SUPPLEMENTAL INFORMATION

Polyphosphate: A Conserved Modifier of Amyloidogenic Processes

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Supplemental Material and Methods

Proteins and Protein Purifications

Purification of CsgA was adapted from Zhou et al. (Zhou et al., 2012). Briefly, *E. coli* strain NEB3016 containing a pET11b vector carrying signal sequence-less His_6-CsgA (Hammer et al., 2012) was grown in lysogeny broth (LB) medium supplemented with 200 µg/ml ampicillin at 37°C. Expression of CsgA was induced at OD_{600} of 0.8-0.9 by addition of 0.5 mM isopropyl 1-thio-β-d-galactopyranoside (IPTG) for 1 h. Cells were harvested by centrifugation and lysed in 8 M guanidine hydrochloride (Gdn-HCl), 50 mM potassium phosphate (KPi) buffer, pH 7.4 overnight (o.n.) at 4°C. After centrifugation at 10,000 × *g* for 20 min at room temperature (RT), the supernatant was sonicated 3 x 15 sec with a Micro Ultrasonic cell disruptor (Kontes) set to 70%. The lysate was then incubated with Ni-NTA resin (Qiagen) at RT for 1 h and loaded onto a disposable polypropylene column. Unbound proteins were washed off with 50 mM KPi, pH 7.4, 10 mM imidazole. Bound CsgA was eluted with 50 mM KPi, pH 7.4 containing 125 mM imidazole. The purified protein was applied onto a desalting column (Zeba spin, ThermoScientific) equilibrated with 40 mM KPi, pH 7.5. CsgA dimers and higher oligomers were removed by centrifugation with a 30-kDa centrifugal filter unit (Millipore). All experiments were conducted with freshly prepared monomeric CsgA. To purify α-Synuclein, *E. coli* strain BL21(DE3) containing the α-Synuclein-expressing vector pT7-7 (Jain et al., 2013) was grown in LB, supplemented with 200 µg/ml ampicillin until OD_{600} of 0.8-1.0 was reached. Protein expression was induced with 0.8 mM IPTG for 4 h. Cells were harvested by centrifugation (4,500 *x g*, 20 minutes, 4°C). The protein was purified according to Jain et al. (Jain et al., 2013) with minor modifications. In short, the cell pellet was resuspended in 50 ml lysis buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) with Roche Complete protease inhibitor cocktail. The lysate was boiled for 15-20 minutes and aggregated proteins were removed by centrifugation (13,500 *x g*, 30 minutes). The cleared lysate was supplemented with 136 µl/ml streptomycin sulfate (10% w/v solution) and 228 µl/ml glacial acetic acid. After centrifugation at 13,500 *x g* for 30 minutes, the supernatant was mixed at a 1:1 ratio with saturated ammonium sulfate and incubated at 4°C for 1 h while mixing. After another centrifugation

step at 13,500 x *g* for 30 minutes, the pellet was resuspended in 10 mM Tris-HCl, pH 7.5, and NaOH was used to adjust the pH of the suspension, the solution was then dialyzed against 10 mM Tris-HCl pH 7.5, 50 mM NaCl. The protein solution was filtered and loaded onto two connected 5 ml HiTrap Q HP columns (GE Healthcare). The columns were washed with 10 mM Tris-HCl pH 7.5, 50 mM NaCl and the protein was eluted using a linear gradient to 500 mM NaCl. α-Synuclein-containing fractions were pooled, concentrated and dialyzed against 20 mM KPi, pH 7.5 at 4°C o.n. Higher oligomers were removed by filtering the protein through a 50-kDa cut-off column (Amicon, Millipore). Aliquots of the protein were prepared, lyophilized and stored at -80°C. The expression and purification of $A\beta_{1-40}$ was adapted from (Finder et al., 2010). The plasmid pRSET containing the His_6 - $A\beta_{1-40}$ - $(NANP)_{19}$ fusion peptide was a kind gift from R. Glockshuber (Finder et al., 2010). Freshly transformed E. c*oli* BL21 (DE3) cells harboring pRSET were grown at 37°C in Terrific Broth medium (12 g/l tryptone, 24 g/l yeast extract, 4 g/l glycerol, 17 mM KH_2PO_4, 72 mM K_2HPO_4, pH 7.2) containing 200 µg/ml ampicillin until an OD_{600} of ~3 was reached. Protein expression was induced by addition of 1 mM IPTG for 4 h. The cells were harvested and resuspended in buffer C (20 mM KPi, 6 M Gdn-HCl, and 0.3 M NaCl, pH 8.0), incubated 90 min at 4°C and then centrifuged at 49,000 x *g*, 1 h, 4°C. The supernatant was loaded onto a 5 ml Ni^{2+}-NTA agarose column (GE Healthcare) equilibrated with buffer C. After washing the column with buffer C containing 10 mM imidazole, the fusion protein was eluted with a linear gradient to 250 mM imidazole. Fractions containing the fusion protein were dialyzed against Buffer D (10 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 1 mM DTT) at 4°C. After 2 h of dialysis, the protein was transferred into a dialysis tube with 500 Da cut off (SpectraPor) and tobacco etch virus (Sievers et al.) protease (see below for purification) was added in a molar ratio of 100:1 (Aβ:TEV). Dialysis and cleavage were continued o.n. at 4°C. After cleavage, all aggregated proteins were removed by centrifugation at 16,000 *x g*, 30 min, 4°C. The cleaved peptide was further purified using a reversed-phase high-performance liquid chromatography (HPLC) column (Kromasil 300-5C8 250 x 10 mm) with a linear gradient from 20-60% acetonitrile, 0.1% trifluoroacetic acid with a flow rate of 1.5 ml/min. Fractions containing the $A\beta_{1-40}$ peptide were combined, aliquoted, lyophilized, and stored at − 80°C. $A\beta_{1-42}$ was purchased from AnaSpec Inc, dissolved in HFIP, aliquoted, dried in SDD SpeedVac

