

Supplementary Material

T Cells Limit Accumulation of Aggregate Pathology Following Intrastratal Injection of α -Synuclein Fibrils

Supplementary Table 1. Antibodies for immunohistochemistry and immunofluorescent staining

Target	Host	Dilution	Company/ Catalogue #
Phosphorylated PS129 α -syn	Rabbit	1:10000	Abcam/ ab51253
Iba-1	Rabbit	1:500	WAKO/ 019-19741
Tyrosine hydroxylase	Rabbit	1:1600	Millipore/ 657012
MHC II	Rat	1:500	Thermo Fisher/ 14-5321-85
CD3	Rabbit	1:100	Abcam/ 5690
CD4	Rabbit	1:100	Abcam/ 183685

Supplementary Table 2. Blood 45.2+ T cells/ μ L.

Wt Saline	NSG Saline	Wt PFF	NSG PFF	NSG PFF T	NSG PFF B
6.98807041	0	0.77498813	0	81.429836	0.01666669
53.3447376	0	0.0068192	0	58.4725604	0
79.2676231	0	45.2502458	0	118.065161	0
16.3735777	0	0	0	111.806386	0.03000007
23.3698191	0.00978453	30.6157294	0	129.075381	0
3.90130697	0	51.0069921	0	264.912428	0
1.8466642	0	0	0	391.735386	0.08000178
7.54420409	0	62.797442	0		0.01666669
0.63373679		22.0500617	0		2.03889002
		7.39002956			
		16.12			

Supplementary Table 3. Spleen 45.2+ T cells/ μ L.

Wt Saline	NSG Saline	Wt PFF	NSG PFF	NSG PFF T	NSG PFF B
1174.79603	0	17.6009681	0	432.646304	0.39444423
1103.75477	0	0	0	398.799287	15.7752153
1843.37388	0	479.408967	0	79.1175488	78.5223669
2508.49033	0	0.03987263	0	100.603658	0.10000039
57.5440764	0.2566655	2194.87586	0.28604174	101.766581	0.19000684
3631.56889	0	1073.17289	0.45556574	1.83335044	2.09002153
3151.63434	0	1157.26475	0.03114698	7.2000096	0.3200001
2103.87271	0	2244.67741	0.20181989		0
1813.58301		2409.89932	0		5.96849999
1705.5594		3235.13059			
		3315.29106			

