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

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Supplementary Table 1. List of antibodies used

| 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 | | | |
| 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 | CellSignaling | 7074 | Western, dot blot 1:2000 |
| Horseradish peroxidase (HRP)-linked Goat anti-Mouse IgG | CellSignaling | 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 | <u>Condition x Time:</u> $F(4,96) = 1.857; P = 0.1243$ <u>Time factor:</u> $F(4,96) = 4.490; P = 0.0023$ <u>Condition factor:</u> $F(1,96) = 22.72; P < 0.0001$ | 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 | | Two-way ANOVA | <u>Condition x Time:</u> $F(4,96) = 3.980; P = 0.0050$ <u>Time factor:</u> $F(4,96) = 1.808; P = 0.1136$ <u>Condition factor:</u> $F(1,96) = 6.438; P = 0.0128$ | 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 = 6, 7, 6, 6, 6 α-Syn PFF = 7, 8, 9, 9, 8 | Two-way ANOVA | <u>Condition x Time:</u> $F(4,62) = 5.264; P = 0.0010$ <u>Time factor:</u> $F(4,62) = 3.042; P = 0.0235$ <u>Condition factor:</u> $F(1,62) = 14.47; P = 0.0003$ | 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-1a: 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-1a, ** 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 | 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 |

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| 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 | 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 <u>For 7, 21, 60, 120 dpi:</u> α -Syn PFF = 6, 5, 4, 4 <u>For 7, 60 dpi:</u> 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 | <u>Genotype x Treatment</u> F(1,16) = 1.272; P = 0.2761 <u>Genotype factor:</u> F(1,16) = 10.63; P = 0.0049 <u>Treatment factor:</u> 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 | <u>Genotype x Treatment</u> F(1,16) = 3.685; P = 0.0729 <u>Genotype factor:</u> F(1,16) = 49.90; P < 0.0001 <u>Treatment factor:</u> 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 | <u>For 0, 7, 21, 60 dpi:</u> WT = 17, 12, 11, 8 ASO = 13, 12, 11, 10 | Fecal pellets: Two-way ANOVA | <u>Genotype x Time</u> F(3,86) = 1.160; P = 0.3296 <u>Genotype factor:</u> F(1,86) = 13.80; P = 0.0004 <u>Time factor:</u> F(3,86) = 2.084; P = 0.1082 | 0 dpi: WT vs. ASO, ** p = 0.0078 |
| | | Pellet weight: Two-way ANOVA | <u>Genotype x Time</u> F(3,85) = 1.612; P = 0.1926 <u>Genotype factor:</u> F(1,85) = 14.14; P = 0.0004 <u>Time factor:</u> F(3,85) = 2.084; P = 0.1082 | 0 dpi: WT vs. ASO, ** p = 0.0061 |

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| 2k | For 0, 7, 21, 60 dpi: WT = 17, 12, 11, 8 ASO = 13, 12, 11, 10 | Proportion water weight: Two-way ANOVA | <u>Genotype x Time</u> F(3,85) = 6.410; P = 0.0006 <u>Genotype factor:</u> F(1,85) = 54.34; P < 0.0001 <u>Time factor:</u> F(3,85) = 0.6193; P = 0.6044 | 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 |
| | | Whole gut transit time: Two-way ANOVA | <u>Genotype x Time</u> F(3,86) = 0.7794; P = 0.5087 <u>Genotype factor:</u> F(1, 86) = 19.15; P < 0.0001 <u>Time factor:</u> F(3,86) = 0.4554; P = 0.7142 | 0 dpi: WT vs. ASO, * p = 0.0123 |
| 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 |
| | | | Area under the curve F(5,932) = 55.90; 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 |
| | | | Area under the curve F(5,167) = 8.613; P < 0.0001 | WT, stim vs. no stim, **** p < 0.001 WT vs. PFF 7 dpi, * p = 0.0488 WT vs. PFF 60 dpi, * p = 0.0214 |
| 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 | <u>Peak ΔF/F</u> <u>Genotype x Time</u> F(2,563) = 10.01; P < 0.0001 <u>Genotype factor</u> F(1, 563) = 68.91 <u>Time factor</u> 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 |
| | | | <u>Area under the curve</u> <u>Genotype x Time</u> F(2,72) = 1.407; P = 0.2516 <u>Genotype factor</u> F(1,72) = 48.71; P < 0.0001 <u>Time factor</u> 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 |

