Gut-seeded α-synuclein fibrils promote gut dysfunction and brain pathology specifically in aged mice

Collin Challis¹, Acacia Hori¹⁴, Timothy R. Sampson¹²⁴, Bryan B. Yoo¹, Rosemary C. Challis¹, Adam M. Hamilton⁰², Sarkis K. Mazmanian⁰¹, Laura A. Volpicelli-Daley³ and Viviana Gradinaru⁰¹*

¹Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. ²Department of Physiology, Emory University School of Medicine, Atlanta, GA, USA. ³Center for Neurodegeneration and Experimental Therapeutics, University of Alabama at Birmingham, Birmingham, AL, USA. *These authors contributed equally: Acacia Hori, Timothy R. Sampson. *e-mail: viviana@caltech.edu
| Primary Antibodies                                                                 | Manufacturer          | Cat. #   | Usage notes          |
|-----------------------------------------------------------------------------------|-----------------------|----------|----------------------|
| Mouse IgG2a monoclonal anti-α-synuclein phospho (Ser129) (Clone81A)             | BioLegend             | MMS-5091 | Duodenum IHC 1:300   |
|                                                                                  |                       |          | Brain IHC 1:500      |
| Rabbit polyclonal anti-α-synuclein phosphor (Ser129)                             | Abcam                 | ab59264  | Western 1:500        |
| Rabbit monoclonal anti-Alpha-synuclein filament antibody [MJFR-14-6-4-2] - Conformation-Specific | Abcam                 | ab209538 | Dot blot 2ng/mL      |
| Rabbit polyclonal anti-Protein gene product 9.5 (PGP9.5)                         | Millipore             | AB1761-I | Duodenum IHC 1:300   |
|                                                                                  |                       |          | Nodose IHC 1:100     |
| Chicken polyclonal anti-Protein gene product 9.5 (PGP9.5)                       | ThermoFisher Scientific | PA1-10011 | Duodenum IHC 1:300   |
| Chicken polyclonal anti-Glial fibrillary acidic protein (GFAP)                   | Millipore             | AB5541   | Duodenum IHC 1:300   |
| Goat polyclonal anti-Choline acetyltransferase (ChAT)                            | Millipore             | AB144P   | Brain IHC 1:500      |
| Rabbit polyclonal anti-Tyrosine hydroxylase (TH)                                 | Millipore             | AB152    | Brain IHC 1:500      |
| Chicken polyclonal anti-Green fluorescent protein (GFP)                          | Aves Labs             | GFP-1010 | Brain IHC 1:1000     |
| Rabbit polyclonal anti-Red fluorescent protein (RFP)                             | Rockland              | 600-401-379 | Brain IHC 1:1000     |