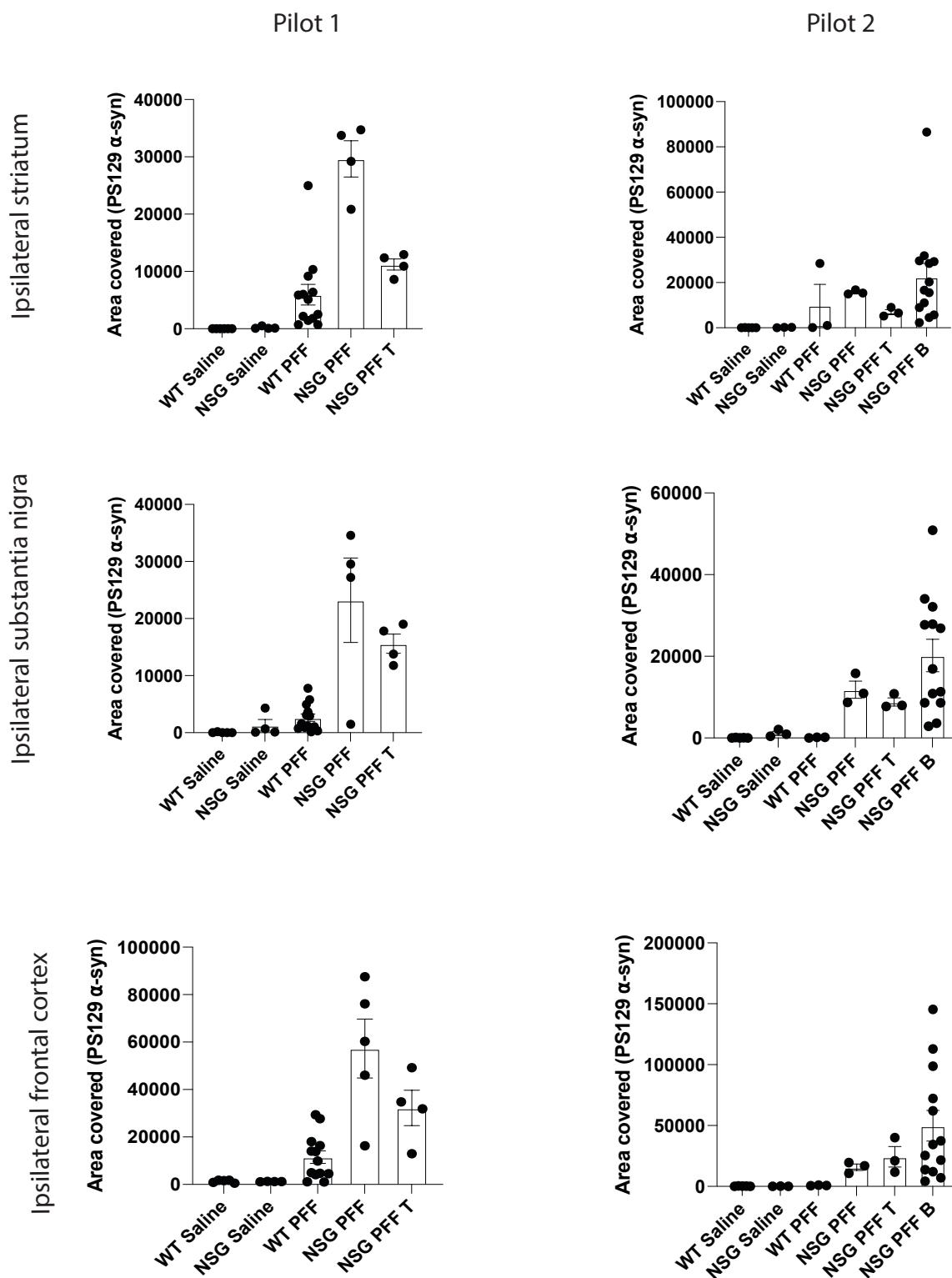
Supplementary Table 4. Blood 45.2+ B cells/ μ L.

Wt Saline	NSG Saline	Wt PFF	NSG PFF	NSG PFF T	NSG PFF B
11.4114736	0	0	0.00977272	0.00378624	0.00555556
138.560982	0	0.020457595	0	0.02060124	0.02222221
191.679895	0	0	0	0.14499326	0.31001066
32.4318999	0	0	0	0.43173392	0.16999966
48.3228804	0.01956907	87.40201985	0	3.14818002	0.86000189
45.4832793	0	116.9003043	0	0.84667513	0.54000335
14.604807	0	0.18780583	0	0.38556632	0.0065802
5.86999217	0	83.49281814	0		0.55001227
11.0672742		44.15012362	0		0.02222221
3.57975646		10.66004264			
		25.34			

Supplementary Table 5. Spleen 45.2+ B cells/ μ L.

Wt Saline	NSG Saline	Wt PFF	NSG PFF	NSG PFF T	NSG PFF B
2855.21995	0.0111111	0	0	0.2352333	3.08888717
2061.65407	0.00555559	3.599575329	0	2.00118821	20.9411589
4495.30058	0	0.232421929	0	4.42521883	0.97309349
5662.86905	0	0	0	0	20.9411589
383.771036	3.29387387	4571.753346	0.47673623	0	39.3301534
6711.56972	0	2195.239609	0.65080821	0	86.7531231
5192.73954	0	2134.984594	0.09344094	0.00666668	78.7208108
2756.16932	0	5168.746601	0.40363978		36.2900109
3158.05255		5605.790099	0		102.496437
2116.05835		6673.148054			
		5458.283659			