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|--------------|---|---------------|---|---|
| 3j jR/ChR | 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 | <u>Peak $\Delta F/F$</u> <u>Genotype x Time</u> F (2, 490) = 7.103; P = 0.0009 <u>Genotype factor</u> F (1, 490) = 61.42; P < 0.0001 <u>Time factor</u> F (2, 490) = 1.595; P = 0.2040 | 0 dpi: WT vs. ASO, **** p < 0.0001 7 dpi: WT vs. ASO, *** p = 0.0006 |
| | | | <u>Area under the curve</u> <u>Genotype x Time</u> F (2, 72) = 1.586; P = 0.2117 <u>Genotype factor</u> F (1, 72) = 10.55; P = 0.0018 <u>Time factor</u> F (2, 72) = 3.074; P = 0.0523 | WT vs. ASO, * p = 0.0398 |
| 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 | <u>For 0, 60, 120 dpi:</u> α -Syn mon. = 9, 6, 4 α -Syn PFF. = 10, 7, 6 | Two-way ANOVA | <u>Treatment x Time</u> F(2,36) = 2.635; P = 0.0855 <u>Time factor</u> F(2,36) = 9.165; P = 0.0006 <u>Treatment factor</u> 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 | <u>Treatment x Time</u> F(2,36) = 3.713; P = 0.0342 <u>Time factor</u> F(2,36) = 2.426; P = 0.1027 <u>Treatment factor</u> 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 | <u>Treatment x Time</u> F(2,29) = 0.9799; P = 0.3874 <u>Time factor</u> F(2,29) = 4.684; P = 0.0173 <u>Treatment factor</u> F(1,29) = 1.089; P = 0.3053 | PFF: 0 dpi vs. 120 dpi, * p = 0.0343 |

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|----|---|---------------|--|--|
| 4g | | Two-way ANOVA | <u>Treatment x Time</u> $F(2,36) = 8.681; P = 0.0008$ <u>Time factor</u> $F(2,36) = 4.437; P = 0.0190$ <u>Treatment factor</u> $F(1,36) = 31.53; P < 0.0001$ | 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 | <u>For 0, 60, 120 dpi:</u> α -Syn mon. = 9, 6, 4 α -Syn PFF. = 10, 7, 6 | Two-way ANOVA | <u>Treatment x Time</u> $F(2,36) = 2.229; P = 0.1223$ <u>Time factor</u> $F(2,36) = 2.760; P = 0.0767$ <u>Treatment factor</u> $F(1,36) = 3.591; P = 0.0661$ | PFF: 0 dpi vs. 120 dpi, * $p = 0.0442$ |
| 4i | | Two-way ANOVA | <u>Treatment x Time</u> $F(2,36) = 1.724; P = 0.1927$ <u>Time factor</u> $F(2,36) = 10.32; P = 0.0003$ <u>Treatment factor</u> $F(1,36) = 1.917; P = 0.1747$ | PFF: 0 dpi vs. 120 dpi, *** $p = 0.0006$ |
| 4l | All conditions = 4 | Two-way ANOVA | <u>Treatment x Time</u> $F(2,18) = 4.220; P = 0.0314$ <u>Treatment factor</u> $F(1,18) = 15.22; P = 0.0005$ <u>Time factor</u> $F(2,18) = 12.10; P = 0.0010$ | PFF: 0 dpi vs 120 dpi, *** $p = 0.0005$ 120 dpi: PFF vs. mon., * $p = 0.0102$ |
| 4m | All conditions = 4 | Two-way ANOVA | <u>Treatment x Time</u> $F(2,18) = 0.7729; P = 0.4764$ <u>Treatment factor</u> $F(1,18) = 10.55; P = 0.0045$ <u>Time factor</u> $F(2,18) = 5.665; P = 0.0124$ | 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., ** $p = 0.0017$ |