| Rabbit polyclonal anti-GBA1                                                      | Abcam                 | ab175869 | Western 1:1000       |
| Rabbit polyclonal anti-Interleukin 6 (IL6)                                       | Abcam                 | ab7737   | Western 1:500        |
| Rabbit polyclonal anti-Iba1                                                      | Wako                  | 016-20001 | Western 1:1000       |
| Rabbit polyclonal anti-β-Tubulin                                                 | Abcam                 | ab6046   | Western 1:1000       |
| Mouse IgG2b anti-β-actin                                                         | Cell Signaling        | 3700     | Western 1:1000       |

| Secondary Antibodies                                                              | Manufacturer          | Cat. #   | Usage notes          |
|-----------------------------------------------------------------------------------|-----------------------|----------|----------------------|
| AlexaFluor 488 Goat anti-Mouse IgG2a                                               | ThermoFisher Scientific | A-21131 | IHC 1:300-1000       |
| AlexaFluor 488 Donkey anti-Mouse IgG                                               | ThermoFisher Scientific | A-21202 | IHC 1:300-1000       |
| AlexaFluor 488 Donkey anti-Chicken IgY                                              | Jackson ImmunoResearch | 703-545-155 | IHC 1:300-1000       |
| AlexaFluor 555 Donkey anti-Rabbit IgG                                              | ThermoFisher Scientific | A-31572 | IHC 1:300-1000       |
| AlexaFluor 555 Donkey anti-Goat IgG                                                | ThermoFisher Scientific | A-21432 | IHC 1:300-1000       |
| AlexaFluor 633 Goat anti-Rabbit IgG                                                | ThermoFisher Scientific | A-21071 | IHC 1:300-1000       |
| AlexaFluor 633 Goat anti-Chicken IgY                                                | ThermoFisher Scientific | A-21103 | IHC 1:300-1000       |
| Horseradish peroxidase (HRP)-linked Goat anti-Rabbit IgG                           | Cell Signaling        | 7074     | Western, dot blot 1:2000 |
| Horseradish peroxidase (HRP)-linked Goat anti-Mouse IgG                            | Cell Signaling        | 7076     | Western 1:2000       |
| Horseradish peroxidase (HRP)-linked Goat anti-Mouse IgG2a                         | Abcam                 | ab97245  | Western 1:2000       |
## Supplementary Table 2. Statistics and quantification

### Main figures

| Figure | Number of subjects | Test | Summary | Key comparisons |
|--------|--------------------|------|---------|-----------------|