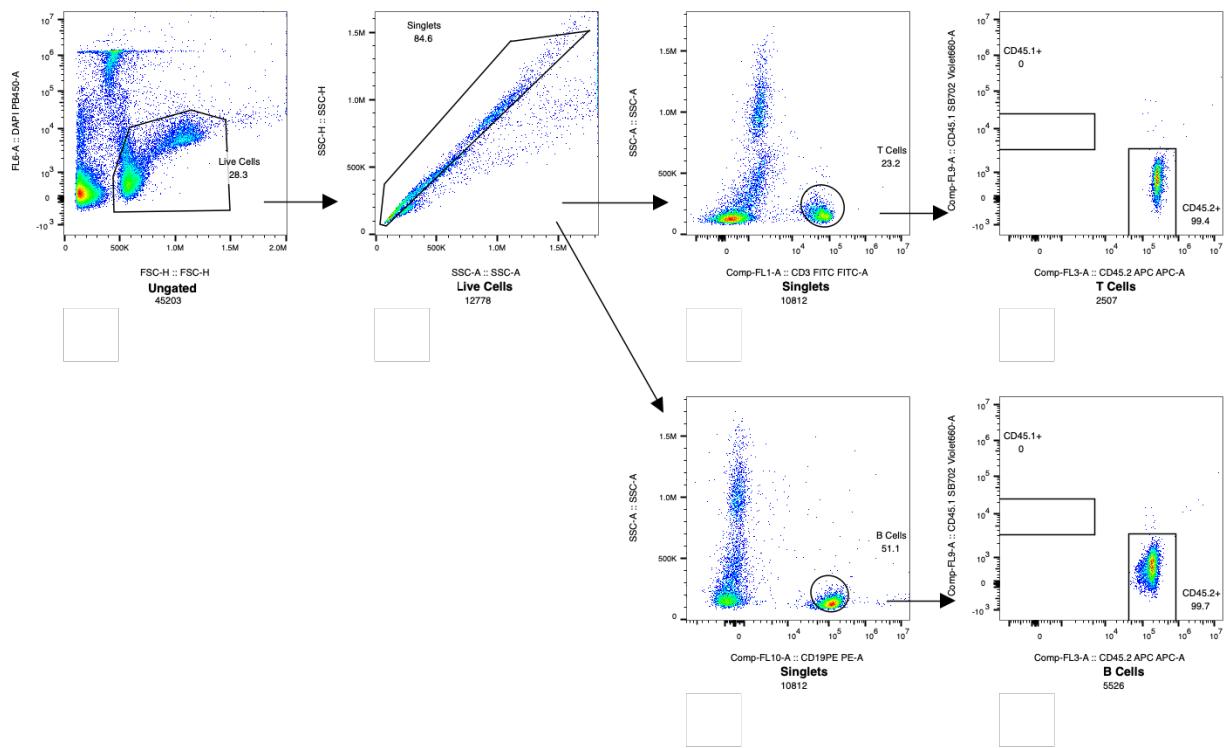
Supplementary Figure 1. Phosphorylated α -syn in separate pilot studies. Pilot 1 and Pilot 2 independent studies measuring the area covered of phosphorylated α -syn stain in the striatum, substantia nigra and frontal cortex.



