Supplementary Information

Uncovering the role of c-Fos in the bidirectional relationship between depression/anxiety behaviors and α-synuclein propagation in Parkinson's disease

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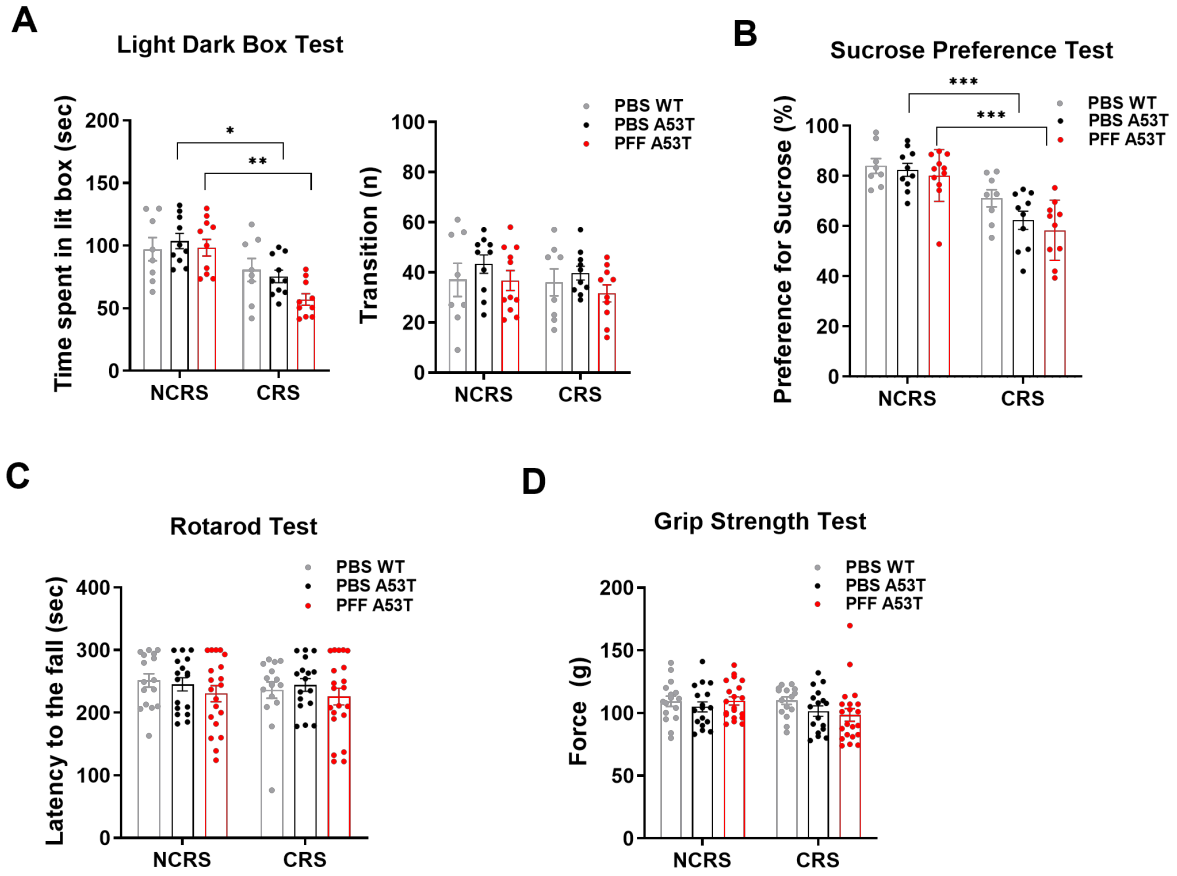
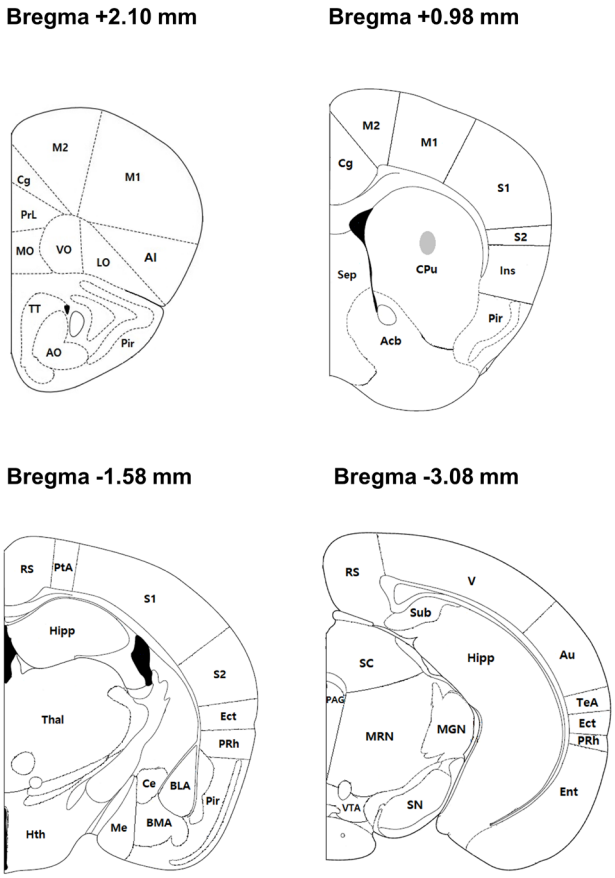