| 1b     | For 0, 7, 21, 60, 120 dpi: α-Syn mon = 9, 9, 9, 8, 8 α-Syn PFF = 16, 14, 14, 11, 8 | Two-way ANOVA | Condition x Time: F(4,96) = 1.857; P = 0.1243 Time factor: F(4,96) = 4.490; P = 0.0023 Condition factor: F(1,96) = 22.72; P < 0.0001 | PFF: 0 dpi vs. 21 dpi, ** p = 0.00 PFF: 0 dpi vs. 60 dpi, ** p = 0.0027 60 dpi: α-Syn PFF vs. α-Syn mon, * p = 0.0187 |
| 1c     | For 0, 7, 21, 60, 120 dpi: α-Syn mon = 6, 7, 6, 6, 6 α-Syn PFF = 7, 8, 9, 9, 8 | Two-way ANOVA | Condition x Time: F(4,96) = 3.980; P = 0.0050 Time factor: F(4,96) = 1.808; P = 0.1136 Condition factor: F(1,96) = 6.438; P = 0.0128 | PFF: 0 dpi vs. 120 dpi, p = 0.0745 60 dpi: α-Syn PFF vs. α-Syn mon, * p = 0.0259 |
| 1d     | For 0, 7, 21, 60, 120 dpi: α-Syn mon = 4 α-Syn PFF = 4 | Two-way ANOVA | Condition x Time: F(4,62) = 5.264; P = 0.0010 Time factor: F(4,62) = 3.042; P = 0.0235 Condition factor: F(1,62) = 14.47; P = 0.0003 | PFF: 0 dpi vs. 120 dpi, ** p = 0.0042 60 dpi: α-Syn PFF vs. α-Syn mon, ** p = 0.0012 120 dpi: α-Syn PFF vs. α-Syn mon, * p = 0.0239 |
| 1e     | α-Syn mon = 4 α-Syn PFF = 4 | Student’s t-test, one-tailed | Fractalkine: t = 6.976, df = 3 IL-1α: t = 5.172, df = 3 IL-6: t = 2.562, df = 3 IL-7: t = 2.606, df = 3 MCP-1: t = 2.564, df = 3 MCSF: t = 2.430, df = 3 MIG: t = 2.572, df = 3 TECK: t = 2.711, df = 3 TIMP-2: t = 2.608, df = 3 | Fractalkine, ** p = 0.0030 IL-1α, ** p = 0.0070 IL-6, * p = 0.0415 IL-7, * p = 0.0400 MCP-1, * p = 0.0416 MCSF, * p = 0.0467 MIG, * p = 0.0412 TECK, * p = 0.0365 TIMP-2, * p = 0.0400 |
| 1f     | All conditions = 4 | One-way ANOVA | F(7,24) = 29.13, P < 0.0001 | WT vs. PFF 21 dpi, * p = 0.0366 WT vs. PFF 60 dpi, **** p < 0.0001 WT vs. PFF 120 dpi, * p = 0.0245 WT vs. ASO, **** p < 0.0001 PFF 60 dpi vs. mon. 60 dpi, *** p = 0.0001 |
| 1h     | WT = 6 ASO = 6 For 7, 21, 60, 120 dpi: α-Syn PFF = 6, 6, 5, 5 For 7, 60 dpi: α-Syn mon = 5, 5 | One-way ANOVA | F(8,45) = 1.519, P = 0.1776 | WT vs. PFF 7dpi, * p = 0.0425 |
| 1i     | For 7, 60 dpi: α-Syn mon = 5, 5 | One-way ANOVA | F(8,45) = 2.501, P = 0.0269 | WT vs. PFF 60 dpi, * p = 0.0329 WT vs. PFF 120 dpi, * p = 0.0232 |
| 1k | All conditions = 4 | One-way ANOVA | F(8,27) = 6.622, P < 0.0001 | WT vs. PFF 21 dpi, ** p = 0.0039  
WT vs. PFF 60 dpi, * p = 0.0149  
WT vs. PFF 120 dpi, * p = 0.0467  
WT vs. ASO, * p = 0.0182 |
| 2b | All conditions = 4 | One-way ANOVA | F(8,27) = 3.230, P = 0.0116 | WT vs. PFF 60 dpi, ** p = 0.0026  
WT vs. PFF 120 dpi, * p = 0.0155  
WT vs. ASO, * p = 0.0032 |
| 2d | All conditions = 4  
Except:  
WT = 6, ASO = 5  
For 7, 21, 60, 120 dpi:  
α-Syn PFF = 6, 5, 4, 4  
For 7, 60 dpi:  
α-Syn mon = 4, 4 | One-way ANOVA | F(8, 30) = 4.993, P = 0.0005 | WT vs. PFF 60 dpi, ** p = 0.0042  
WT vs. ASO, ** p = 0.0057 |
| 2e | WT = 11  
ASO = 6  
For 7, 21, 60, 120 dpi:  
