Supplementary Information for

Wild-type α-synuclein inherits the structure and exacerbated neuropathology of E46K mutant fibril strain by cross-seeding

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METHODS

Atomic force microscopy (AFM). Fibril samples were mounted on a freshly cleaved mica for 3 min and gently rinsed with Milli-Q water to remove unbound fibrils. Nitrogen flow was used to dry samples. The images were acquired by using Nanoscope V Multimode 8 (Bruker) on ScanAsyst air mode. Scanning was conducted by using a SNL-10 probe with a constant of 0.35 N m^{-1}. Images were recorded at 512x512 pixels with rate at 1.5 Hz. The images were analyzed on the Nanoscope software.

ThT kinetic assay. Cross-seeding of either hWT or mWT monomer by the hE46K α-syn PFFs was tested by ThT assay. 50 μM α-syn WT monomer (hWT or mWT protein, in 50 mM Tris, pH 7.5, 150 mM KCl, 0.05% NaN_3) was incubated with the hE46K α-syn PFFs (5 mol%, relative to α-syn monomer) with 10 μM Thioflavin-T (ThT) in the reaction mixture. The comparison of the seeding capabilities of the hWT_{cs} PFFs and hWT PFFs was conducted by adding 5 mol% PFFs (hWT_{cs} PFFs or hWT PFFs, relative to α-syn monomer) to 50 μM α-syn hWT monomer protein with 10 μM ThT in the reaction mixture. Reactions were performed in a 384 well optical plate (Thermo Scientific) in triplicate. A Fluoroskan Ascent microplate reader (Thermo Scientific) was used. The fluorescent intensities were monitored by using 440 nm excitation wave-length and 485 nm emission wave-length, with a bottom read. Graphing was performed with GraphPad Prism 6. For all ThT experiments, at least three

independent experiments were performed to confirm the reproducibility. The data shown in each ThT experiment are mean ± s.d., n=3 independent samples.

50 μl fibrillation samples at 9 h, 22 h and 80 h after shaking were concentrated by centrifugation (14,462 × *g*, 25 °C, 45 min), respectively. 45 μl supernatant was boiled with SDS-loading buffer for 10 min. The pellet was washed by PBS and resuspended in 45 μl buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Triton X-100, 2% SDS). The solution was sonicated for 5 min and boiled for 30 min, followed by being boiled in the SDS-loading buffer for 10 min. The supernatant and dissolved pellet samples were loaded on 4%-20% Bis-Tris gels (GenScript), separately. The gels were stained by Coomassie brilliant blue and images were acquired and analyzed with Image Lab 3.0 (Bio-Rad).

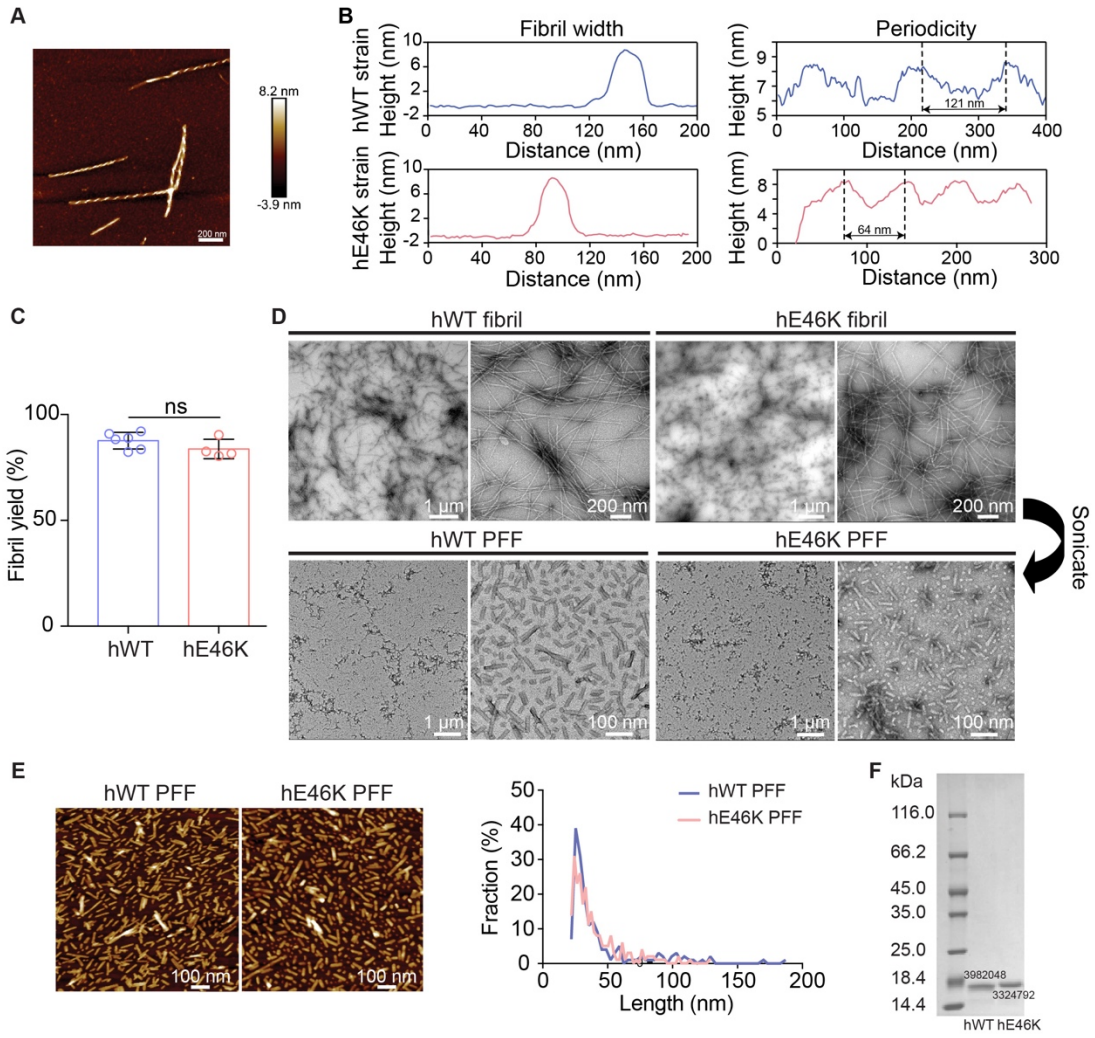


Fig. S1. Quantification and characterization of hWT and hE46K PFFs injected into mice.