Additional file 1: Figure S1 Behavioral results of PFF-injected mice under CRS treatment in the light-dark box, sucrose preference, rotarod, and grip strength tests.

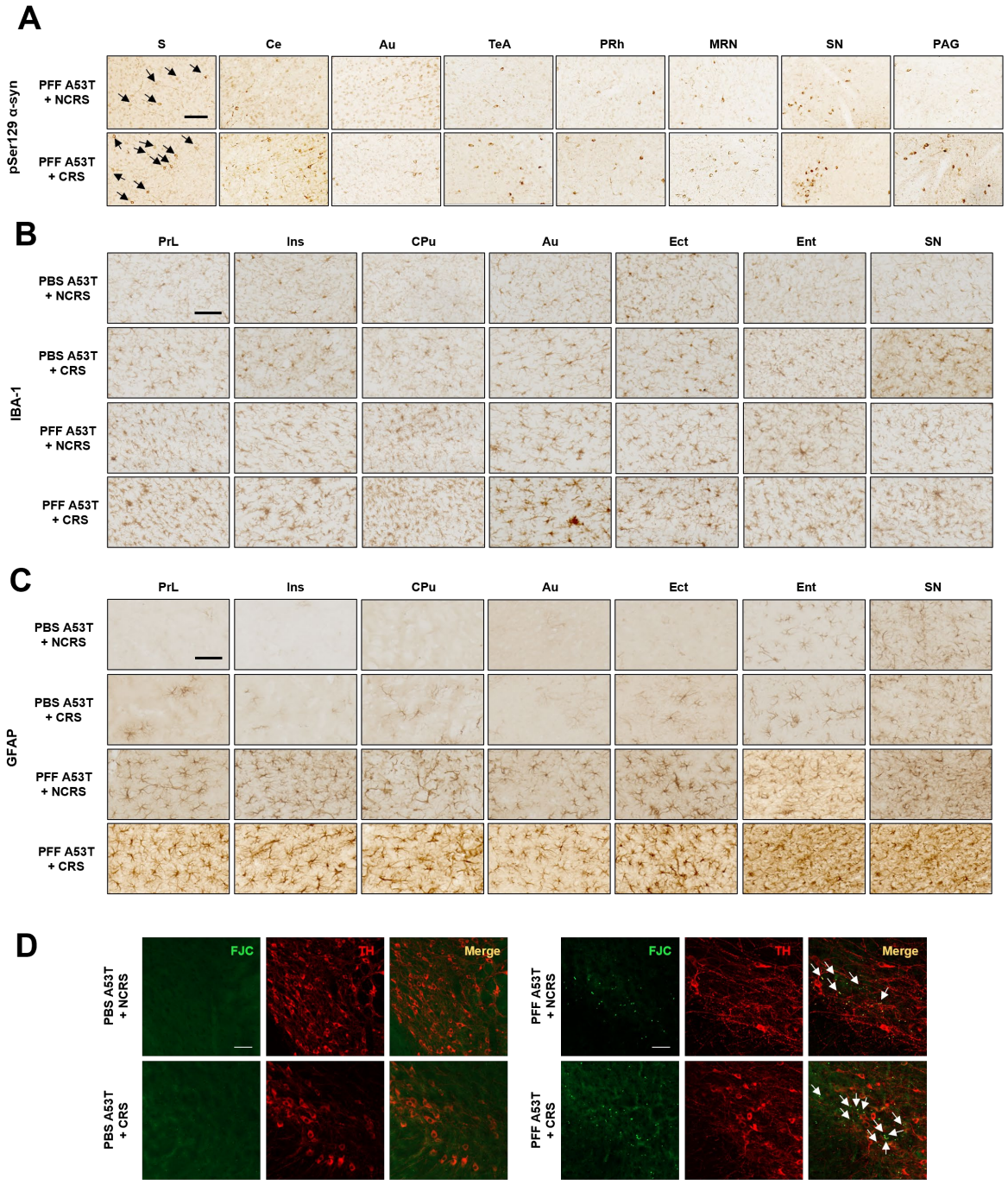
(A) Quantification of the total time spent in the light compartment and transition of the light-dark box. **(B)** Anhedonic behavior in the sucrose preference test is shown. **(C)** In the rotarod test, the latency to fall off the rod, which evaluates motor coordination and balance, is presented. **(D)** In the forelimb grip strength test, the force in grams used to measure neuromuscular strength is recorded. n=8-11 per group for A and B, n=16-21 per group for C and D. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, two-way ANOVA with, Tukey's multiple comparison tests.