α-Syn PFF = 6, 5, 4, 4  
For 7, 60 dpi:  
α-Syn mon = 4, 4 | One-way ANOVA | F(8,40) = 4.697, P = 0.0004 | WT vs. PFF 7dpi, * p = 0.0202  
PFF 7 dpi vs. PFF 120 dpi, * p = 0.0415  
WT vs. ASO, ** p = 0.0035  
ASO vs. PFF 120 dpi, * p = 0.0109 |
| 2i | All conditions = 5 | Two-way ANOVA | Genotype x Treatment  
F(1,16) = 1.272; P = 0.2761  
Genotype factor:  
F(1,16) = 10.63; P = 0.0049  
Treatment factor:  
F(1,16) = 66.58; P < 0.0001 | WT/GFP vs. WT/GBA1, **** p < 0.0001  
WT/GFP vs. ASO/GBA1, * p = 0.0192  
ASO/GFP vs. ASO/GBA1, *** p = 0.0008  
WT/GBA1 vs. ASO/GBA1, * p = 0.0410 |
| 2j | All conditions = 5 | Two-way ANOVA | Genotype x Treatment  
F(1,16) = 3.685; P = 0.0729  
Genotype factor:  
F(1,16) = 49.90; P < 0.0001  
Treatment factor:  
F(1,16) = 3.801; P = 0.0690 | WT/GFP vs. ASO/GFP, **** p < 0.0001  
WT/GFP vs. ASO/GBA1, * p = 0.0139  
ASO/GFP vs. ASO/GBA1, p = 0.0879 |
| 2k | For 0, 7, 21, 60 dpi:  
WT = 17, 12, 11, 8  
ASO = 13, 12, 11, 10  
Fecal pellets:  
Two-way ANOVA | Genotype x Time  
F(3,86) = 1.160; P = 0.3296  
Genotype factor:  
F(1,86) = 13.80; P = 0.0004  
Time factor:  
F(3,86) = 2.084; P = 0.1082 | 0 dpi: WT vs. ASO, ** p = 0.0078 |
| 2l | Pellet weight:  
Two-way ANOVA | Genotype x Time  
F(3,85) = 1.612; P = 0.1926  
Genotype factor:  
F(1,85) = 14.14; P = 0.0004  
Time factor:  
F(3,85) = 2.084; P = 0.1082 | 0 dpi: WT vs. ASO, ** p = 0.0061 |
| 2k | Proportion water weight: Two-way ANOVA | Whole gut transit time: Two-way ANOVA | 0 dpi: WT vs. ASO, **** p < 0.0001 7 dpi: WT vs. ASO, *** p = 0.0003 ASO: 0 dpi vs. 60 dpi, * p = 0.0265 |
|----|--------------------------------------|-------------------------------------|--------------------------------|
| For 0, 7, 21, 60 dpi: WT = 17, 12, 11, 8 ASO = 13, 12, 11, 10 | Genotype x Time F(3,85) = 6.410; P = 0.0006 Genotype factor: F(1,85) = 54.34; P < 0.0001 Time factor: F(3,85) = 0.6193; P = 0.6044 | Genotype x Time F(3,86) = 0.7794; P = 0.5087 Genotype factor: F(1, 86) = 19.15; P < 0.0001 Time factor: F(3,86) = 0.4554; P = 0.7142 | |
| 3i jR+ | Baseline = 2(63) WT = 3(183) PFF 7 dpi = 4(355) PFF 60 dpi = 3(110) Mon 7 dpi = 3(110) Mon 60 dpi = 3(90) | One-way ANOVA Peak ΔF/F F(5, 949) = 60.52; P < 0.0001 | WT, stim vs. no stim, **** p < 0.0001 WT vs. PFF 7 dpi, **** p < 0.0001 WT vs. PFF 60 dpi, **** p < 0.0001 |
| 3i jR/ChR | Baseline = 2(12) WT = 3(28) PFF 7 dpi = 4(55) PFF 60 dpi = 3(40) Mon 7 dpi = 3(23) Mon 60 dpi = 3(15) | One-way ANOVA Peak ΔF/F F(5, 157) = 23.49; P < 0.0001 | WT, stim vs. no stim, **** p < 0.0001 WT vs. PFF 7 dpi, **** p < 0.0001 WT vs. PFF 60 dpi, **** p < 0.0001 |