(Thermo Electron), and stored at − 80°C. Purification of hTau40 from *E. coli* BL21(DE3) strains carrying the expression vector pNG2 (pET3b vector containing the hTau40 gene) (Barghorn et al., 2005) was performed as described previously (Sievers et al., 2011). pNG2 carrying hTau40 was a kind gift from M. Ivanova. Purified Tau was concentrated using a 10-kDa centrifugal filter unit (Millipore) and dialyzed o.n. against 40 mM KPi, 50 mM KCl, pH 7.5. Aliquots were stored at -80°C. The TEV protease purification was modified from Keogh et al.(Keogh et al., 2006). His_6-tagged TEV^{S219N} protease was expressed from a pTPSN plasmid (Lucast et al., 2001) in *E. coli* BL21(DE3) cells. Cells were grown in LB medium and expression was induced with 0.1 mM IPTG once OD_{600} of 0.4 was reached. After 4 h incubation at RT, cells were harvested and resuspended in buffer E (50 mM Tris, 150 mM NaCl, 10 mM imidazole, 5 mM β-mercaptoethanol, 150 µM PMSF, pH 8.0). Then, 10 µl DNase I (1 mg/ml) was added. Cells were lysed using a french press cell (2 cycles at 1,300 PSI) and the lysate was cleared of debris by centrifugation (36,000 x g, 30 min, 4°C). The supernatant was applied onto a 5 ml Ni^{2+}-NTA agarose column (GE Healthcare) equilibrated with buffer E. The column was washed with buffer E containing 20 mM imidazole and TEV protease was eluted with 500 mM imidazole in buffer E. Imidazole was removed by dialysis against buffer E before the protein was re-applied onto a 5 ml Ni^{2+}-NTA agarose column (GE Healthcare) equilibrated with buffer E. Unspecific bound protein was washed off with a step-wise gradient (50, 75, 100 mM imidazole in buffer E) before the protease was eluted with 500 mM imidazole in buffer E. TEV protease was dialyzed against 50 mM Tris, pH 8, 5 mM DTT, 400 mM NaCl,1 mM EDTA, 5% Glycerol, aliquoted and stored at -80°C. PPK purification was performed as described in Gray et al. (Gray et al., 2014).

Polyphosphates and polyphosphate labeling

Short heterogeneous polyP ($polyP_{SH}$; average length ~50 P_i) (Acros) was dissolved in water or nematode growth media before use. For terminal labeling of polyP chains, 250 µM $polyP_{300}$ chains were incubated with 2.5 mM Alexa Fluor 647 cadaverine (Life Technologies) and 150 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDAC) (Invitrogen) in water for 2 hr at 60°C. Labeled polyP

(polyP$_{300}$-AF^{647}) was separated from unlabeled polyP and free dye on a NAP-5 column (GE Healthcare) equilibrated with 40 mM KP$_i$, pH 7.5 (Choi et al., 2010).

Determination of CsgA (curli) concentration *in vivo*

For quantification of *in vivo* curli formation, western blot analysis of CsgA were performed as described in Zhou et al. (Zhou et al., 2013) with the following modifications: bacteria were scraped off the YESCA plates containing either no polyP or 0.1 mM polyP$_{SH}$-chains. HFIP was used to solubilize CsgA fibrils, proteins were separated on a 12% TGX SDS-Page and transferred on nitrocellulose membrane, and westernblot analysis using anti-CsgA antibody were performed to visualize CsgA. The housekeeping σ^{70}-factor was detected using anti-σ^{70} antibody and used as loading control.

Circular dichroism

Secondary structure of proteins was monitored using far-UV circular dichroism (CD) spectroscopy. In general, about 0.2 mg/ml of the test proteins were incubated in 20 mM KPi pH 7.5 buffer and spectra were recorded using a Jasco-J810 spectropolarimeter. To determine the conformational changes in citrate synthase upon temperature increase, the spectra were recorded at either 30°C or after heating (1°C/min) of the sample to 60°C. In addition, one heated sample was centrifuged (20,000 x g, 20 min, 4°C) and the soluble supernatant was analyzed. The secondary structure content was determined using CDSSTR (Dichroweb) using reference set 4 for analysis. All spectra were buffer corrected.

Thioflavin T fluorescence to monitor fibril formation

15 µM CsgA was incubated in 40 mM KPi, pH 7.5 at 25°C. The samples were shaken for 15 sec before each reading. 100 µM α-Synuclein was incubated in 40 mM KPi, 100 mM KCl, pH 7.5 at 37°C under continuous shaking using two 2 mm borosilicate glass beads (Aldrich) as nucleation source. Aβ_{1-40} and Aβ_{1-42} were first treated with hexafluoroisopropanol (HFIP) to disrupt any pre-existing oligomers. After removal of HFIP using a SDD SpeedVac (Thermo Electron), the Aβ_{1-40} and Aβ_{1-40} peptides were dissolved in 10 mM NaOH. Polymerization was conducted using 20 µM Aβ_{1-40} or 8 µM Aβ_{1-42} in 40 mM KPi, 50 mM KCl, pH 7.5. The samples were shaken for 15 sec before each reading at

37°C. Fibrillation of hTau40 was induced by incubating 100 µM Tau in 40 mM KPi, 50 mM KCl, pH 7.5. The samples were shaken for 15 sec before each reading at 37°C. The effects of polyP, heparin from porcine intestinal mucosa (molecular weight 17–20 kDa; Sigma-Aldrich), DNA (Sigma-Aldrich, herring testes), RNA (Sigma-Aldrich, TypeVI from torula yeast), or arginine (MPbiomedicals) were determined by adding them to the polymerization reactions prior to incubation. Fluorescence measurements were set up in black 96-well polystyrene microplates with clear bottoms (Greiners), or 384 black polystyrene microplates (NUNC) and fluorescence was detected in a Synergy HT Multi-Mode Microplate Reader (Biotec) exciting at 430 nm and measuring emission at 485 nm.