Extended Data Figures

| Figure | Number of subjects | Test | Summary | Key comparisons |
|--------|--|---------------------------------|---|---|
| e1a | WT = 42 ASO = 20 Aged = 19 | One-way ANOVA | $F(2,78) = 7.080$; $P = 0.0015$ | WT vs. ASO, ** $p = 0.022$ ASO vs. Aged, ** $p = 0.0075$ |
| e1b | | One-way ANOVA | $F(2,78) = 25.12$; $P < 0.0001$ | WT vs. ASO, **** $p < 0.0001$ WT vs. Aged, *** $p = 0.0009$ ASO vs. Aged, **** $p < 0.0001$ |
| e1c | | One-way ANOVA | $F(2,78) = 27.16$; $P < 0.0001$ | WT vs. ASO, **** $p < 0.0001$ ASO vs. Aged, **** $p < 0.0001$ |
| e1d | WT = 13 ASO = 9 Aged = 12 | One-way ANOVA | $F(2,31) = 14.69$; $P < 0.0001$ | WT vs. ASO, **** $p < 0.0001$ ASO vs. Aged, ** $p = 0.0038$ |
| e1e | WT = 42 ASO = 20 Aged = 19 | One-way ANOVA | $F(2,78) = 116.9$; $P < 0.0001$ | WT vs. Aged, **** $p < 0.0001$ ASO vs. Aged, **** $p < 0.0001$ |
| e1f | WT = 3 ASO = 4 Aged = 4 | One-way ANOVA | $F(2, 8) = 2.541$; $P = 0.1399$ | |
| e1g | See Fig. 1e | | | |
| e1i | All conditions = 4 | One-way ANOVA | $F(7, 24) = 4.712$; $P = 0.0019$ | WT vs. ASO, * $p = 0.0304$ WT vs. PFF 60 dpi, * $p = 0.0327$ 60 dpi: PFF vs. mon., * $p = 0.0480$ |
| e2d | WT = 4 All PFF = 5 Mon. 7 dpi = 4 Mon. 60 dpi = 5 | One-way ANOVA | Neurons per crypt $F(8, 45) = 0.07617$; $P = 0.9997$ EGCs per crypt $F(8, 39) = 2.501$; $P = 0.0269$ | EGCs, WT vs. PFF 60 dpi, * $p = 0.0329$ EGCs, WT vs. PFF 120 dpi, * $p = 0.0232$ |
| e2f | α -Syn mon. = 4 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 PFF 7 dpi = 5 PFF 21 dpi = 5 PFF 60 dpi = 5 PFF 120 dpi = 5 Mon. 7 dpi = 4 Mon. 60 dpi = 4 ASO = 8 Aged = 5 | One-way ANOVA | $F(8, 40) = 5.132$; $P = 0.0002$ | WT vs. ASO, ** $p = 0.0092$ |
| e3d | All conditions = 3 except BSA, 50 = 4 | One-way ANOVA | $F(4, 11) = 7.188$; $P = 0.0042$ | BSA vs. PFF, 50, ** $p = 0.0080$ BSA vs. PFF, 100, *** $p = 0.0009$ Mon., 50 vs. PFF, 50, * $p = 0.0178$ Mon., 100 vs. PFF, 100, ** $p = 0.0078$ |