| 3j jR+ | WT 0 dpvi = 3(131) WT 7 dpvi = 3(78) WT 60 dpvi = 3(131) ASO 0 dpvi = 3(78) ASO 7 dpvi = 3(80) ASO 60 dpvi = 3(71) | Two-way ANOVA Peak ΔF/F Genotype x Time F(2,563) = 10.01; P < 0.0001 Genotype factor: F(1, 563) = 68.91 Time factor: F(2,563) = 3.847; P = 0.0219 | WT vs. ASO, **** p < 0.0001 WT vs. ASO 60 dpvi, * p = 0.0296 ASO vs. ASO 60 dpvi, *** p = 0.0007 ASO 7 dpvi vs. ASO 60 dpvi, ** p = 0.0068 |
| 3j jR+ | Baseline = 2(63) WT = 3(131) WT 7 dpvi = 3(78) WT 60 dpvi = 3(131) ASO 0 dpvi = 3(78) ASO 7 dpvi = 3(80) ASO 60 dpvi = 3(71) | Area under the curve Genotype x Time F(2,72) = 1.407; P = 0.2516 Genotype factor F(1,72) = 48.71; P < 0.0001 Time factor F(2, 72) = 0.8416; P = 0.4352 | WT vs. ASO, **** p < 0.0001 WT vs. ASO 60 dpvi, *** p = 0.0006 WT 7 dpvi vs. ASO 7 dpvi, ** p = 0.0031 ASO vs. ASO 60 dpvi, p = 0.0808 |
|   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|
| 3j | WT 0 dpvi = 3(131) WT 7 dpvi = 3(78) WT 60 dpvi = 3(131) ASO 0 dpvi = 3(78) ASO 7 dpvi = 3(80) ASO 60 dpvi = 3(71) | Two-way ANOVA | Peak ΔF/F | Genotype x Time | F (2, 490) = 7.103; P = 0.0009 Genotype factor | F (1, 490) = 61.42; P < 0.0001 Time factor | F (2, 490) = 1.595; P = 0.2040 0 dpi: WT vs. ASO, **** p < 0.0001 7 dpi: WT vs. ASO, *** p = 0.0006 |
|   |   |   |   |   |   |   |   |
| 4a | WT = 11 Aged = 5 ASO = 6 | One-way ANOVA | F(2,19) = 24.13; P < 0.0001 | WT vs Aged, ** p = 0.0022 WT vs. ASO, **** p < 0.0001 |
| 4b | WT = 6 Aged = 4 ASO = 5 | One-way ANOVA | F(2,12) = 14.09; P = 0.0007 | WT vs. ASO, *** p = 0.0006 Aged vs. ASO, * p = 0.0202 |
| 4c | WT = 4 Aged = 4 ASO = 6 | One-way ANOVA | F(2,11) = 4.298; P = 0.0418 | WT vs. ASO, * p = 0.0468 |
| 4d | For 0, 60, 120 dpi: α-Syn mon. = 9, 6, 4 α-Syn PFF. = 10, 7, 6 | Two-way ANOVA | Treatment x Time | F(2,36) = 2.635; P = 0.0855 Time factor | F(2,36) = 9.165; P = 0.0006 Treatment factor | F(1,36) = 2.148; P = 0.1514 PFF: 0 dpi vs. 60 dpi, * p = 0.0300 PFF: 0 dpi vs. 120 dpi, *** p = 0.0007 |
| 4e |   | Two-way ANOVA | Treatment x Time | F(2,36) = 3.713; P = 0.0342 Time factor | F(2,36) = 2.426; P = 0.1027 Treatment factor | F(1,36) = 18.63; P = 0.0001 PFF: 0 dpi vs. 120 dpi, * p = 0.0209 120 dpi: mon. vs. PFF, * p = 0.0125 |
| 4f |   | Two-way ANOVA | Treatment x Time | F(2,29) = 0.9799; P = 0.3874 Time factor | F(2,29) = 4.684; P = 0.0173 Treatment factor | F(1,29) = 1.089; P = 0.3053 PFF: 0 dpi vs. 120 dpi, * p = 0.0343 |
| 4g  | For 0, 60, 120 dpi:  
α-Syn mon. = 9, 6, 4  
α-Syn PFF. = 10, 7, 6 | Two-way ANOVA | Treatment x Time  
F(2,36) = 8.681; P = 0.0008  
Time factor  
F(2,36) = 4.437; P = 0.0190  
Treatment factor  
F(1,36) = 31.53; P < 0.0001 | PFF: 0 dpi vs. 60 dpi, * p = 0.0110  
PFF: 0 dpi vs. 120 dpi, *** p = 0.0002  
60dpi: mon. vs. PFF, ** p = 0.0044  
120 dpi: mon. vs. PFF, *** p = 0.0004 |