(A) An AFM image of human prion protein (PrP) fibril as a control for the handedness of fibrils. The structure of the PrP fibril has been determined by cryo-EM to an atomic resolution of 2.70 Å (1) and the handedness is certain as left-handed. (B) The fibril width and periodicity of the hWT (blue) and hE46K (red) fibrils measured by AFM. Supplementary to Fig 1A. (C) The yield of hWT and hE46K fibrils prepared in vitro. Fibril yield was calculated as the total amount of α-syn monomer subtracting the amount of residual soluble α-syn after pelleting the fibrils. Data are shown as mean ± s.d., n is independent samples for hWT (n=6) and hE46K (n=4). (D) Negative-staining TEM images of the hWT and the hE46K fibrils (top) and PFFs after sonication (bottom). Images are shown with two magnifications for each sample. (E) Size distribution

of hWT and hE46K PFFs measured by AFM. Scale bar: 100 nm. The percentage for fibril length was processed and analyzed by NanoScope Analysis software (version 1.5). (F) SDS-PAGE gel of the hWT and hE46K PFFs injected into mice. The gel was stained by coomassie brilliant blue. The intensities of the protein bands were analyzed by Image Lab 3.0 (Bio-Rad).

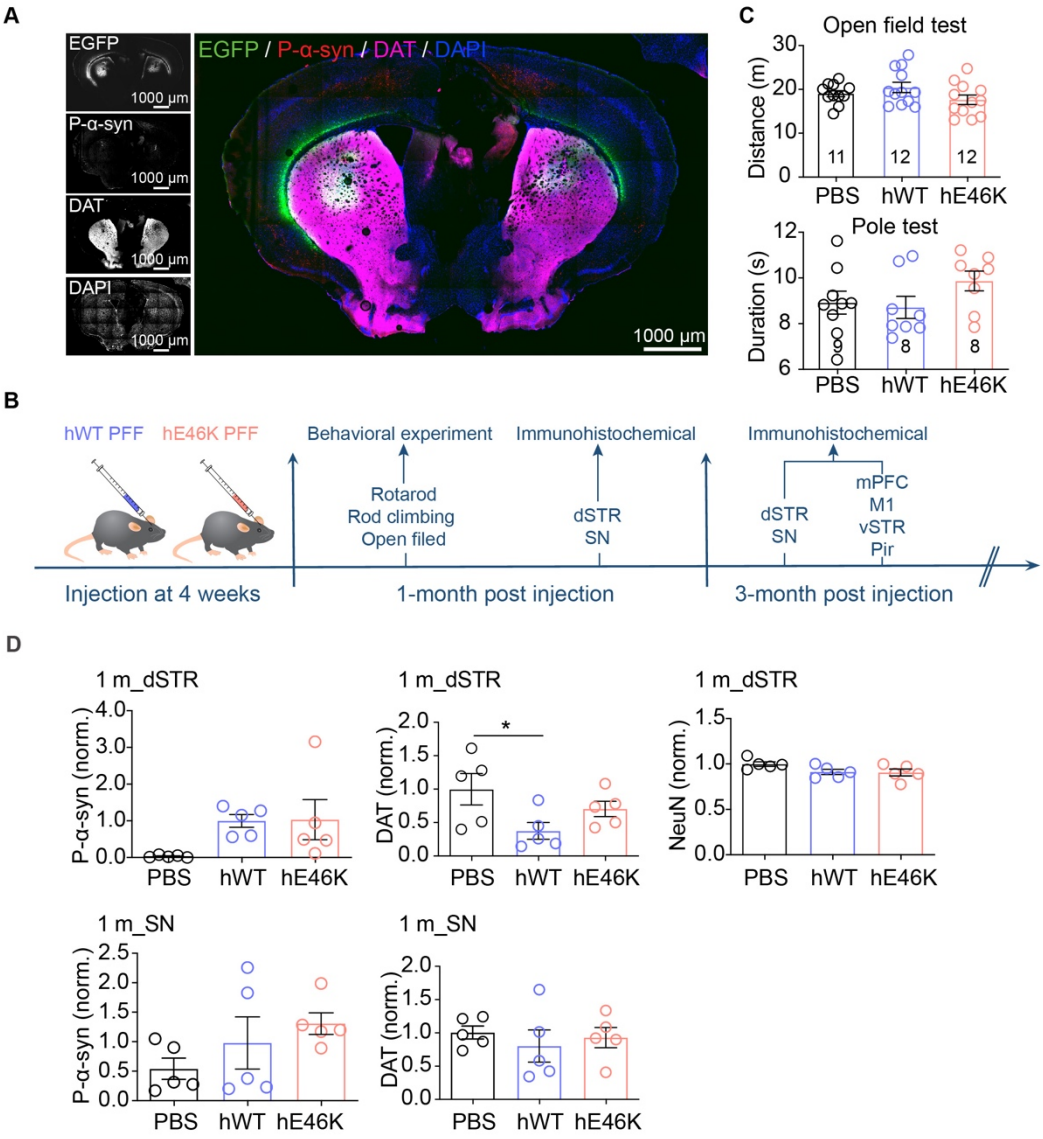


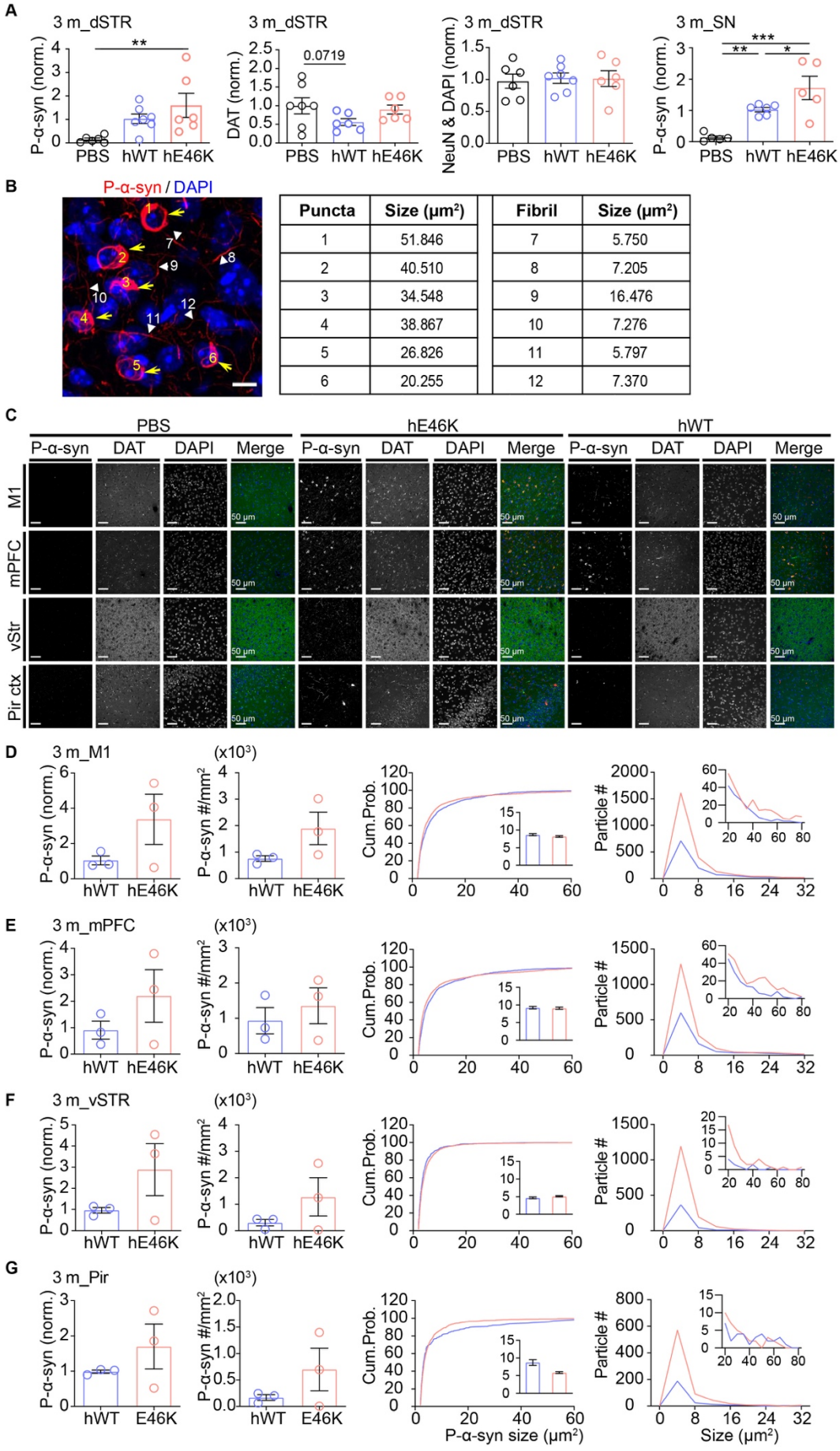
Fig. S2. Tests and characterizations of mice injected with α-syn PFFs 1-month post inoculation.

(A) Immunofluorescence images of the coronal brain slice containing striatum from mouse co-injected with α-syn PFF and aav-hsyn-GFP. Slice was stained with p-α-syn antibody (red), DAT antibody (magenta) and DAPI (blue). (B) Experiment flowchart of the behavior and histological tests on mice injected with α-syn PFFs. (C) Measurement of the locomotor