TEM Analysis

Samples were imaged under low dose conditions using a G2 Spirit transmission electron microscope (FEI) operated at 120 keV. Micrographs were taken at 52,000x magnification with 2.16 Å per pixel using a 4k x 4k CCD camera (Gatan). Individual filaments were boxed out of micrographs and aligned using the helixboxer program in EMAN2 (Tang et al., 2007). 838 α-Synuclein particles and 1432 α-Synuclein^{polyP} particles were used to generate 15 and 20 2D class averages, respectively using EMAN2. The width of the fibril in the class average was measured and reported based on the fraction of total particle images that went into each average.

Proteinase K stability of α-Synuclein fibrils

α-Synuclein fibrils (200 µM) in the absence of ThT were produced in the absence or presence of 40 µM polyP$_{SH}$. Fibrils were collected by centrifugation at 20,000 x g, 20 min, RT. The pellet was washed twice with 40 mM KPi, 50 mM KCl, pH 7.5 to remove smaller oligomers. After the final spin, the pellet was resuspended in 40 mM KPi, 50 mM KCl, pH 7.5. A small aliquot was taken and the fibrils were dissolved in 8 M Gdn-HCl in 40 mM KPi, 50 mM KCl, pH 7.5 to determine protein concentration. To determine proteinase K stability of the fibrils, 1.4 mg/ml amyloid fibrils were incubated with 3.18 µg/ml proteinase K (ICN Biomedicals) in 20 mM Tris-HCl, 1 mM EDTA, pH 7.5 (Bousset et al., 2013). At various time points, 10 µl of the sample was withdrawn, boiled in the presence of 2 x SDS-Tricine reducing buffer for 10 min, and analyzed using 16% SDS-tricine peptide gels (Schagger, 2006).

Quantification of ThT fluorescence, protein solubility, and polyP segregation of α-Synuclein

100 µM α-Synuclein was incubated in the presence of various concentration of $polyP_{SH}$ for 24 h at 37°C. After polymerization, the soluble and insoluble material was separated by centrifugation (20,000 x g, 20 min, RT). The pellet fraction was resuspended in an equal amount of buffer compared to the supernatant. For ThT measurements, 10 µl of the sample were supplemented with 10 µM ThT in 40 mM KPi, pH 7.5, 50 mM KCl in black 96-well polystyrene microplates with clear bottoms (Greiners), and fluorescence was measured in a Synergy HT Multi-Mode Microplate Reader (Biotec) exciting at 430 nm and measuring emission at 485 nm. For quantification of solubility, 15 µl of each sample was supplemented with 2 x SDS-reducing buffer, analyzed on SDS-Page, and proteins were visualized by Coomassie blue staining. Band intensity was determined with a LI-COR biosciences system. The polyP content in each sample was determined upon converting polyP to ATP and measuring ATP levels using a luciferase based assay (see below).

***Caenorhabditis elegans* strains, cultivation, polyP uptake and analysis of paralysis in GM101**

The following *C. elegans* strains were used in this study: wild-type N2, GMC101 (dvIs100 [unc-54p::$A\beta_{1-42}$::unc-54 3'-UTR + mtl-2p::GFP]) and its transgenic control strain CL2122 (dvIs15 [pPD30.38 (unc-54 vector) + mtl-2::GFP (pCL26)]) (McColl et al., 2012), SJ4005 zcls4 ([hsp-4::GFP; lin-15(n765)]) and CL2017 dvls70 ([hsp-16.2p::GFP + rol-6(su1006)]). If not stated otherwise, worms were propagated at 20°C. Synchronization was performed using alkaline hypochlorite solution, and arrested L1 larvae were transferred onto NGM (O'Nuallain et al.) plates covered with a lawn of 10^{10} cells/ml *E. coli* OP50 to continue development and growth. For experiments involving polyP treatments, heat-killed OP50 (1 h in ~80°C water bath) were used. To determine polyP uptake, about 12 µl of worm pellet containing synchronized N2 wild-type L3 larvae was incubated in 100 µl NGM buffer with or without 1 µM $polyP_{300}$-AF^{647} for 30 min at 20°C and constant mixing at 1,000 rpm. Worms were washed twice with NGM buffer to remove unincorporated $polyP_{300}$-AF^{647}. Five µl of worm pellet was mounted on an objective slide using 4 µl thermoreversible CyGEL (BioStatus; Fisher Scientific) and 2 µl 50 mM levamisole for immobilization. Z-stack imaging was performed on a Leica

SP5 laser scanning microscope (Leica GmbH) on a DM6000B microscope base using LAS AF v2.4.1 build 6384 software, with a triple-dichroic 488/561/633 beam splitter and a 633 nm HeNe laser with 640–777 nm spectral detection, and using a 40X objective. The z-stacks were assembled in ImageJ and the z-projection was performed with all the z-stacks in which Alexa647 signal was visible; the same z-stacks were used for the corresponding brightfield images. Brightness and contrast were adjusted to be the same in worms treated with or without $polyP_{300}$-AF^{647}. To determine the effects of polyP on worms expressing $A\beta_{1-42}$, GMC101 and its transgenic control strain CL2122 were grown at 20°C on NGM plates seeded with live OP50 bacteria. At L4 larval stage/young adult stage, worms were transferred into 1.5 ml Eppendorf tubes with fresh NGM media (total volume: 500 µl) supplemented with 100 µl OP50 and 0, 1, or 5 mM $polyP_{SH}$ and incubated for 5.5 h at 25°C under constant shaking (1,000 rpm). After incubation, worms were placed onto 6 cm NGM plates containing a lawn of OP50. After 24 h of incubation at 25°C, the movement ability of the worms was assessed. Worms were scored as paralyzed when they failed to respond with movement after tapping the plate or gently prodding them. The percent that were paralyzed was determined for the untreated and polyP-treated worms. The control strain CL2122 was used to assess the effects of polyP on movement ability. To determine the effect of polyP on the heat shock response *C. elegans* L3-L4 larvae of the SJ4005 zcIs4 (Hsp4) or adult dvls70 (Hsp16) reporter strain were incubated in NGM-buffer with or without 5 mM $polyP_{SH}$-chains for 5 h at 20°C. After the incubation, worms were imaged using an Olympus fluorescence microscope. The mean GFP-fluorescence of the whole worm was determined. To monitor the influence of polyP on the lifespan of N2 wild type worms, synchronized L4 larvae were incubated in 500 µl M9 media supplemented with heat killed OP50 in the absence or presence of 1 mM polyP for 12h. After the treatment, the worms were transferred onto NGM plates containing 20 mg/ml 5'-fluoro-2'-deoxyuridine (FUdR). Life span was followed at 20°C and animals that did not react to a poke with the worm pick were considered to be dead. Results were analyzed with a Log-Rank test.