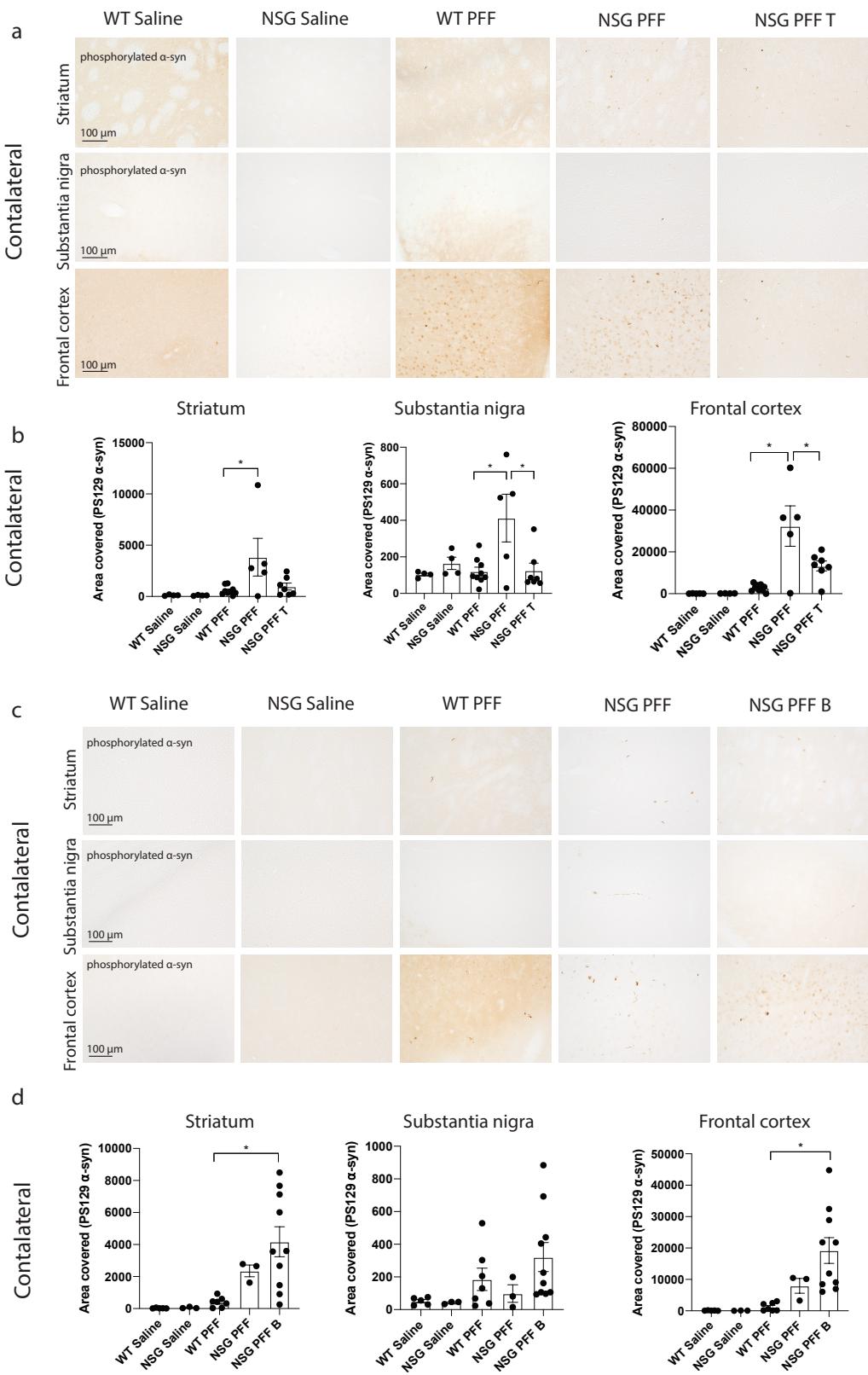
Supplementary Figure 2. Flow cytometry gating strategy. Live single cells were gated for T cells based on CD3 or B cells using CD19. The WT-mouse origin of cells was confirmed by the presence of CD45.2. NSG-derived cells would express CD45.1.