activity by open field test is shown on the left. PBS = 1906±70.20 cm, n = 11; hWT = 2044±117.90 cm, n = 12; hE46K = 1761±107.90 cm, n = 12; One-way ANOVA, F (2,32) = 1.96, p = 0.1574. The climb down duration in pole test is shown on the right. PBS = 8.92±0.50 s, n = 9; hWT = 8.71±0.49 s, n = 8; hE46K = 9.87±0.43 s, n = 8; One-

way ANOVA, F (2,22) = 1.62, p = 0.2210. (D) Quantitative analyses of the immunofluorescence imaging 1-month post inoculation, supplementary to Fig 1. From left to right, first row to the second row: normalized p-α-syn signal intensity in dSTR (PBS = 0.03508±0.01414, n = 5; hWT = 1±0.1749, n = 5; hE46K = 1.036±0.5453, n = 5; normalized to hWT; one-way ANOVA F (2,12) = 2.948, p = 0.0909); DAT signal intensity in dSTR (PBS = 1±0.233, n = 5; hWT = 0.377±0.127, n = 5; hE46K = 0.706±0.116, n = 5; normalized to PBS; one-way ANOVA, F (2,12) = 3.47, p = 0.0647); normalized NeuN signal intensity in dSTR (PBS = 1±0.02536, n = 5; hWT = 0.9143±0.02928, n = 5; hE46K = 0.9077±0.03907, n = 5; normalized to PBS; one-way ANOVA, F(2,12) = 2.628, p=0.1131); normalized p-α-syn signal intensity in SN (PBS = 0.5434±0.1807, n = 5; hWT = 0.9804±0.4410, n = 5; hE46K = 1.308±0.1824, n = 5; normalized to hWT; one-way ANOVA, F (2,12) = 1.696, p = 0.2245); DAT signal intensity in SN (PBS = 1.01±0.0983, n = 5; hWT = 0.804±0.241, n = 5; hE46K = 0.931±0.152, n = 5; normalized to PBS; one-way ANOVA, F (2,12) = 0.345, p = 0.7148).

n numbers listed here represent mouse number. Only significant or close to significant pairwise comparisons were labeled in the figures. Fisher's LSD post hoc test. Data shown are mean ± SEM. The level of significance was set as

* p<0.05; ** p<0.01; *** p<0.001, **** p<0.0001.