α-Synuclein and Aβ – Expression, and Secretion

CHO cells stable expressing APPswe/PS1ΔE9 were kindly provided by Y. Wang (University of Michigan) and were cultured in F12K media, containing 10% FBS, 1% P/S. The media was exchanged every 2 days. Cells were cultured in a humidified incubator at 37°C, 5% CO_2 and split when about 90% confluence was reached. To capture secreted Aβ, cells were split into 6 well plates. Once the cells reached about 70% confluence, the media was exchanged and reduced to 1.25 ml. Then, secretion of Aβ was allowed for 48 h in the absence or presence of 20 µM $polyP_{SH}$. Afterwards, the supernatant was recovered and cleared by centrifugation (1,000 x g, 5 min, RT) for subsequent cytoxicity assays. HeLa were cultured in DMEM, containing 10% FBS, 1% P/S using a humidified incubator at 37°C, 5% CO_2. The media was exchanged every 2 days. Cells were split when 90% confluence was reached. For expression of α-Synuclein, HeLa cells were transiently transfected with pCDNA3.1, a plasmid harboring the α-Synuclein sequence (a kind gift of T. Outerio, University of Göttingen), using the lipofectamine 3000 method (ThermoFisher Scientific) and following the manufacturer's protocol. Efficiency of transfection was tested and verified to be around 85%. 24 h after transfection, the media was replaced and secretion of α-Synuclein was allowed for 48 h in the absence or presence of 20 µM $polyP_{SH}$. Afterwards, the spent media was recovered and cleared by centrifugation (1,000 x g, 5 min, RT) for subsequent cytoxicity assays. To ascertain that presence of $polyP_{SH}$ in the media does not affect α-Synuclein secretion, samples were taken at the indicated time points and spent media was prepared as before. The samples were then separated on TGX gels (BioRad) and α-Synuclein was visualized using mouse anti-α-Synuclein antibodies (BD; 1:1500). Ponceau red staining was conducted as loading control.

Cytotoxicity Assays using differentiated SH-SY5Y cells

Human neuroblastoma cells SH-SY5Y cells (ATCC CRL-2266) were cultured in DMEM/F12 medium supplemented with 10% (v/v) fetal bovine serum, 1% (w/v) penicillin/streptomycin, using a humidified incubator at 37°C, 5% CO_2. Media was exchanged every 2–3 days and cells were split 1–2 times per week. For differentiation, 1.25 x 10^5 cells/ml were cultured overnight in complete media. Media was

subsequently exchanged for DMEM/F12 media, 3% FBS, 1% P/S, and 10% all trans retinoic acid. Differentiation was achieved over 4-5 days, with media changes every day. To determine the toxicity of secreted α-Synuclein or Aβ in the absence or presence of polyP (see Supplemental Experimental Procedures for α-Synuclein or Aβ expression and secretion), the cleared spent media was added to the differentiated cells so that the spent media was 1/5th of the total media volume. Cells were incubated for 40 h, after which survival was determined. To determine the toxicity of pre-formed or defibrillated α-Synuclein, α-Synuclein fibrils were formed for 24 h in the absence or presence of 20 μM polyP$_{SH}$. Fibrils were collected by centrifugation, and washed twice to remove soluble material. Defibrillation was conducted at a concentration of 50 μM fibrils in terms of monomer in the presence or absence of 20 μM polyP$_{SH}$ at 4°C for 24 h. 10 μM of either pre-formed fibrils or defibrillated species were added to differentiated human cells SH-SY5Y and toxicity was determined after 40 h. To determine survival, cells were harvested by centrifugation (10 min, 200 x*g*, RT), and the cell pellet was dissolved in 20 μl complete cell culture medium. Then, 20 μl of a trypan blue solution (HyClone) was added and cells were counted in a haemocytometer 2–5 min later. Most of the counting was performed blinded.

Amyloid cytotoxicity assays with PC-12 cells

Rat adrenal gland cells (PC-12) were cultured in RPMI-1640 medium (Life Technologies) supplemented with 15% (v/v) fetal bovine serum, 2% (v/v) horse serum, 1% (w/v) penicillin/streptomycin, using a humidified incubator at 37°C, 5% CO_2. Media was exchanged every 2–3 days and cells were split 1–2 times per week. For determination of the cytotoxicity of amyloids, cells were split 24 h before treatment into 96-well cell culture plates (Corning) with a density of 12,000 cells/well. To determine the toxicity of α-Synuclein on PC-12 cells, fibrils were formed in the absence or presence of polyP$_{SH}$ as described above, and added at the indicated concentrations to cells pre-incubated in 96-well plates for 24 h prior to the treatment with amyloids. To investigate the effects of polyP$_{SH}$ pre-incubation on cell survival, cells were treated for 1 h with the indicated concentrations of polyP$_{SH}$ before amyloidogenic proteins were added. The cytotoxic effect of amyloids was monitored

40 h after amyloid treatment by trypan blue staining. Cells were harvested by centrifugation (10 min, 200 *g*, room temperature) and the cell pellet was dissolved in 20 µl complete cell culture medium. 20 µl of a trypan blue solution (HyClone) was added and the cells were counted in a haemocytometer 2–5 min later. Most of the counting was performed as a blinded experiment.

Alzheimer's Disease mouse model

The hemizygous transgenic PDAPP [hAPP(J20)] mice carrying the Swedish and Indiana familial Alzheimer's disease mutations ($hAPP_{Sw,Ind}$) in a human APP minigene driven by a neuron-specific promoter activated at embryonic day 14 and non-transgenic littermate (control) mice were generated by crosses with C57Bl/6J mice (Jackson Laboratories). Hemizygous crosses were set up such that the transgenic animal was the dam or the sire in ~50% of the breeding pairs to minimize confounds related to potential effects of transgene expression during gametogenesis, or imprinting effects.

