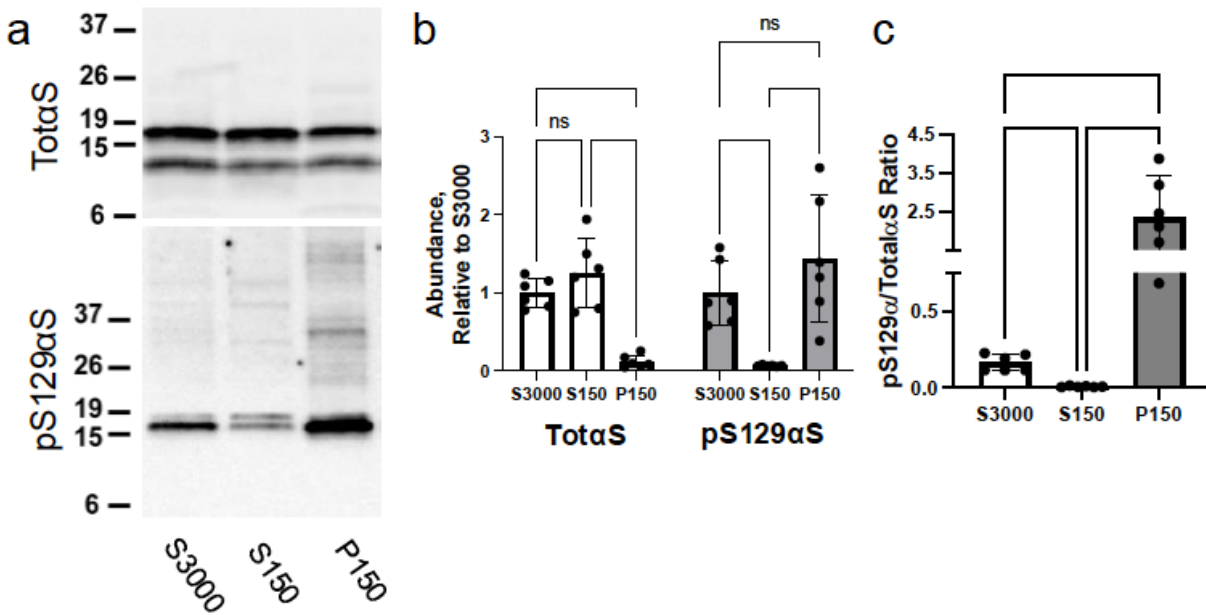


Figure S6. Immunoblot analysis of ESL, S150, and P150 injected fractions for α S. **a)** Immunoblot analysis of ESL (S3000), S150, and P150 fractions for total α S (Tot α S) and pS129 α S. 5 μ g of total protein from each fraction were analyzed. Tot α S enriched in S3000 and S150 while pS129 α S is most abundant in P150. **b)** Quantitative analysis of Tot α S and pS129 α S in S3000, S150, and P150. The abundance was normalized to the average levels in S3000. $n=6$ animals per group; $**p<0.01$, $***p<0.001$, $****p<0.0001$; Two-way ANOVA, Tukey's *post hoc* test for multiple comparisons. **c)** Relative abundance of pS129 α S normalized to Tot α S. $n=6$ animals per group; $*p<0.05$, $**p<0.01$, One-way ANOVA, Tukey's *post hoc* test for multiple comparisons.

