Supplementary Material

T Cells Limit Accumulation of Aggregate Pathology Following Intrastriatal 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/μL.

|                | 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| 0.0300007 |           |
| 23.3698191     | 0.00978453| 30.6157294 | 0      | 129.075381| 0         |           |
| 3.90130697     | 51.0069921| 0          | 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          | 0      | 2.03889002|           |           |
|                |           |            |        | 7.39002956|           | 16.12     |

**Supplementary Table 3.** Spleen 45.2+ T cells/μL.

|                | 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          | 0      | 5.96849999|           |           |
| 1705.5594      | 3235.13059|            |       |           |           |           |
|                |           |            |        | 3315.29106|           |           |
**Supplementary Table 4.** Blood 45.2+ B cells/μL.

| 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          | 0.02045795 | 0       | 0.02060124 | 0.02222221 |
| 191.679895 | 0          | 0      | 0.14499326 | 0.31001066 | 0.16999966 |
| 32.4318999 | 0          | 0      | 0.43173392 | 0.00555556 | 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 | 0.0065802 |
| 11.0672742 | 44.15012362 | 0    | 0.30888717 | 0.00555559 | 0.02222221 |
| 3.57975646 | 10.6604264 | 0    | 25.34    | 0.00555559 | 0.02222221 |

**Supplementary Table 5.** Spleen 45.2+ B cells/μL.

| 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 | 0      | 0       | 2.00118821 | 20.9411589 |
| 4495.30058 | 0         | 0.23242192 | 0     | 4.42521883 | 0.97309349 |
| 5662.86905 | 0         | 0      | 0       | 0         | 20.9411589 |
| 383.771036 | 3.29387387 | 0      | 0       | 0         | 39.3301534 |
| 6711.56972 | 0         | 2195.239609 | 0     | 0.65080821 | 86.7531231 |
| 5192.73954 | 0         | 2134.984594 | 0     | 0.09344094 | 78.7208108 |
| 2756.16932 | 0         | 5168.746601 | 0.40363978 | 0.00666668 | 36.2900109 |
| 3158.05255 | 5605.790099 | 0    | 102.496537 | 0.00666668 | 36.2900109 |
| 2116.05835 | 6673.148054 | 0    | 5458.283659 | 0.00666668 | 36.2900109 |
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.

Ipsilateral substantia nigra

Ipsilateral striatum

Ipsilateral frontal cortex

Pilot 1

Pilot 2

Area covered (PS129-α-syn)
**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 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 µm.
**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 µm.
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.
Supplementary Figure 5

Striatum
Substantia nigra
Frontal cortex

Contalateral

Ipsilateral

Average process length/cell

Contalateral