PolyP determination after fibril formation and in AD mouse brain homogenates

The level of polyP in brain LN_2 homogenates was determined using a modified method originally developed by Kumble and Kornberg 1995 (Kumble and Kornberg, 1995). Tissue samples were added to 260 µl GITC buffer (4 M guanidine isothiocyanate, 50 mM Tris-HCl, pH 7.0), and the samples were homogenized using a PowerGen 125 (Fisher Scientific) for 30 sec, boiled 10 min at 95°C, and sonicated for 15 sec using a Micro Ultrasonic cell disruptor (Kontes) set to 70%. To remove insoluble material, the samples were centrifuged (20,000 x g, for 30 min, RT). A 10 µl aliquot of the cleared supernatant was used to determine the protein concentration (BioRad). The remaining sample was used to measure the polyP content. To extract polyP, 15 µl 10% (w/v) SDS, 0.25 ml 95% (v/v) ethanol, and 7.5 µl glass milk (Sigma) (Boyle and Lew, 1995) were added to the samples. Between each addition, the samples were heavily vortexed. The solution was transferred into an EconoSpin column (Epoch Lifescience) and centrifuged (3,000 x g, 5 min, RT). The flow-through was discarded and 0.6 ml NW Buffer (5 mM Tris-HCl, pH 7.5, 50 mM NaCl, 5 mM EDTA, 50% (v/v) ethanol) was added. The solution was centrifuged (3000 x g, 5 min RT). The washing step was repeated twice. To dry the membrane, a fast spin was performed (8,500 x g, 3 min, RT). The column was transferred into

a 1.5 ml microcentrifuge tube and polyP was eluted by incubating the filter in 40 µl 50 mM Tris-HCl, pH 8.0 for 15 minutes at RT. PolyP was collected using a final spin at 8,500 x g, 5 min, RT. To digest polyP into ATP, 10 µl of the sample was incubated in a total volume of 40 µl in 50 mM Hepes, pH 7.5, 50 mM ammoniumsulfate, 5 mM $MgCl_2$, 30 µM ADP in the presence or absence of 50 nM *E. coli* PPK (purified as described(Gray et al., 2014)) at 37°C for 60 min. The solution was then heated to 95°C to inactivate PPK. Generated ATP was detected using a luciferase/luciferin-based system. Here, 10 µl sample was transferred into white 96 well plates (Corning) and 90 µl luciferase buffer (50 mM Tricine buffer, pH 7.8, 10 mM $MgSO_4$, 0.2 mM EDTA, 0.2 mM sodium azide, 1 mM DTT, 100 µM luciferin, 25 nM luciferase) was injected into each well. The samples were shaken for 5 sec and luminescence was detected using an Omega Fluorstar plate reader. To calculate the amount of polyP in each sample, values obtained from samples digested in the absence of PPK were subtracted from the values of samples digested in the presence of PPK. A polyP standard, which was processed the same way as the sample (including extraction) was used to calculate the polyP concentration. Finally, the amount of polyP was normalized to the amount of protein in the sample.

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Supplemental Table

Table S1: Influence of defined-length polyP chains on $T_{1/2}$ of fiber formation (related to Figure 2).

A. α-Synuclein (100 µM)

Concentration [µM chain]	T½ [h] in $polyP_{16}$	T½ [h] in $polyP_{60}$	T½ [h] in $polyP_{130}$	T½ [h] in $polyP_{300}$
0	21.0 +/- 2.3	21.0 +/- 2.3	21.0 +/- 2.3	21.0 +/- 2.3
1	14.5 +/-0.9	3.2 +/-0.3	2.4 +/- 0.2	2.0 +/- 0.7
5	6.5 +/- 0.8	3.1 +/- 0.2	2.7 +/- 0.2	3.5 +/- 0.8
10	5.5 +/- 0.5	2.7 +/- 0.2	2.5 +/- 0.2	3.9 +/- 1.2

B. $A\beta_{1-40}$ (20 µM)

Concentration [µM chain]	T½ [h] in $polyP_{16}$	T½ [h] in $polyP_{60}$	T½ [h] in $polyP_{130}$	T½ [h] in $polyP_{300}$
0	14.1 +/- 0.6	14.1 +/- 0.6	14.1 +/- 0.6	14.1 +/- 0.6
1	10.7 +/- 1.4	10.1 +/- 0.4	11.9 +/- 0.6	12.8 +/- 0.8
5	10.7 +/- 0.8	7.8 +/- 1.0	8.6 +/- 1.3	11.6 +/- 0.1
10	10.4 +/- 0.6	8.1 +/- 1.3	7.9 +/- 1.0	11.7 +/- 0.7

C. $A\beta_{1-42}$ (8 µM)

Concentration [µM chain]	T½ [h] in $polyP_{16}$	T½ [h] in $polyP_{60}$	T½ [h] in $polyP_{130}$	T½ [h] in $polyP_{300}$
0	14.2 +/- 2.3	14.2 +/- 2.3	14.2 +/- 2.3	14.2 +/- 2.3
1	12.2 +/- 3.1	9.5 +/- 2.6	7.6 +/- 1.8	7.7 +/- 1.8
5	12.1 +/- 3.7	7.8 +/- 1.6	7.0 +/- 1.9	7.0 +/- 1.7
10	12.1 +/- 5.1	8.5 +/- 0.15	6.2 +/- 1.7	5.9 +/- 1.2