Supplementary Figure 2

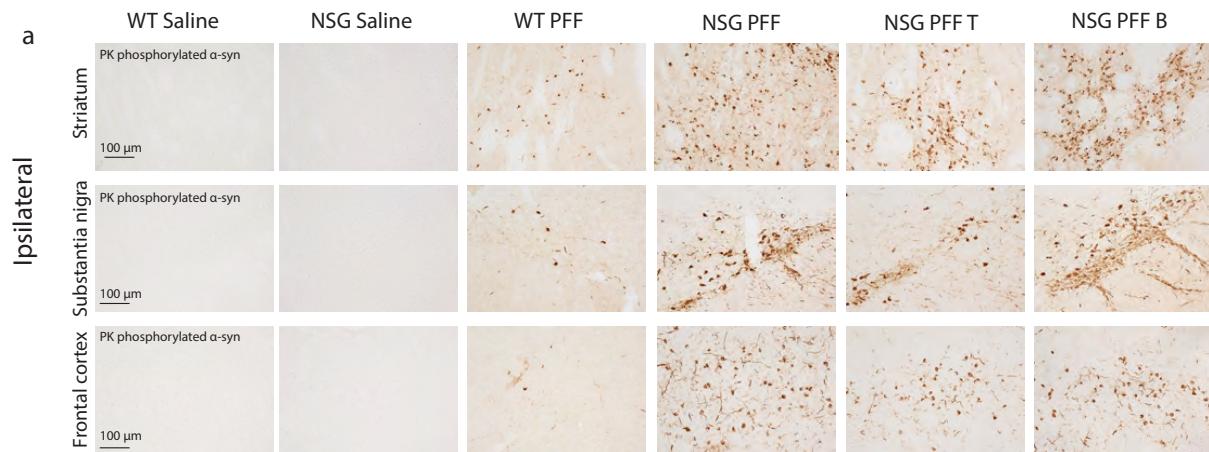
Example Gating Strategy using WT PBS-Treated Mouse