Puncta	Size (μm²)	Fibril	Size (μm²)
1	51.846	7	5.750
2	40.510	8	7.205
3	34.548	9	16.476
4	38.867	10	7.276
5	26.826	11	5.797
6	20.255	12	7.370

Fig. S3. α-Syn pathology induced by hE46K and hWT PFF 3-month post inoculation.

(A) Quantitative analyses of the immunofluorescence imaging 3-month post inoculation, supplementary to Fig 2. From left to right: normalized p-α-syn signal intensity in dSTR (PBS = 0.148±0.0572, n = 6; hWT = 1.03±0.202, n = 7; hE46K = 1.6±0.515, n = 6; Normalized to hWT; One-way ANOVA, F (2, 16) = 5.27, p=0.0174); DAT signal intensity in dSTR (PBS = 1±0.215, n = 7; hWT = 0.562±0.0953, n=6; hE46K=0.898±0.122, n=6; Normalized to PBS; One-way ANOVA, F (2,16) = 1.98, p = 0.1705); normalized NeuN or DAPI signal intensity in dSTR 3-month post inoculation. (PBS = 0.9737±0.1125, n = 6; hWT = 1.024±0.07987, n = 7; hE46K = 1.016±0.01252, n = 6; normalized to PBS; One-way ANOVA, F (2,16) = 0.06437, p=0.9379); normalized p-α-syn signal intensity in SN (PBS = 0.134±0.0544, n = 5; hWT = 1.03±0.0696, n = 6; hE46K = 1.72±0.377, n = 5; Normalized to hWT, One-way ANOVA, F (2,13) = 13.5, p=0.0007). (B) Representative image from mPFC of hE46K-injected mouse (left). Scale bar, 10 μm. Large deposits are pointed by arrows; small deposits are pointed by arrowheads. The deposits are numbered, and their sizes are listed in (B). (C) Representative images from 4 different brain regions. Scale bar, 50 μm. M1: primary motor cortex; mPFC: medial prefrontal cortex; vSTR: ventral striatum; Pir: piriform cortex. Quantitative analyses are shown in (D-G). (D) From left to right: mean p-α-syn signal intensity in M1 3 mpi (hWT = 1.047±0.2553, n = 3; hE46K = 3.376±1.425, n = 3; Normalized to hWT; Welch's t test, p = 0.2414), p-α-syn aggregate density in M1 3 mpi (hWT = 757.8±109.2 /mm^2, n = 3; hE46K = 1897±615.4 /mm^2, n = 3; Welch's t test, p = 0.2023), the size cumulative probability curve (hWT vs hE46k, p<0.0001, K-S test) with the average aggregate size inserted (hWT (blue) = 8.703±0.294 μm^2, n = 3; hE46K (red) = 8.213±0.2314 μm^2, n = 3, and the distribution curve of the aggregate size using 4 μm^2 as the bin size with the zoom in distribution curve for bin size ranging from 20-80 inserted; (E) From left to right: mean p-α-syn signal intensity

in mPFC 3mpi (hWT = 0.9112±0.3367, n = 3; hE46K = 2.204±0.9969, n = 3; Normalized to hWT; Welch's t test, p = 0.3241), p-α-syn aggregate density in mPFC 3 mpi (hWT = 935.2±371.2 /mm^2, n = 3; hE46K = 1360±508.3 /mm^2, n = 3; Welch's t test, p = 0.5402), the size cumulative probability curve (hWT vs hE46k, p<0.0001, K-S test) with the average aggregate size inserted (hWT (blue) = 9.278±0.358 μm^2, n = 3; hE46K (red) = 9.117±0.291 μm^2, n = 3, and the distribution curve of the aggregate size using 4 μm^2 as the bin size with the zoom in distribution curve for bin size ranging from 20-80 inserted. (F) From left to right: mean p-α-syn signal intensity in vStr 3mpi (hWT = 0.9670±0.1341, n = 3; hE46K = 2.888±1.226, n = 3; Normalized to hWT; Welch's t test, p = 0.2569), p-α-syn aggregate density in vStr 3 mpi (hWT = 305±125.4 /mm^2, n = 3; hE46K = 1278±726.5 /mm^2, n = 3; Welch's t test, p = 0.3114), the size cumulative probability curve (hWT vs hE46k, p=0.0157, K-S test) with the average aggregate size inserted (hWT (blue) = 4.672±0.235 μm^2, n = 3; hE46K (red) = 5.145±0.142 μm^2, n = 3, and the distribution curve of the aggregate size using 4 μm^2 as the bin size with the zoom in distribution curve for bin size ranging from 20-80 inserted. (G) From left to right: mean p-α-syn signal intensity in Pirctx 3mpi (hWT = 0.9887±0.04594, n = 3; hE46K = 1.700±0.6380, n = 3; Normalized to hWT; Welch's t test, p = 0.3809), p-α-syn aggregate density in vStr 3 mpi (hWT = 173.3±54.78 /mm^2, n = 3; hE46K = 700.5±402.4 /mm^2, n = 3; Welch's t test, p = 0.3198), the size cumulative probability curve (hWT vs hE46k, p=0.1005, K-S test) with the average aggregate size inserted (hWT (blue) = 8.722±0.828 μm^2, n = 3; hE46K (red) = 5.895±0.266 μm^2, n = 3, and the distribution curve of the aggregate size using 4 μm^2 as the bin size with the zoom in distribution curve for bin size ranging from 20-80 inserted.

n numbers listed here represent mouse number. Only significant or close to significant pairwise comparisons were labeled in the figures. Fisher's LSD post hoc test. Data shown are mean ± SEM. The level of significance was set as

* p<0.05; ** p<0.01; *** p<0.001, **** p<0.0001.