Sup. Figure 1

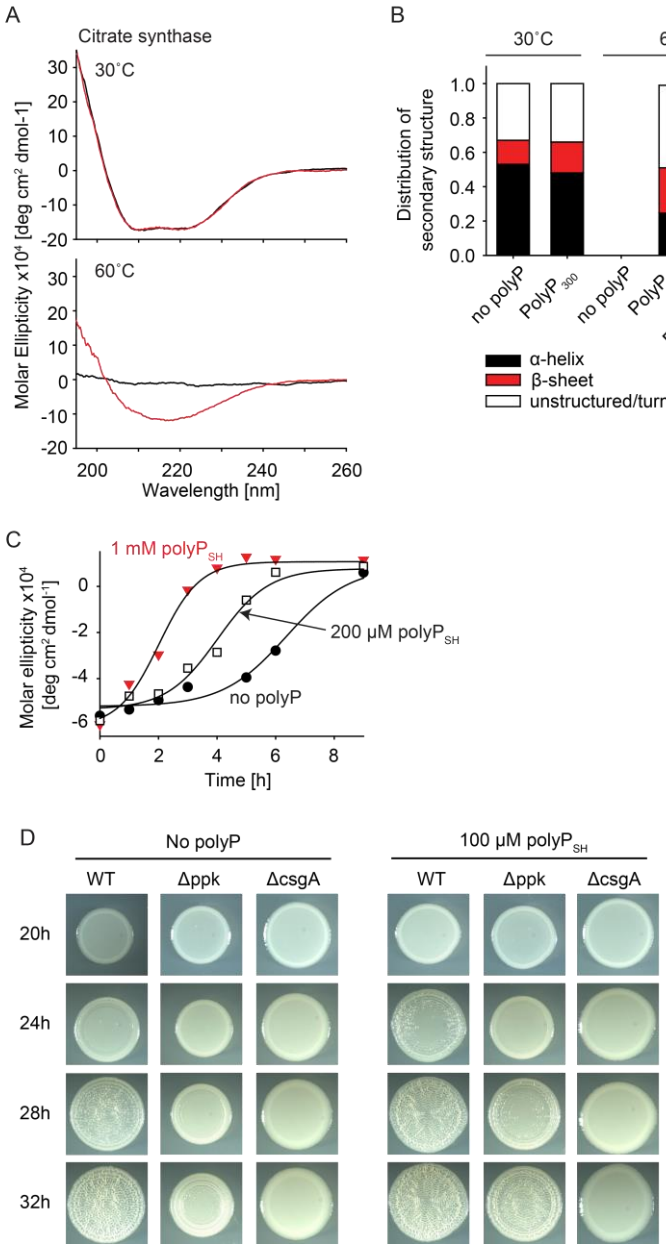

Figure S1. Influence of polyP on protein stability, β-sheet formation and biofilms (related to Figure 1).

(A) Secondary structure of citrate synthase (CS) in the absence (black line) or presence of 50 µM $polyP_{300}$-chains (red line) was monitored by CD spectroscopy at either 30°C or upon heating (1°C/min) to 60°C. (B) Secondary structure content of CS at 30°C or 60°C in the presence or absence of 50 µM $polyP_{300}$ as calculated by Dichroweb CDSSTR analysis. In addition to the samples shown in Fig. 1A, we also incubated CS in the presence of polyP for 20 min at 60°C and removed any insoluble material by centrifugation. The supernatant was then used for CD spectroscopy and subsequent secondary structure analysis ($polyP_{300}$ (SN)). (C) Changes in CsgA's secondary structure during fibril formation at 26°C. The CD signal at 220 nm of 15 µM CsgA in the absence of additives (black circles) or in the presence of either 0.2 mM $polyP_{SH}$ (open square) or 1 mM $polyP_{SH}$ (red triangle) was monitored over time. (D) Macrocolony biofilm formation of *E. coli* UTI89 wild-type, Δ*ppk* or Δ*csgA* mutant strains in the absence or presence of 100 µM $polyP_{SH}$ after the indicated time at 26°C.