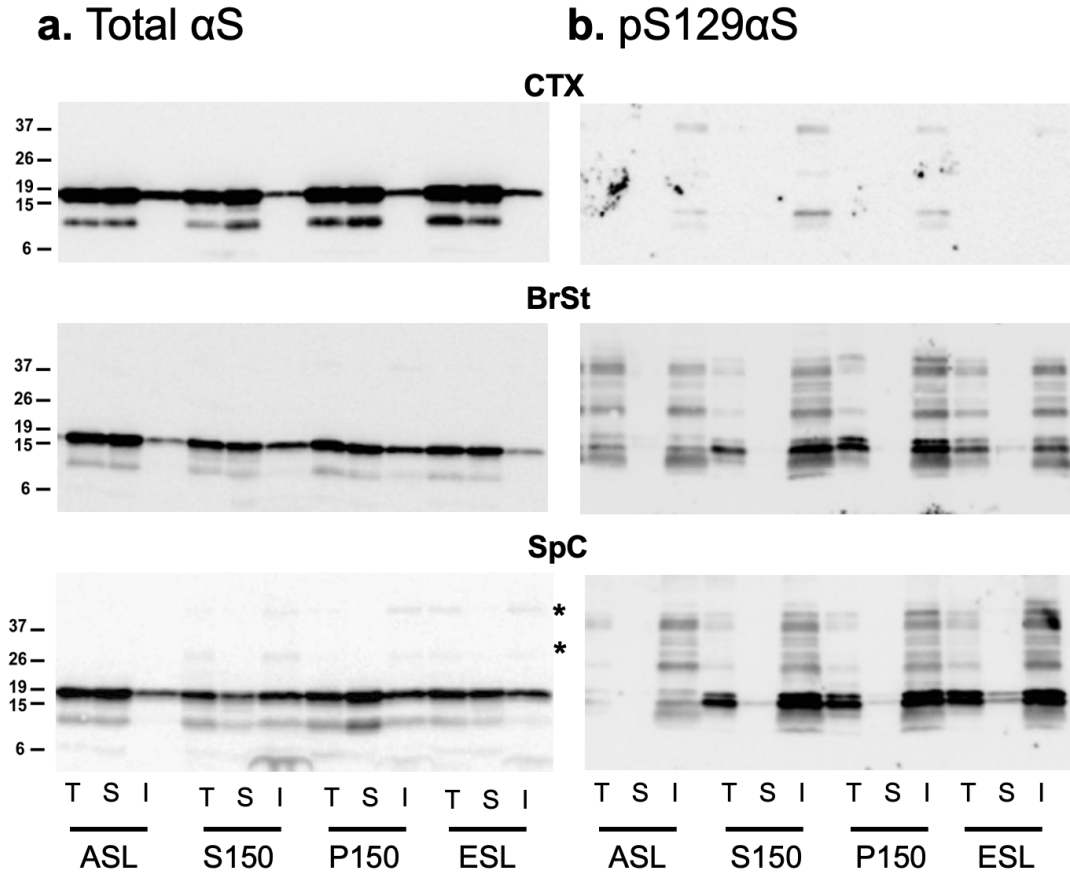


Figure S7. Immunoblot analysis of α S in ASL, ESL, S150, and P150 injected *TgA53T* mice. Whole tissue lysates (T) from CTX, BrSt, and SpC were further fractionated into 1% Tx-100 soluble (S) and insoluble (I) fractions. Equal amounts of proteins were analyzed for Total α S (**a**) and pS129 α S (**b**). Virtually all the pS129 α S accumulates in the insoluble fractions of BrSt and SpC, along with HMW α S (*). While the highest levels of total α S is expressed in the CTX, the level of pS129 α S is minimal and do not increase with injection of pathogenic lysates. No signs of α S aggregation is seen with the ASL. Abbreviations: α -synuclein, α S; asymptomatic lysate, ASL; end-stage lysate, ESL; cortex, CTX; brainstem, BrSt; spinal cord, SpC; high molecular weight, HMW.