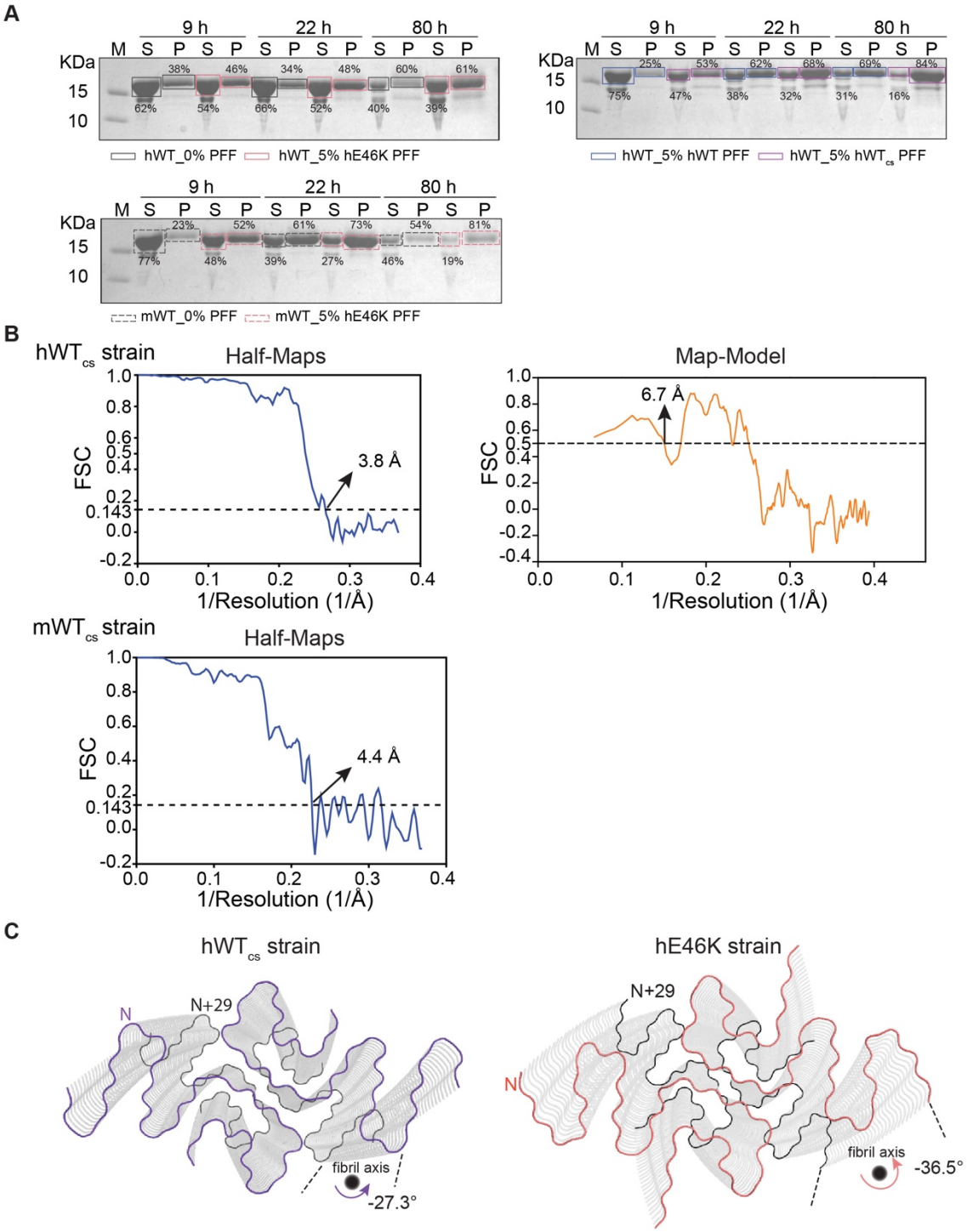


Fig. S4. Various cross-seeding of the amyloid fibril formation of α-syn.

(A) Gel electrophoresis of the samples collected during the ThT kinetics assay. The intensities of the protein bands were analyzed by Image Lab 3.0 (Bio-Rad). The relative percentages of proteins in the supernatant and pellet were calculated. S, supernatant; P, pellet; M, protein marker. (B) Gold-standard

Fourier shell correlation (FSC) curves of the hWT_{cs} and mWT_{cs} fibrils. (C) Structural models of hWT_{cs} (PDB ID：7C1D) and hE46K (PDB ID：6L4S) fibril strains. The rotation angles are between layers N and N+29 perpendicular to the fibril axis, showing the subtle difference in the subunit packing along the fibril axis in the two fibril structures.