Sup. Figure 2

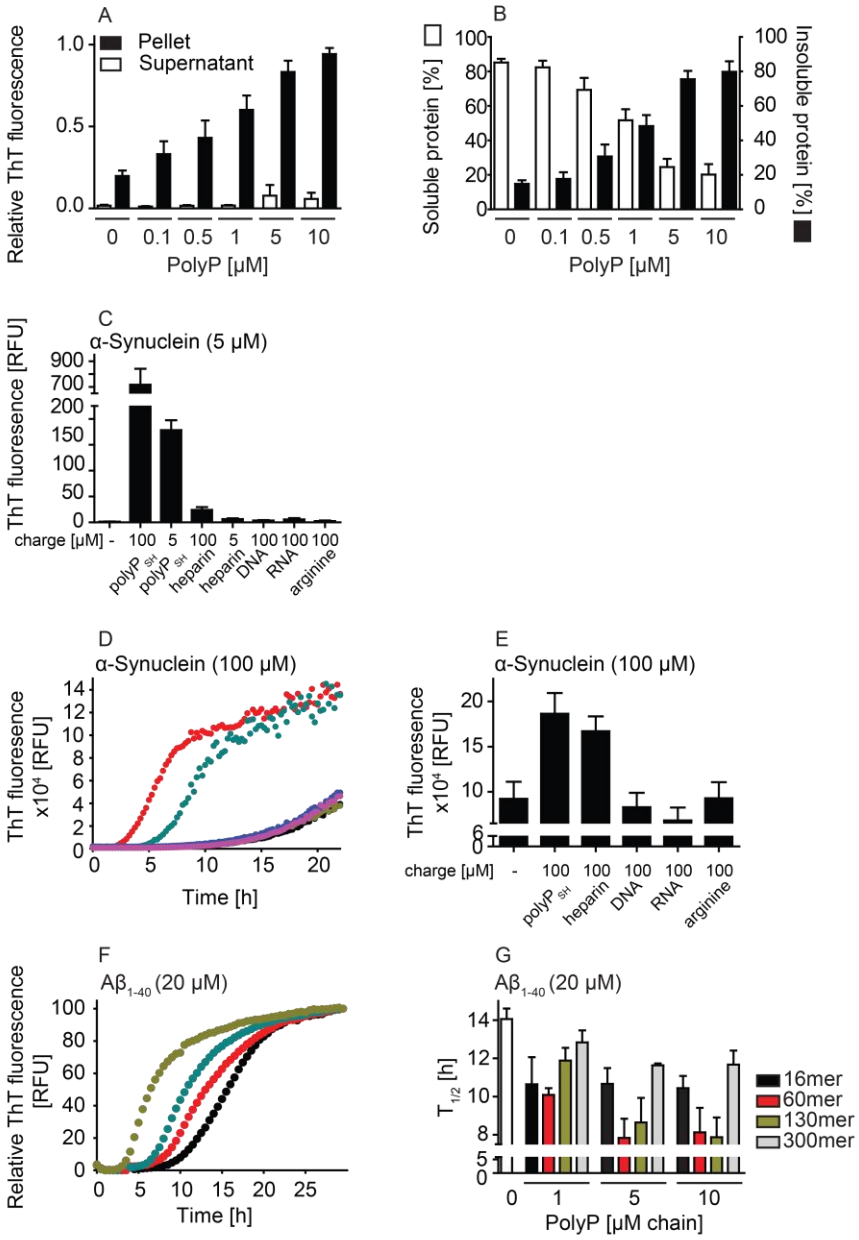

Figure S2. Influence of polyP and other polyanions on in vitro fibril formation (related to Figure 2). α-Synuclein (100 µM) was incubated in the presence of the indicated concentration of $polyP_{SH}$-chains for 24 h. Then, the soluble (SN) and insoluble (P) fractions were separated by centrifugation. Pellet fractions were re-suspended in an equal volume of buffer. (A) Samples from SN and P were taken and 10 µM thioflavinT was added. Then, thioflavinT measurements were conducted. (B) Samples of SN and P were analyzed on SDS-PAGE. Quantification of the band intensity was performed with the LI-COR imaging system. (C) ThT fluorescence of 5 µM α-Synuclein after 24h of fibrillation in the presence or absence of either 5 or 100 µM negative ($PolyP_{SH}$, heparin, DNA, RNA) or positive (arginine) charges. (D) ThT fluorescence of 100 µM α-Synuclein in the absence (black) or in the presence of 100 µM polyanions; $polyP_{SH}$ (red), heparin (cyan), DNA (green), RNA (blue), or the chemical chaperone arginine (pink). (E) Quantification of the ThT signal of 100 µM α-Synuclein after 24h of fibrillation in the presence or absence of either 100 µM negative ($PolyP_{SH}$, heparin, DNA, RNA) or positive (arginine) charges. (F) 20 µM $Aβ_{1-40}$ incubated in the absence (black) or presence of 1 µM

(red), 5 µM (cyan) or 10 µM (light green) $polyP_{60mer}$; (G) Half-time ($T_{1/2}$) of fibril formation of 20 µM $A\beta_{1-40}$ in the presence of the indicated concentrations of $polyP_{16}$ (black), $polyP_{60}$ (red), $polyP_{130}$ (green), or $polyP_{300}$ (grey) (see also Table S1). n = 4 independent experiments.

Sup. Figure 3

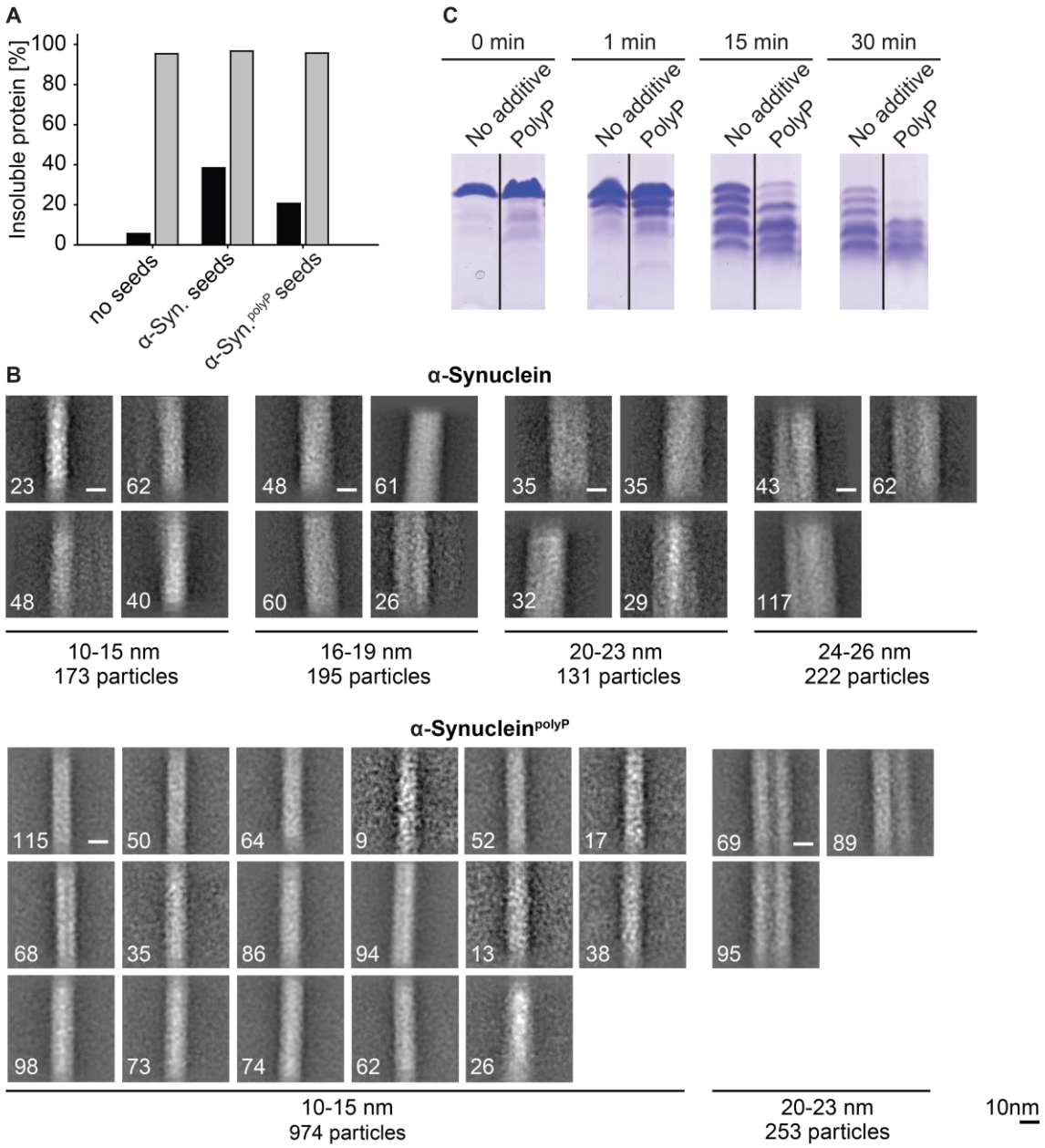

Fig. S3. PolyP-Fibril Association and the Influence of PolyP on Seeding Efficiency and Fibril Morphology (related to Figure 3).