| 4h  | For 0, 60, 120 dpi:  
α-Syn mon. = 9, 6, 4  
α-Syn PFF. = 10, 7, 6 | Two-way ANOVA | Treatment x Time  
F(2,36) = 2.229; P = 0.1223  
Time factor  
F(2,36) = 2.760; P = 0.0767  
Treatment factor  
F(1,36) = 3.591; P = 0.0661 | PFF: 0 dpi vs. 120 dpi, * p = 0.0442 |
| 4i  | All conditions = 4 | Two-way ANOVA | Treatment x Time  
F(2,36) = 1.724; P = 0.1927  
Time factor  
F(2,36) = 10.32; P = 0.0003  
Treatment factor  
F(1,36) = 1.917; P = 0.1747 | PFF: 0 dpi vs. 120 dpi, *** p = 0.0006 |
| 4l  | All conditions = 4 | Two-way ANOVA | Treatment x Time  
F(2,18) = 4.220; P = 0.0314  
Treatment factor  
F(1,18) = 15.22; P = 0.0005  
Time factor  
F(2,18) = 12.10; P = 0.0010 | PFF: 0 dpi vs 120 dpi, *** p = 0.0005  
120 dpi: PFF vs. mon., * p = 0.0102 |
| 4m  | All conditions = 4 | Two-way ANOVA | Treatment x Time  
F(2,18) = 0.7729; P = 0.4764  
Treatment factor  
F(1,18) = 10.55; P = 0.0045  
Time factor  
F(2,18) = 5.665; P = 0.0124 | PFF: 0 dpi vs 120 dpi, p = 0.0771  
Mon 0 dpi vs PFF 120 dpi, ** p = 0.0049 |
| 4n  | For 0, 60, 120 dpi:  
WT = 4, 4, 4  
Aged PFF = 5, 5, 6  
For 60, 120 dpi:  
Aged mon. = 4, 4  
ASO young = 4  
ASO 12 m.o. = 6 | One-way ANOVA | F(9,36) = 6.176; P < 0.0001 | Aged PFF: 0 dpi vs. 120 dpi, * p = 0.0487  
ASO young vs. ASO 12 m.o., ** p = 0.0017 |
| Figure | Number of subjects | Test | Summary | Key comparisons |
|--------|-------------------|------|---------|-----------------|
| **e1a** | **WT = 42**<br>**ASO = 20**<br>**Aged = 19** | One-way ANOVA | **F(2,78) = 7.080; P = 0.0015** | **WT vs. ASO, ** p = 0.022**<br>**ASO vs. Aged, ** p = 0.0075** |
| **e1b** | **WT = 13**<br>**ASO = 9**<br>**Aged = 12** | One-way ANOVA | **F(2,78) = 7.080; P = 0.0015** | **WT vs. ASO, **** p < 0.0001**<br>**WT vs. Aged, *** p = 0.0009**<br>**ASO vs. Aged, **** p < 0.0001** |
| **e1c** | **WT = 13**<br>**ASO = 9**<br>**Aged = 12** | One-way ANOVA | **F(2,78) = 7.080; P = 0.0015** | **WT vs. ASO, **** p < 0.0001**<br>**WT vs. Aged, *** p = 0.0009**<br>**ASO vs. Aged, **** p < 0.0001** |
| **e1d** | **WT = 3**<br>**ASO = 4**<br>**Aged = 4** | One-way ANOVA | **F(2,8) = 2.541; P = 0.1399** | **WT vs. Aged, **** p < 0.0001**<br>**ASO vs. Aged, **** p < 0.0001** |
| **e1e** | **WT = 42**<br>**ASO = 20**<br>**Aged = 19** | One-way ANOVA | **F(2,78) = 7.080; P = 0.0015** | **WT vs. ASO, **** p < 0.0001**<br>**ASO vs. Aged, **** p < 0.0001** |
| **e1f** | **WT = 3**<br>**ASO = 4**<br>**Aged = 4** | One-way ANOVA | **F(2,8) = 2.541; P = 0.1399** | **WT vs. Aged, **** p < 0.0001**<br>**ASO vs. Aged, **** p < 0.0001** |
| **e1g** | See Fig. 1e | | | |
| **e1i** | **All conditions = 4** | One-way ANOVA | **F(7, 24) = 4.712; P = 0.0019** | **WT vs. ASO, * p = 0.0304**<br>**WT vs. PFF 60 dpi, * p = 0.0327**<br>**60 dpi: PFF vs. mon., * p = 0.0480** |