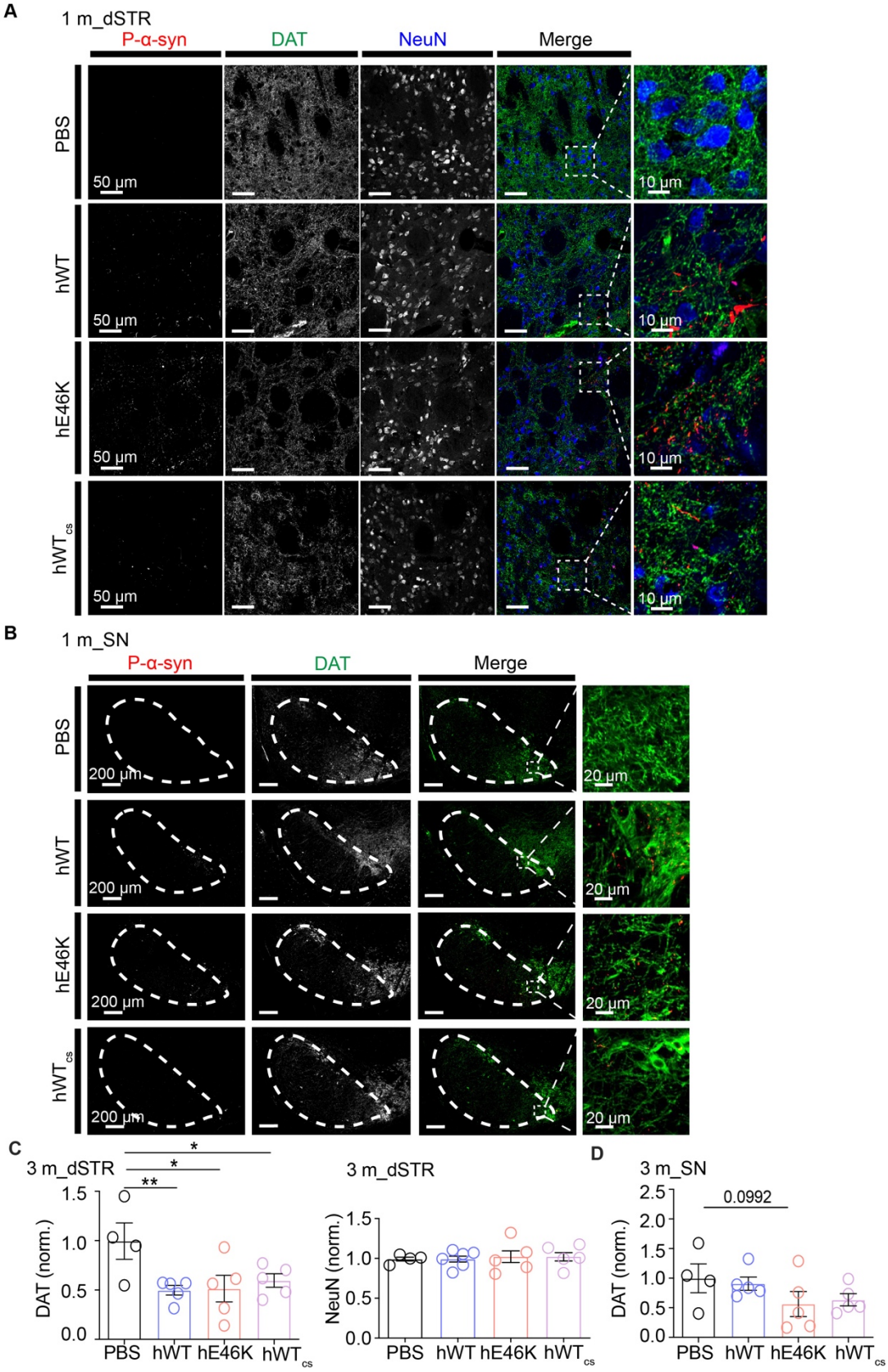


Fig. S5. Mice injected with hWT_{cs} PFF develops pathology resembling those induced by hE46K PFF.

(A) Representative immunofluorescence images of dSTR 1-month post fibril inoculation. Antibodies were used to stain p-α-syn (red), DAT (green), NeuN (blue). Scale bar: 50 μm. Zoom-in of the merged images are shown on the right. Scale bar: 10 μm. (B) Representative immunofluorescence images of SN 1-month post fibril inoculation. Scale bar: 200 μm. Zoom-in of the merged images are shown on the right. Scale bar: 20 μm. (C) Left, DAT signal intensity in dSTR 3-month post inoculation, supplementary to Fig 5C (PBS=1±0.185, n=4; hWT=0.498±0.0495, n=5; hE46K=0.514±0.136, n=5; hWT_{cs}=0.596±0.0686, n=5; normalized to PBS; ANOVA F (3, 15) = 3.77, p=0.0337). Right, NeuN signal intensity normalized to PBS group in dSTR 3 m post inoculation. (PBS = 0.9907±0.02345, n = 4; hWT = 0.9910±0.03628, n = 6; hE46K = 1.022±0.07392, n = 5; hWT_{cs} = 1.021±0.05192, n = 5; normalized to PBS; One-way ANOVA, F (3,16) = 0.1212, p = 0.9463). (D) DAT signal intensity in SN 3-month post inoculation, supplementary to Fig 5F (PBS=1±0.243, n=4; hWT=0.908±0.109, n=5; hE46K=0.565±0.210, n=5; hWT_{cs}=0.634±0.103, n=5; normalized to PBS; One-way ANOVA F (3, 15) = 1.49, p=0.2575).

n numbers listed here represent mouse number. Only significant or close to significant pairwise comparisons were labeled in the figures. Fisher's LSD post hoc test. Data shown are mean ± SEM. The level of significance was set as

* $p<0.05$.