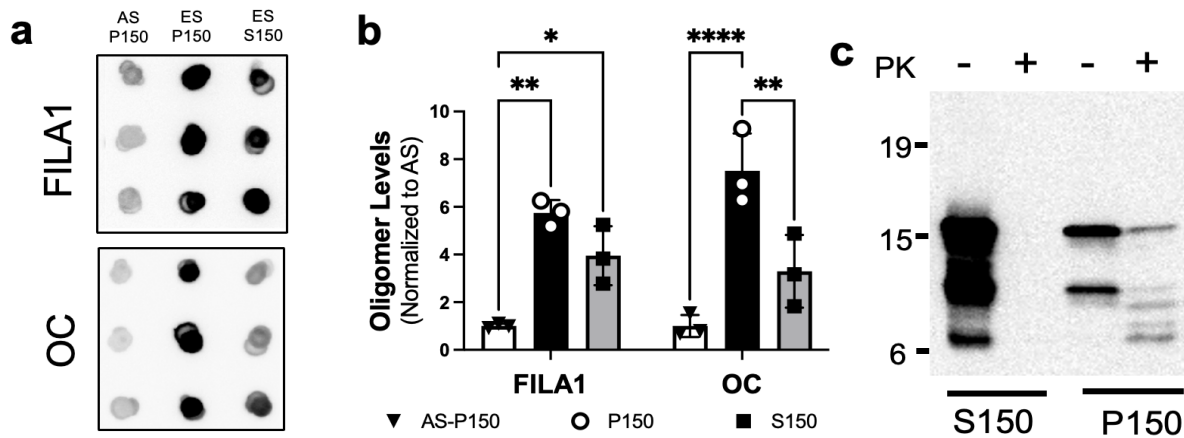


Figure S8. Analysis of α S oligomers in S150 and P150 fractions. **a)** Dot blot analysis of S150 and P150 for FILA1 and OC immunoreactivity. Equal amount of total protein (2 μ g) from each fraction were dotted on to nitrocellulose membrane and probed with FILA1 antibody and OC antibody. **b)** Quantitative analysis of dot blots shown in **a**. Levels of immunoreactivity was normalized to AS-P150. In P150, both FILA1 and OC immunoreactivity are higher than in AS-P150. In S150, FILA1 levels are higher but the OC levels are not different than in AS-150. $n=3$, $*p<0.05$, $**p<0.01$, $****p<0.0001$, One-way ANOVA, Tukey's *post hoc* test for multiple comparisons. **c)** S150 and P150 containing equal amount of protein (5 μ g) were incubated with 50 μ g/ml proteinase K (PK) and 1% Triton x-100 for 20 min on ice. The reaction was stopped by the addition of 2mM (final concentration) of phenylmethylsulfonyl fluoride. The fractions were immunoblotted for total α S. Virtually all the α S in S150 is degraded by PK while both full length and partially proteolyzed α S survive PK treatment in P150. Abbreviations: alpha-synuclein, α S; asymptomatic, AS; end-stage, ES.

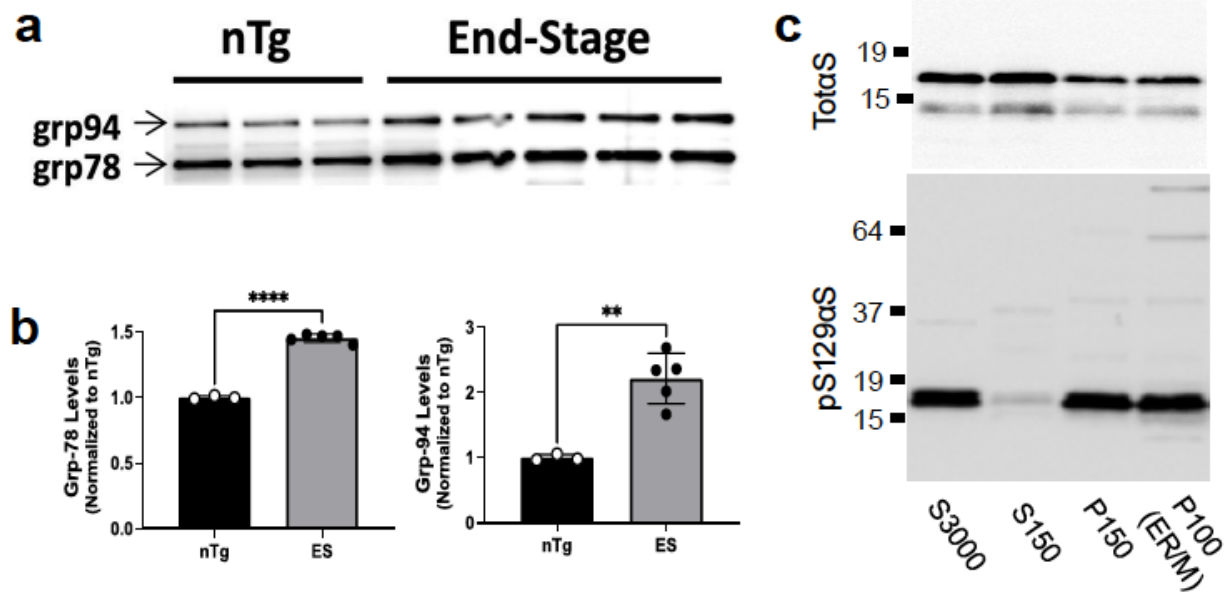


Figure S9. Increased ER chaperones in ES mice injected with ESL into BrSt. **a)** Whole SpC lysates from nTg and End-Stage *TgA53T* mice injected with ESL were immunoblotted using anti-KDEL antibody (2D6, Invitrogen) that recognizes both Grp78 and Grp94. **b)** Quantitative analysis of **a** shows that both ER chaperones are increased in *TgA53T* mice. $**p < 0.01$, $****p < 0.0001$, unpaired *t*-test, two-tailed. nTg, *n*=3; Tg-End Stage, *n*=5. **c)** Immunoblot comparison of total αS and pS129αS in end-stage lysates and fractions [S3000, S150, P150, and P100(ER/M)]. Equal amount of total protein (5 μg) from each fraction were analyzed. Despite the lower levels of total αS, pS129αS is enriched in P150 and P100. Abbreviations: endoplasmic reticulum, ER; end-stage, ES; end-stage lysate, ESL; nontransgenic, nTg; transgenic, Tg.