Additional file 1: Figure S2 Schematic images of the regions annotated for pathology measures and used in subsequent analysis.

Bolded regions were analyzed. Abbreviations : Cg = cingulate cortex; PrL = prelimbic cortex; MO = medial orbital cortex; M1 = motor cortex 1; M2 = motor cortex 2; AI = agranular insular cortex; LO = lateral orbital cortex; VO = ventral orbital cortex; AO = anterior olfactory nucleus; TT = tenia tecta; S1 = somatosensory cortex 1; Pir = piriform cortex; S2 = somatosensory cortex 2; Ins = insular cortex; Cpu = caudate putamen (striatum); Acb = nucleus accumbens; Sep = septal; RS = retrosplenial cortex; PtA = parietal association cortex; Ect = ectorhinal cortex; PRh = perirhinal cortex; Ce = central amygdaloid nucleus; Me = medial amygdaloid nucelus; BLA = basolateral amygdaloid nucleus; BMA = basomedial amygdaloid nucelus; Thal = thalamic nuclei; Hth = hypothalamus; Hipp = hippocampus; V = visual corterx; Au = auditory cortrex; TeA = temporal association cortex; Ent = entorhinal cortex; Sub = subiculum; SC

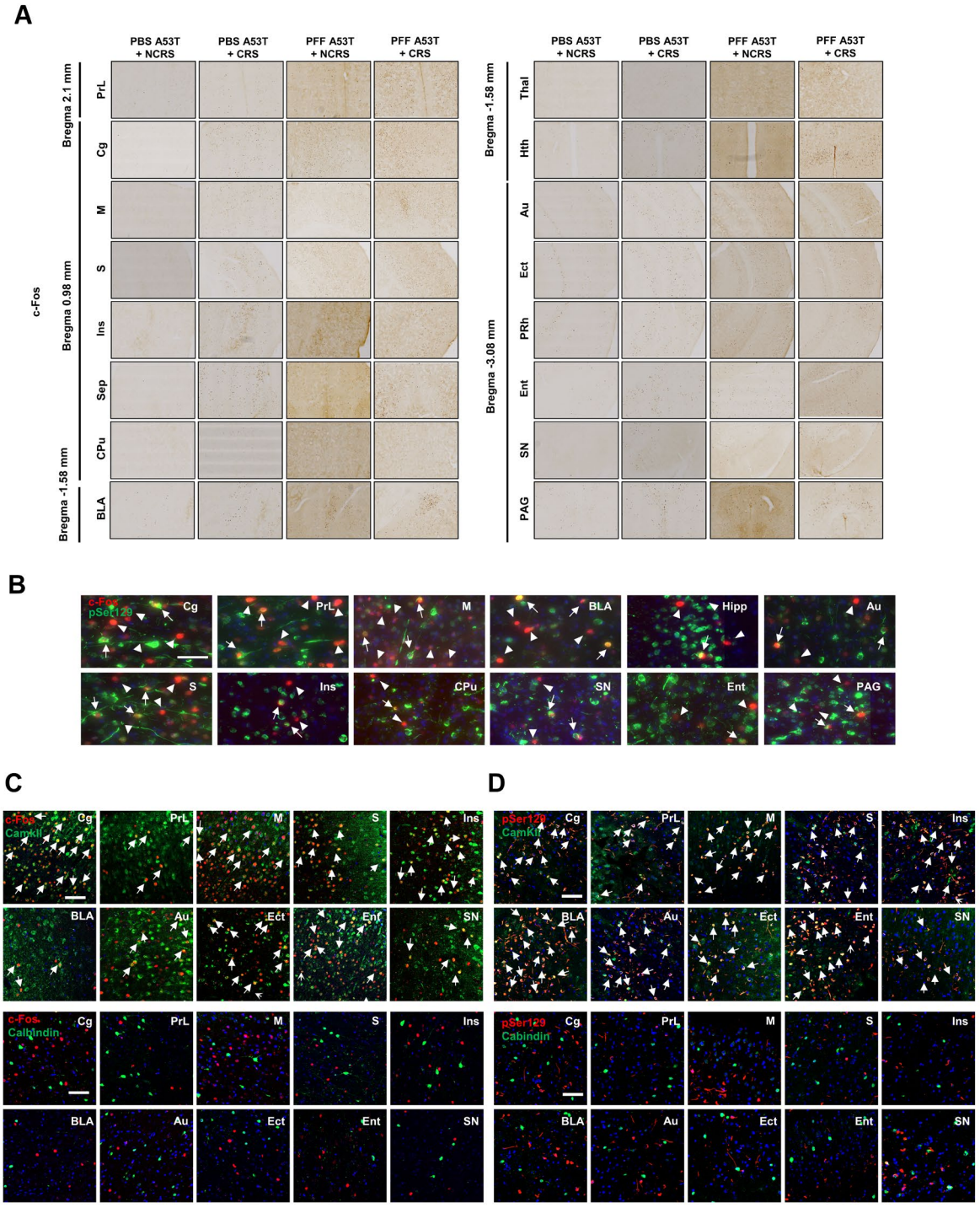
= superior colliculus; MRN = midbrain red nucleus; MGN = medial geniculate nucleus; PAG = periaqueductal gray; VTA = ventral tegment area; SN = substantia nigra



Additional file 1: Figure S3 CRS triggers α-syn propagation, microgliosis, and astrogliosis induced by PFF injection.