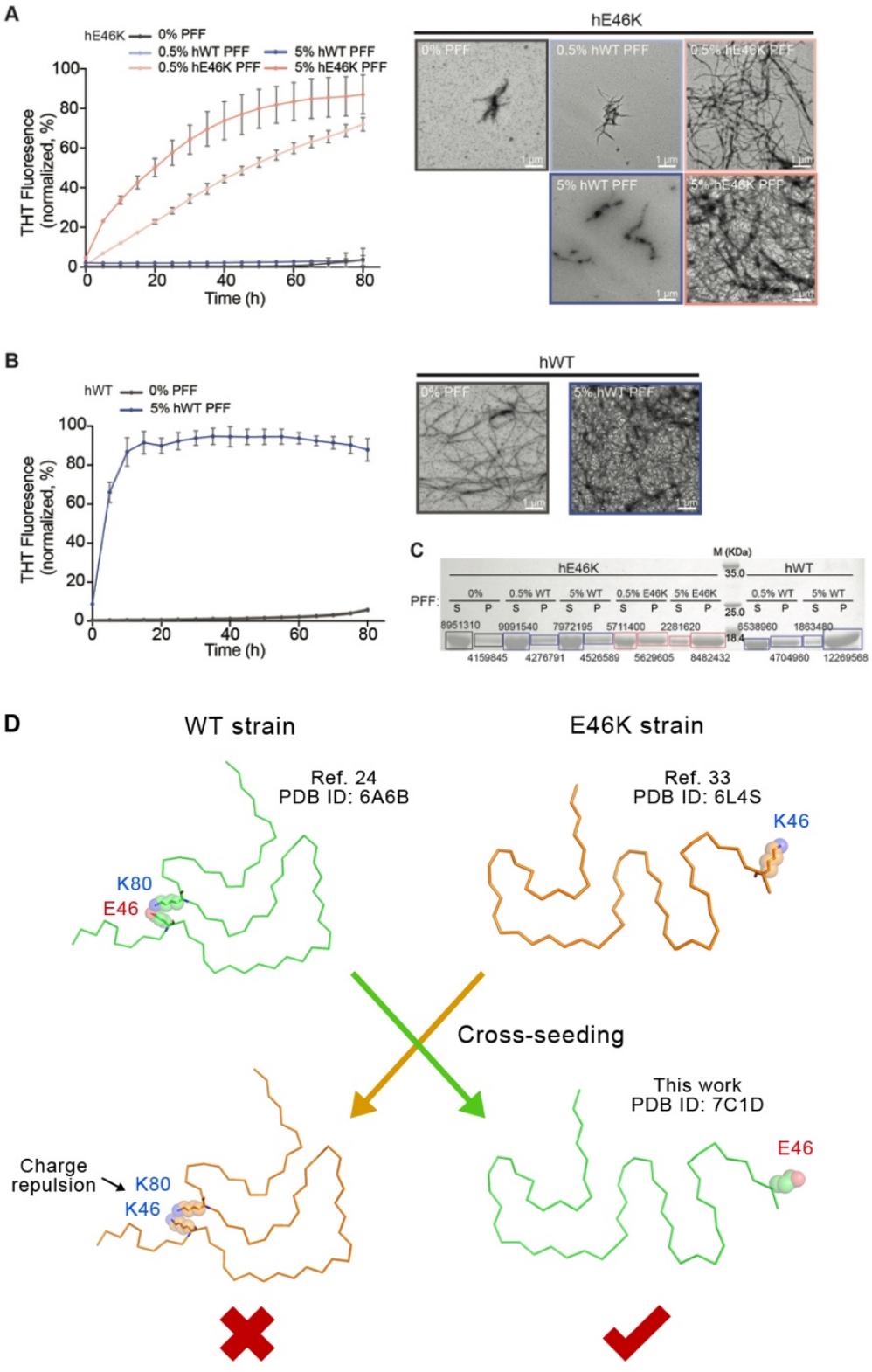


Fig. S6. Mutual seeding of WT and E46K α-syn.

(A) ThT kinetic assay for human E46K α-syn monomer seeded by the hWT and hE46K PFFs, respectively (left). The concentrations of PFFs are indicated. Data

shown are mean ± s.d., n=3 individual independent samples. The experiment was repeated for at least 3 times. The samples collected in the end of the ThT assay were imaged by negative-staining TEM (right). Scale bar: 1 μm. (B) ThT kinetic assay for human WT α-syn monomer self-seeded by the hWT PFF (left). The experiment was performed as a control for (A) to confirm the activity of the hWT PFF. The concentrations of PFFs are indicated. Data shown are mean ± s.d., n=3 individual independent samples. The samples collected in the end of the ThT assay were imaged by negative-staining TEM (right). Scale bar: 1 μm. (C) SDS-PAGE for the fibril samples collected in the end of the ThT assays in (A) and (B). The intensities of the protein bands were analyzed with Image Lab 3.0 (Bio-Rad). S, supernatant; P, pellet; M, marker. (D) Structural demonstration of the mutual seeding of WT and E46K α-syn. The WT monomer is colored in green; the E46K monomer is in orange. Overall structures are shown in ribbon. E46 (the mutation site) and K80 (the residue that forms a salt bridge with E46) are shown in sticks and spheres. Residue labels: blue for positive charge; red for negative charge.

Table S1 Statistical information for Figure 1.

Figure 1			
Figure 1B, left	PBS	hWT	hE46K
P summary	Two-way ANOVA, F (2,32) = 9.44, p=0.0006		
Figure 1B, right	PBS	hWT	hE46K
n	11	10	11
mean±SEM	100.00±7.31	102.60±8.33	64.42±8.00
P summary	One-way ANOVA, F (2,29) = 7.439, p = 0.0025 Data were normalized to PBS		
Figure 1C	PBS	hWT	hE46K
n	9	8	8
mean±SEM	1.89±0.08 s	2.15±0.13 s	2.88±0.21 s
P summary	One-way ANOVA, F (2,22) = 12.78, p = 0.0002		
Figure 1E	PBS	hWT	hE46K
n	5	5	5
mean±SEM	1.35±0.633 /mm^2	332±33.4 /mm^2	298±158 /mm^2
P summary	One-way ANOVA, F (2,12) = 3.83, p = 0.0519		
Figure 1F, insert		hWT	hE46K
n		5	5
mean±SEM		4.403 ±0.3604 μm^2	3.76±0.1846 μm^2
Figure 1H	PBS	hWT	hE46K
n	5	5	5
mean±SEM	1.45±0.666 /mm^2,	14.8±4.34 /mm^2	35.4±5.42 /mm^2
P summary	One-way ANOVA, F (2,12) =18.0, p=0.0002		
Figure 1I, insert		hWT	hE46K
n		5	5
mean±SEM		5.415±0.6782 μm^2	6.153±0.5510 μm^2
Figure 1J	PBS	hWT	hE46K
n	5	5	5
mean±SEM	54.39±2.425	37.47±2.985	39.4±2.312
P summary	One-way ANOVA, F (2,12) = 12.79, p = 0.0011		