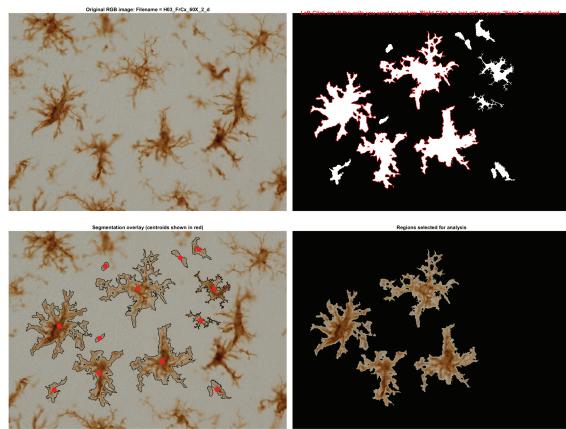
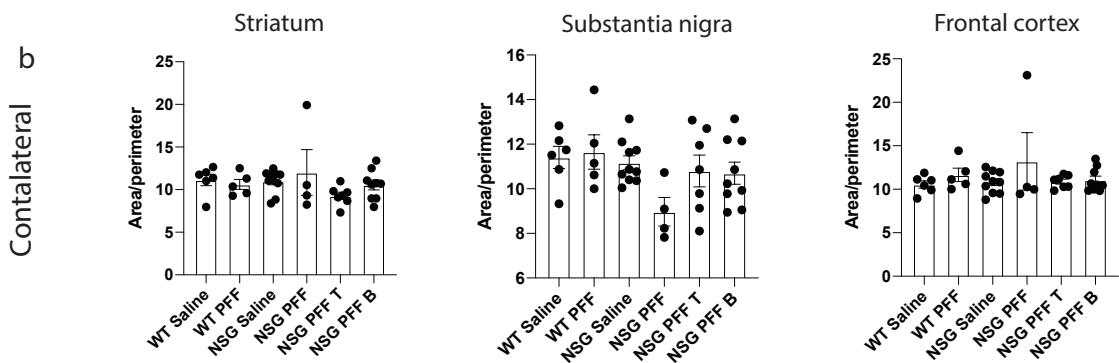
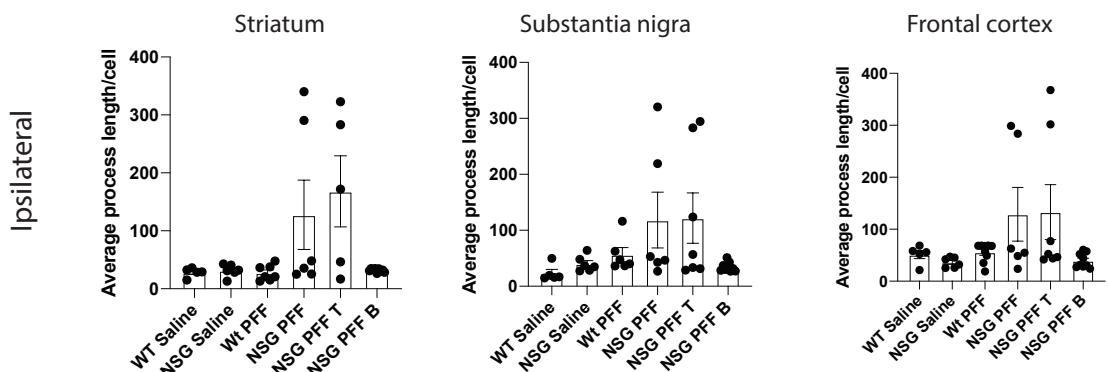
Supplementary Figure 3. Phosphorylated α -syn is found in the contralateral hemisphere in PFFs-injected mice. a) Tissue from the contralateral hemisphere of the striatum, substantia nigra and frontal cortex from wildtype saline, NSG saline, wildtype PFF, NSG PFF, and NSG PFF T mice were all positive for phosphorylated α -syn. b) Densitometry of 5-7 mice per group to determine the fold change in phosphorylated α -syn levels in the contralateral striatum, substantia nigra and frontal cortex. Wildtype Saline, n = 5; NSG Saline n = 5, wildtype PFFs, n = 9; NSG PFFs, n = 4; NSG PFF T n = 7). c) Tissue from the contralateral hemisphere of the striatum, substantia nigra and frontal cortex from wildtype saline, NSG saline, wildtype PFF, NSG PFF, and NSG PFF B mice were all positive for phosphorylated α -syn. d) Densitometry of 5-7 mice per group to determine the fold change in phosphorylated α -syn levels in the contralateral striatum, substantia nigra and frontal cortex. Wildtype Saline, n = 5; NSG Saline n = 5, wildtype PFFs, n = 9; NSG PFFs, n = 4; NSG PFF B n = 7). The error bars represent S.E.M. Statistical analyses were performed by Kruskal-Wallis test. ** p < 0.01. Scale bar: 100 μ m.



Supplementary Figure 4. Proteinase K resistant phosphorylated α -syn is found in PFFs-injected mice. Tissue from the striatum, substantia nigra and frontal cortex from wildtype saline, NSG saline, wildtype PFF, NSG PFF, NSG PFF T, and NSG PFF B mice were all positive for phosphorylated α -syn following proteinase K treatment. Scale bar: 100 μ m.



Supplementary Figure 5. Microglia analysis in immunocompromised mice following adoptive transfer of T and B cells. a) Example of Iba-1 immunoreactive microglia analyzed by Matlab algorithm. Individual microglia are identified and selected by blind investigator and the area/perimeter measurement generated. b) Area/perimeter analysis of microglia in the contralateral hemisphere in striatum, substantia nigra and frontal cortex. c) Analysis of microglia average process length per microglial cell in the ipsilateral hemisphere from the striatum, substantia nigra and frontal cortex. d) Analysis of microglia average process length per microglial cell in the contralateral hemisphere from the striatum, substantia nigra and frontal cortex. Wildtype Saline, n = 5; NSG Saline n = 5, wildtype PFFs, n = 9; NSG PFFs, n = 6; NSG PFF T n = 7; NSG PFF B n = 9). The error bars represent S.E.M. Statistical analyses were performed by Kruskal-Wallis test.

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