(A) Pathology (pSer129) detected in numerous brain regions (S, Ce, Au, TeA, PRh, MRN, SN, and PAG regions) of PFF + NCRS, and PFF + CRS groups in ipsilateral structures. Abbreviations are available in Figure S2. Scale bar, 50μm. **(B)** Representative immunostaining

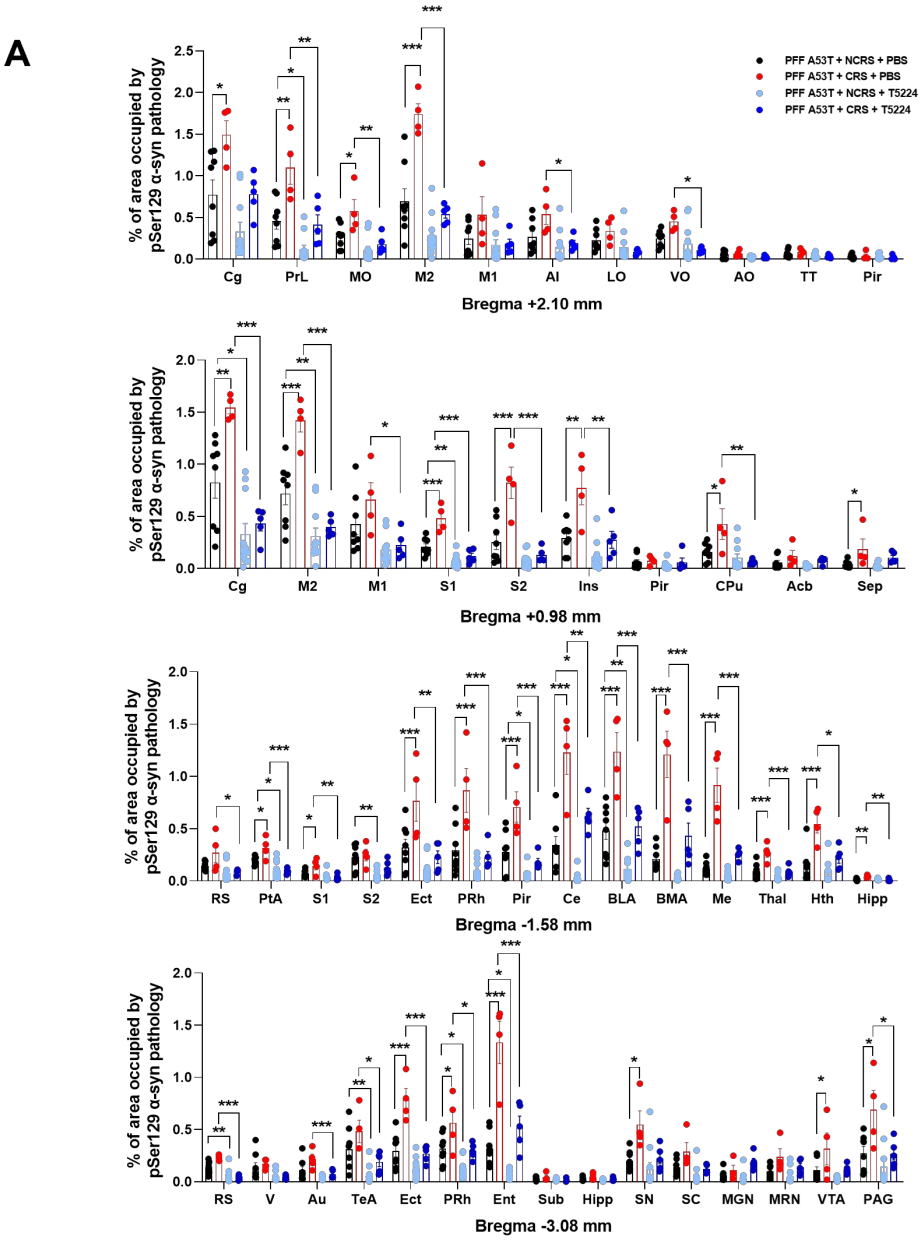
for Iba1 in the ipsilateral PrL, Ins, CPu, Au, Ect, Ent, and SN regions of PBS + NCRS, PBS + CRS, PFF + NCRS, and PFF + CRS groups. Scale bar, 50μm. **(C)** Representative immunostaining for GFAP in the ipsilateral PrL, Ins, CPu, Au, Ect, Ent, and SN regions of PBS + NCRS, PBS + CRS, PFF + NCRS, and PFF + CRS groups. Scale bar, 50μm. **(D)** Double immunofluorescence staining of TH (red) and FJC (green) in the SN of PBS + NCRS, PBS + CRS, PFF + NCRS, and PFF + CRS groups; merged images at 4Ws of CRS slightly induced neurodegeneration in the SN of A53Tg mice injected with PFF.