| **e2d** | **WT = 4**<br>All PFF = 5<br>Mon. 7 dpi = 4<br>Mon. 60 dpi = 5 | One-way ANOVA | Neurons per crypt<br>**F(8, 45) = 0.07617; P = 0.9997**<br>EGCs per crypt<br>**F(8, 39) = 2.501; P = 0.0269** | **EGCs, WT vs. PFF 60 dpi, * p = 0.0329**<br>**EGCs, WT vs. PFF 120 dpi, * p = 0.0232** |
| **e2f** | **α-Syn mon. = 4**<br>α-Syn PFF. = 5 | Student’s t-test, one-tailed | **F(4,3) = 58.59; P = 0.0071** | **Mon. vs. PFF, * p = 0.0071** |
| **e3b** | **WT = 8**<br>PFF 7 dpi = 5<br>PFF 21 dpi = 5<br>PFF 60 dpi = 5<br>PFF 120 dpi = 5<br>Mon. 7 dpi = 4<br>Mon. 60 dpi = 4<br>ASO = 8<br>Aged = 5 | One-way ANOVA | **F(8, 40) = 5.132; P = 0.0002** | **WT vs. ASO, ** p = 0.0092** |
| **e3d** | **All conditions = 3**<br>except BSA, 50 = 4 | One-way ANOVA | **F(4, 11) = 7.188; P = 0.0042** | **BSA vs. PFF, 50, ** p = 0.0080**<br>**BSA vs. PFF, 100, *** p = 0.0009**<br>**Mon., 50 vs. PFF, 50, * p = 0.0178**<br>**Mon., 100 vs. PFF, 100, ** p = 0.0078** |
### Table 1: Statistical Analysis

| Conditions | ANOVA Type | Effect | F Value | P Value |
|------------|------------|--------|---------|---------|
| Treatment x Genotype | Two-way ANOVA | F(2,18) = 0.8512; P = 0.4434 | 0.8512 | 0.4434 |
| Time factor | Two-way ANOVA | F(2,18) = 0.4326; P = 0.6554 | 0.4326 | 0.6554 |
| Genotype factor | Two-way ANOVA | F(1,18) = 12.10; P = 0.0027 | 12.10 | 0.0027 |
| Treatment x Genotype | Two-way ANOVA | F(2,18) = 0.08766; P = 0.9165 | 0.08766 | 0.9165 |
| Time factor | Two-way ANOVA | F(2,18) = 0.8450; P = 0.4459 | 0.8450 | 0.4459 |
| Genotype factor | Two-way ANOVA | F(1,18) = 6.227; P = 0.0225 | 6.227 | 0.0225 |
| Treatment x Genotype | Two-way ANOVA | F(2,18) = 0.5592; P = 0.5813 | 0.5592 | 0.5813 |
| Time factor | Two-way ANOVA | F(2,18) = 0.5461; P = 0.5885 | 0.5461 | 0.5885 |
| Genotype factor | Two-way ANOVA | F(1,18) = 9.940; P = 0.0055 | 9.940 | 0.0055 |
| Treatment x Genotype | Two-way ANOVA | F(2,18) = 0.1810; P = 0.8359 | 0.1810 | 0.8359 |
| Time factor | Two-way ANOVA | F(2,18) = 0.4914; P = 0.6198 | 0.4914 | 0.6198 |
| Genotype factor | Two-way ANOVA | F(1,18) = 41.38; P < 0.0001 | 41.38 | < 0.0001 |

### Table 2: Additional Analysis

| Conditions | ANOVA Type | Effect | F Value | P Value |
|------------|------------|--------|---------|---------|
| WT = 5 For 7, 60, 120 dpi: α-Syn PFF = 5, 5, 4 For 7, 60 dpi: α-Syn mon. = 4, 3 ASO = 5 Aged = 5 | One-way ANOVA | F(7,28) = 5.007; P = 0.0009 | 5.007 | 0.0009 |
| WT vs. ASO | One-way ANOVA | 0.0011 | 0.0011 |
| ASO vs. mon. 7 dpi | One-way ANOVA | 0.0027 | 0.0027 |
| ASO vs. mon. 60 dpi | One-way ANOVA | 0.0176 | 0.0176 |