Table S2 Statistical information for Figure 2.

Figure 2			
Figure 2B	PBS	hWT	hE46K
n	6	7	5
mean±SEM	14±12.1 /mm^2	444±76.3 /mm^2	1007±298 /mm^2
P summary	One-way ANOVA, F (2, 15) =9.96, p = 0.0018		
Figure 2C, insert		hWT	hE46K
n		7	6
mean±SEM		8.087±1.122 μm^2	4.716±0.3665 μm^2
P summary	Mann-Whitney test, p = 0.0140		
Figure 2E	PBS	hWT	hE46K
n	5	6	5
mean±SEM	10±4.08 /mm^2	173±38 /mm^2	255±81.2 /mm^2
P summary	One-way ANOVA F (2,13) = 5.77, p = 0.0161		
Figure 2F, insert		hWT	hE46K
n		6	5
mean±SEM		11.87±0.8087 μm^2	10.02±0.8272 μm^2
Figure 2G	PBS	hWT	hE46K
n	5	6	5
mean±SEM	83.5±6.94	55.1±5.22	48.8±1.97
P summary	One-way ANOVA, F (2,13) = 12.1, p = 0.0011		

Table S3 Statistical information for Figure 6.

Figure 6				
Figure 6A	PBS	hWT	hE46K	hWT_{cs}
n	12	12	12	12
mean±SEM	154.30±22.35 s	115.60±15.46 s	81.48±4.48 s	87.50±7.45 s
P summary	One-way ANOVA, F (3,44) = 5.429, p = 0.0029			
Figure 6B, left	PBS	hWT	hE46K	hWT_{cs}
n	11	12	12	12
mean±SEM	136.3±19.04 s	71.77±4.97 s	77.68±3.011 s	77.84±4.185 s
P summary	One-way ANOVA, F (3,43) = 9.575, p < 0.0001			
Figure 6B, right	PBS	hWT	hE46K	hWT_{cs}
n	11	12	12	12
mean±SEM	2.228±0.0615 s	3.962±0.2242 s	3.334±0.2647 s	4.055±0.2916 s
P summary	One-way ANOVA, F (3,43) = 12.55, p<0.0001			
Figure 6D, left	PBS	hWT	hE46K	hWT_{cs}
n	4	5	5	5
mean±SEM	41.3±7.71 /mm^2	496±160 /mm^2	2386±571 /mm^2	1599±468 /mm^2
P summary	One-way ANOVA, F (3, 15) = 6.87, p = 0.0039			
Figure 6D, right	PBS	hWT	hE46K	hWT_{cs}
n	4	5	5	5
mean±SEM	0.333±0.109	1±0.309	3.71±0.928	2.64±0.710
P summary	One-way ANOVA, F (3, 15) = 5.58, p = 0.0089 Normalized to hWT			
Figure 6E	PBS	hWT	hE46K	hWT_{cs}
P summary	hWT vs hE46K, p<0.0001; hWT vs hWT_{cs}, p<0.0001; hE46K vs hWT_{cs}, p = 0.6026; K-S test			
Figure 6E, insert		hWT	hE46K	hWT_{cs}
n		5	5	5
mean±SEM		6.16±0.204 μm^2	4.65±0.216 μm^2	4.62±0.212 μm^2
P summary	One-way ANOVA F (2, 12) = 17.4, p = 0.0003			
Figure 6G, left	PBS	hWT	hE46K	hWT_{cs}
n	4	5	5	5
mean±SEM	5.51±0.751 /mm^2	179±21.2 /mm^2	485±154 /mm^2	281±86 /mm^2
P summary	One-way ANOVA, F (3, 15) = 4.30, P = 0.0223			
Figure 6G, right	PBS	hWT	hE46K	hWT_{cs}
n	4	5	5	5
mean±SEM	0.0815±0.0128	0.981±0.155	3.41±1.19	2.05±0.503
P summary	One-way ANOVA, F(3, 15) = 4.16, p = 0.0249 Normalized to hWT			
Figure 6H	PBS	hWT	hE46K	hWT_{cs}
P summary	hWT vs hE46K, p < 0.0001; hWT vs hWT_{cs}, p < 0.0001; hE46K vs hWT_{cs}, p <			

	0.0001; K-S test			
Figure 6H, insert		hWT	hE46K	hWT_{cs}
n		5	5	5
mean±SEM		11.2±1.56 μm^2	8.72±0.742 μm^2	10.8±0.582 μm^2
P summary	One-way ANOVA F (2, 12) = 1.55, p = 0.2521			
Figure 6I	PBS	hWT	hE46K	hWT_{cs}
n	4	5	5	5
mean±SEM	125±3.16	89.7±2.31	82.4±1.86	90.0±2.98
P summary	One-way ANOVA F (3, 15) = 48.5, p＜0.0001			

REFERENCE

1. Wang LQ*, et al.* (2020) Cryo-EM structure of an amyloid fibril formed by full-length human prion protein. *Nat Struct Mol Biol* 27(6):598-602.