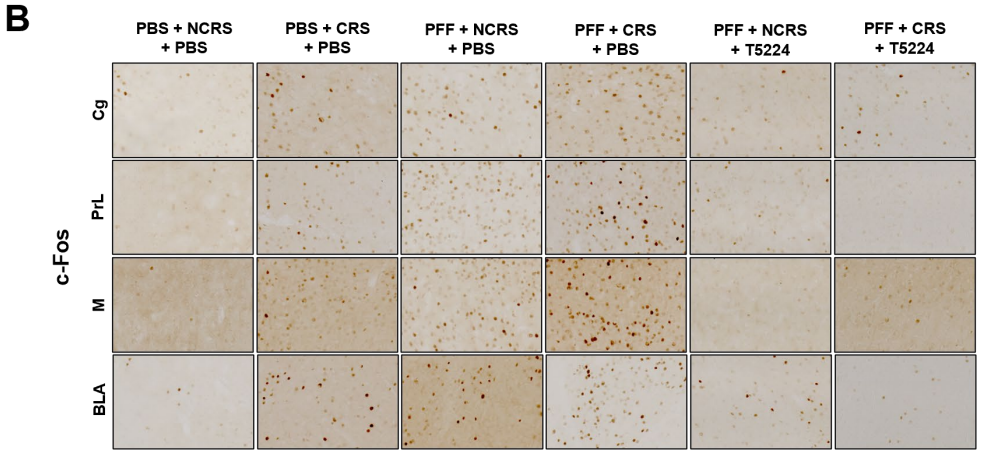


Additional file 1: Figure S4 The hyperactive neurons are likely candidates to trigger α-syn pathology.

(A) Immunohistochemistry of c-Fos in the ipsilateral PrL, Cg, M, S, Ins, Sep, CPu, BLA, Thal, Hth, Au, Ect, PRh, Ent, SN, and PAG regions of PBS + NCRS, PBS + CRS, PFF + NCRS, and PFF + CRS groups. **(B)** Double-labeling immunofluorescence disclosed co-localization of c-

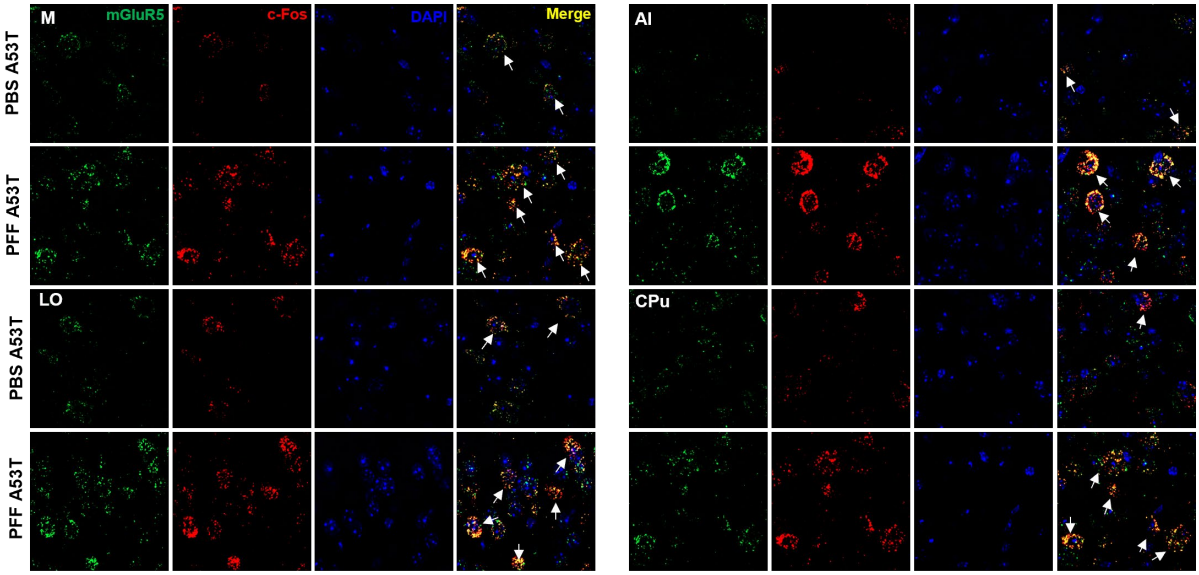
Fos and pser129-positive inclusions in the majority but not all neurons containing Lowy bodies in the brains of PFF-injected A53T mice. **(C)** Representative confocal images showing c-Fos (red), Ca2+/calmodulin-dependent protein kinase II excitatory neurons (CaMKII) (green), and calbindin (CB) inhibitory neurons (green) in the M. Scale bars, 50 μm. c-Fos primarily localized CaMKII and appeared to be excluded from CB neurons. **(D)** pSer129-α-syn positive inclusions were localized almost exclusively to excitatory CaMKII neurons, and the Inhibitory neurons expressing, CB showed minimal to no pSer129-α-syn positive inclusions.