| ASO vs. Aged | One-way ANOVA | 0.0323 | 0.0323 |
| WT = 3 α-Syn PFF, 60 dpi = 3 ASO = 3 | One-way ANOVA | F(2, 6) = 47.90; P = 0.0002 | 47.90 | 0.0002 |
| WT vs. ASO | One-way ANOVA | 0.0003 | 0.0003 |
| PFF 60 dpi vs. ASO | One-way ANOVA | 0.0007 | 0.0007 |
| WT = 3 α-Syn PFF, 60 dpi = 3 ASO = 3 | One-way ANOVA | F(2, 6) = 32.76; P = 0.0006 | 32.76 | 0.0006 |
| WT vs. ASO | One-way ANOVA | 0.0011 | 0.0011 |
| PFF 60 dpi vs. ASO | One-way ANOVA | 0.0014 | 0.0014 |
| e8a | For 0, 7, 21, 60, 90, 120 dpi:  
α-Syn PFF = 16,14,14,11,9,8  
α-Syn mon. = 9,9,9,8,8,8  
BSA =  17,16,11,9,7,7 | Two-way ANOVA | Treatment x time  
F(10,172) = 2.399; P = 0.0108  
Time factor  
F(5, 172) = 2.388; P = 0.0400  
Treatment factor  
F(2, 172) = 18.24; P < 0.0001 | PFF: 0 dpi vs. 60 dpi, ** p = 0.0012  
90 dpi: PFF vs. mon., * p = 0.0265 |
|---|---|---|---|
| e8b | For 0, 7, 21, 60, 90, 120 dpi:  
α-Syn PFF = 16,14,14,11,9,8  
α-Syn mon. = 9,9,9,8,8,8  
BSA =  17,16,11,9,7,7 | Two-way ANOVA | Treatment x time  
F(10,172) = 2.125; P = 0.2844  
Time factor  
F(5, 172) = 8.351; P < 0.0001  
Treatment factor  
F(2, 172) = 8.086; P = 0.0004 | PFF: 0 dpi vs. 60 dpi, * p = 0.0306  
PFF: 0 dpi vs. 120 dpi, * p = 0.0285 |
| e8c | For 0, 7, 21, 60, 90, 120 dpi:  
α-Syn PFF = 16,14,14,11,9,8  
α-Syn mon. = 9,9,9,8,8,8  
BSA =  17,16,11,9,7,7 | Two-way ANOVA | Treatment x time  
F(10,172) = 2.125; P = 0.2844  
Time factor  
F(5, 172) = 8.351; P < 0.0001  
Treatment factor  
F(2, 172) = 8.086; P = 0.0004 | PFF: 0 dpi vs. 90 dpi, ** p = 0.0014  
PFF: 0 dpi vs. 120 dpi, * p = 0.0285  
60 dpi: PFF vs. mon., * p = 0.0193  
90 dpi: PFF vs. mon., * p = 0.0342 |
| e8d | For 0, 7, 21, 60, 90, 120 dpi:  
α-Syn PFF = 8,8,8,12,8,8  
α-Syn mon. = 9,9,9,8,8,8  
BSA =  17,16,11,9,7,7 | Two-way ANOVA | Treatment x time  
F(10,152) = 0.1109; P = 0.9997  
Time factor  
F(5, 152) = 10.52; P < 0.0001  
Treatment factor  
F(2, 152) = 7.809; P = 0.0006 | |
| e8e | For 0, 7, 21, 60, 90, 120 dpi:  
α-Syn PFF = 8,10,7,6,6  
α-Syn mon. = 9,9,9,8,8,8  
BSA =  17,16,11,9,7,7 | Two-way ANOVA | Treatment x time  
F(10,145) = 0.5406; P = 0.8589  
Time factor  
F(5, 145) = 0.8005; P = 0.5510  
Treatment factor  
F(2, 145) = 5.321; P = 0.0059 | |
| e8f | For 0, 7, 21, 60, 90, 120 dpi:  
α-Syn PFF = 8,10,7,6,6  
α-Syn mon. = 9,9,9,8,8,8  
BSA =  17,16,11,9,7,7 | Two-way ANOVA | Treatment x time  
F(10,145) = 1.352; P = 0.2087  
Time factor  
F(5, 145) = 1.628; P = 0.1562  
Treatment factor  
F(2, 145) = 17.66; P < 0.0001 | 60 dpi: PFF vs. BSA, ** p = 0.0023 |
| e8g | Aged baseline: 10  
Aged monomer, 60 dpi: 4  
Aged PFF, 60 dpi: 6 | One-way ANOVA | F(2,17) = 0.07091 | |
| e9d | All conditions = 4 | One-way ANOVA | F(2,9) = 0.1824; P = 0.8362 | |