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|-----|--|---------------|---|---|
| e4a | See Fig. 2k | | | |
| e4b | | | | |
| e4c | | | | |
| e4d | | | | |
| e4e | All conditions = 4 | Two-way ANOVA | <u>Treatment x Genotype</u> $F(2,18) = 0.8512; P = 0.4434$ <u>Time factor</u> $F(2,18) = 0.4326; P = 0.6554$ <u>Genotype factor</u> $F(1,18) = 12.10; P = 0.0027$ | |
| e4f | | Two-way ANOVA | <u>Treatment x Genotype</u> $F(2,18) = 0.08766; P = 0.9165$ <u>Time factor</u> $F(2,18) = 0.8450; P = 0.4459$ <u>Genotype factor</u> $F(1,18) = 6.227; P = 0.0225$ | |
| e4g | | Two-way ANOVA | <u>Treatment x Genotype</u> $F(2,18) = 0.5592; P = 0.5813$ <u>Time factor</u> $F(2,18) = 0.5461; P = 0.5885$ <u>Genotype factor</u> $F(1,18) = 9.940; P = 0.0055$ | |
| e4h | | Two-way ANOVA | <u>Treatment x Genotype</u> $F(2,18) = 0.1810; P = 0.8359$ <u>Time factor</u> $F(2,18) = 0.4914; P = 0.6198$ <u>Genotype factor</u> $F(1,18) = 41.38; P < 0.0001$ | 0 dpi: WT vs. ASO, ** p = 0.0085 60 dpi: WT vs. ASO, * p = 0.0293 |
| e5a | See Fig. 3i | | | |
| e5b | See Fig 3j | | | |
| e6c | WT = 5 <u>For 7, 60, 120 dpi:</u> α-Syn PFF = 5, 5, 4 <u>For 7, 60 dpi:</u> α-Syn mon. = 4, 3 ASO = 5 Aged = 5 | One-way ANOVA | $F(7,28) = 5.007; P = 0.0009$ | WT vs. ASO, ** p = 0.0011 ASO vs. mon. 7 dpi, ** p = 0.0027 ASO vs. mon. 60 dpi, * p = 0.0176 ASO vs. Aged, * p = 0.0323 |
| e7e | WT = 3 α-Syn PFF, 60 dpi = 3 ASO = 3 | One-way ANOVA | $F(2, 6) = 47.90; P = 0.0002$ | WT vs. ASO, *** p = 0.0003 PFF 60 dpi vs. ASO, *** p = 0.0007 |
| e7f | | One-way ANOVA | $F(2, 6) = 32.76; P = 0.0006$ | WT vs. ASO, ** p = 0.0011 PFF 60 dpi vs. ASO, ** p = 0.0014 |

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| e8a | | Two-way ANOVA | <u>Treatment x time</u> $F(10,172) = 2.399; P = 0.0108$ <u>Time factor</u> $F(5, 172) = 2.388; P = 0.0400$ <u>Treatment factor</u> $F(2, 172) = 18.24; P < 0.0001$ | 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 | <u>Treatment x time</u> $F(10,172) = 1.215; P = 0.2844$ <u>Time factor</u> $F(5,172) = 8.351; P < 0.0001$ <u>Treatment factor</u> $F(2,172) = 8.086; P = 0.0004$ | PFF: 0 dpi vs. 60 dpi, * p = 0.0306 PFF: 0 dpi vs. 120 dpi, * p = 0.0285 |
| e8c | | Two-way ANOVA | <u>Treatment x time</u> $F(10,172) = 1.215; P = 0.2844$ <u>Time factor</u> $F(5,172) = 8.351; P < 0.0001$ <u>Treatment factor</u> $F(2,172) = 8.086; P = 0.0004$ | 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 | <u>Treatment x time</u> $F(10,152) = 0.1109; P = 0.9997$ <u>Time factor</u> $F(5,152) = 10.52; P < 0.0001$ <u>Treatment factor</u> $F(2,152) = 7.809; P = 0.0006$ | |
| e8e | For 0, 7, 21, 60, 90, 120 dpi: α -Syn PFF = 8,8,10,7,6,6 α -Syn mon. = 9,9,9,8,8,8 BSA = 17,16,11,9,7,7 | Two-way ANOVA | <u>Treatment x time</u> $F(10,145) = 0.5406; P = 0.8589$ <u>Time factor</u> $F(5,145) = 0.8005; P = 0.5510$ <u>Treatment factor</u> $F(2,145) = 5.321; P = 0.0059$ | |
| e8f | | Two-way ANOVA | <u>Treatment x time</u> $F(10,145) = 1.352; P = 0.2087$ <u>Time factor</u> $F(5,145) = 1.628; P = 0.1562$ <u>Treatment factor</u> $F(2,145) = 17.66; P < 0.0001$ | 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 | $F(2,9) = 0.1824; P = 0.8362$ | |