A
PFF A53T + NCRS + PBS
PFF A53T + CRS + PBS
PFF A53T + NCRS + T5224
PFF A53T + CRS + T5224
% of area occupied by pSer129 α-syn pathology
Cg PrL MO M2 M1 AI LO VO AO TT Pir
Bregma +2.10 mm
Cg M2 M1 S1 S2 Ins Pir CPu Acb Sep
Bregma +0.98 mm
RS PtA S1 S2 Ect PRh Pir Ce BLA BMA Me Thal Hth Hipp
Bregma -1.58 mm
RS V Au TeA Ect PRh Ent Sub Hipp SN SC MGN MRN VTA PAG
Bregma -3.08 mm


B
PBS + NCRS + PBS
PBS + CRS + PBS
PFF + NCRS + PBS
PFF + CRS + PBS
PFF + NCRS + T5224
PFF + CRS + T5224
c-Fos
Cg
PrL
M
BLA

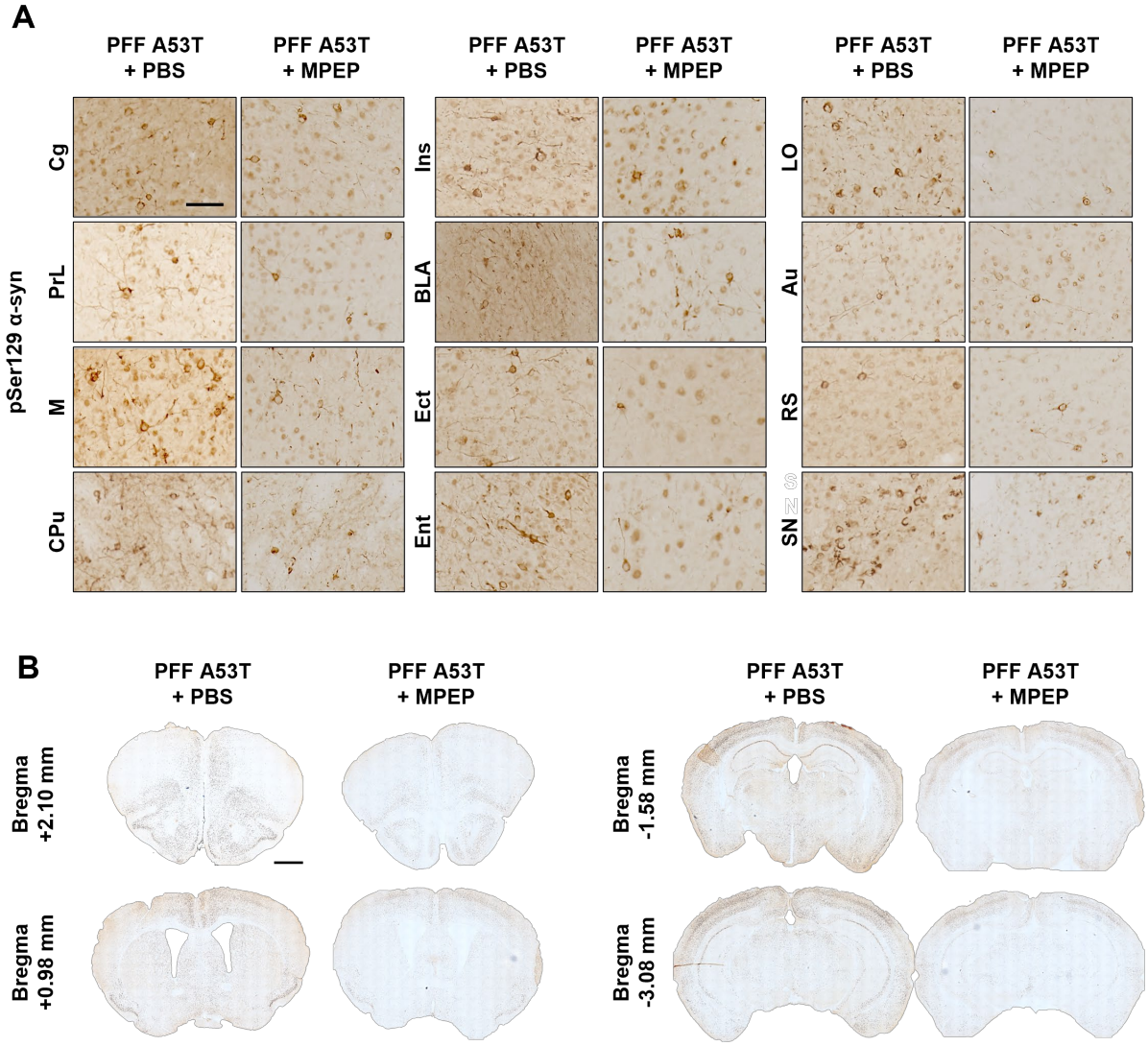
Additional file 5: Figure S5 T5224 alleviates α-syn propagation associated with c-Fos induction in PFF-injected A53 Tg mice.

(A) Quantitative analysis of pSer129 α-syn -immunoreactive inclusions in numerous brain regions of PFF + NCRS + PBS (n=8), PFF + CRS + PBS (n=4), PFF + NCRS +T5224 (n=10) and PFF + CRS + T5224 (n=5) A53Tg mice in ipsilateral structures. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, two-way ANOVA with, Tukey's multiple comparison test**. (B)** Representative c-Fos images are shown in Cg, PrL, M, and BLA of PBS + NCRS + PBS, PBS + CRS + PBS, PFF + NCRS + PBS, PFF + CRS + PBS, PFF + NCRS +T5224 and PFF + CRS + T5224 A53Tg mice.



Additional file 1: Figure S6 mGluR5 mRNA is predominantly expressed in c-Fos-positive cells.

Expression and colocalization of mGluR5 mRNA in M, AI, LO, and CPu. Scale bar, 20 μm.



Additional file 1: Figure S7 MPEP alleviates α-syn propagation associated with c-Fos induction in PFF-injected A53 Tg mice.

(A) Representative pSer129 α-syn images are shown in Cg, PrL, M, CPu, Ins, BLA, Ect, Ent, LO, Au, RS, and SN of PFF + PBS and PFF + MPEP A53Tg mice. **(B)** Representative coronal brain sections from PFF + PBS and PFF + MPEP A53Tg mice at four positions of the brain stained for c-Fos under PBS (left) and MPEP (right) treatment. Scale bars represent 1 mm.