(A) 50 µM α-Synuclein was incubated in the absence (black bars) or presence of 20 µM $polyP_{SH}$ (grey bars) either without any seeds or with 10% w/v α-Synuclein fibrils or α-$Synuclein^{polyP}$ fibrils. After 24h of incubation, insoluble α-Synuclein fibrils were separated from soluble supernatant and the proteins were visualized by SDS-PAGE. Analysis of the band intensity was conducted using a LI-COR imaging system. (B) Time-dependent proteinase K digest of 1.4 mg/ml pre-formed α-Synuclein fibrils. α-Synuclein fibrils were formed in the absence of additives or in the presence of 50 µM $polyP_{SH}$-chains and separated from soluble smaller oligomers by centrifugation. The incubation time for fibril formation was adjusted to yield in similar amounts of total fibrils. (C) 2D projection averages of α-Synuclein alone and α-$Synuclein^{polyP}$ samples, grouped by fibril width. Particle number for each average is shown in the lower left, and the scale bars equal 10 nm.

Sup. Figure 4

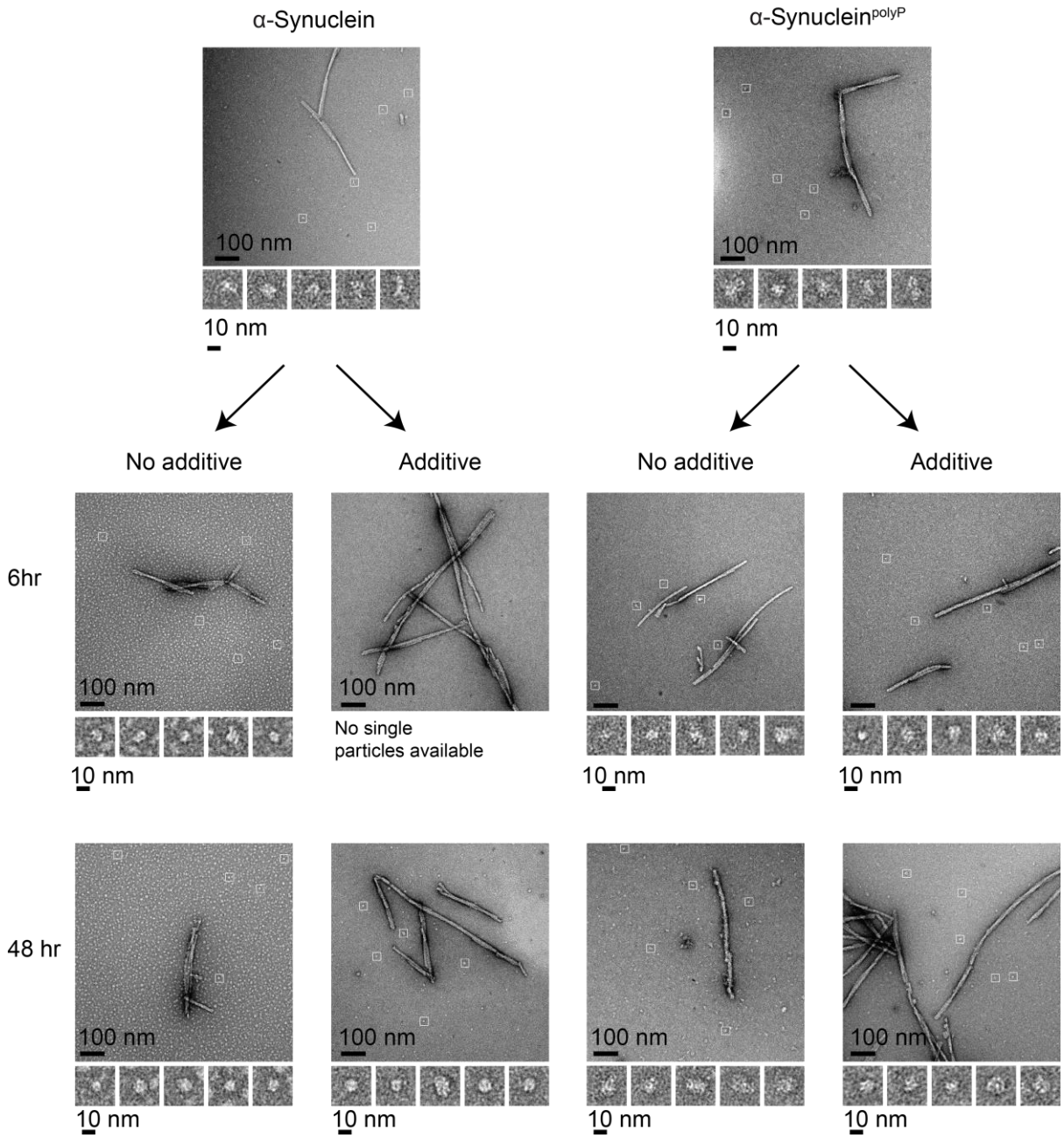

Fig. S4. PolyP Delays Shedding of Mature α-Synuclein Fibrils (related to Figure 4). Monitoring of fibril disassembly of preformed α-Synuclein or α-Synuclein^{polyP} fibrils, which were incubated in the presence or absence of 20 μM polyP$_{SH}$ at 4°C. At the indicated time points, samples were taken and analyzed by TEM. 2D projection averages of samples, grouped by condition. Scale bars equal 100 and 10 nm.

Sup. Figure 5

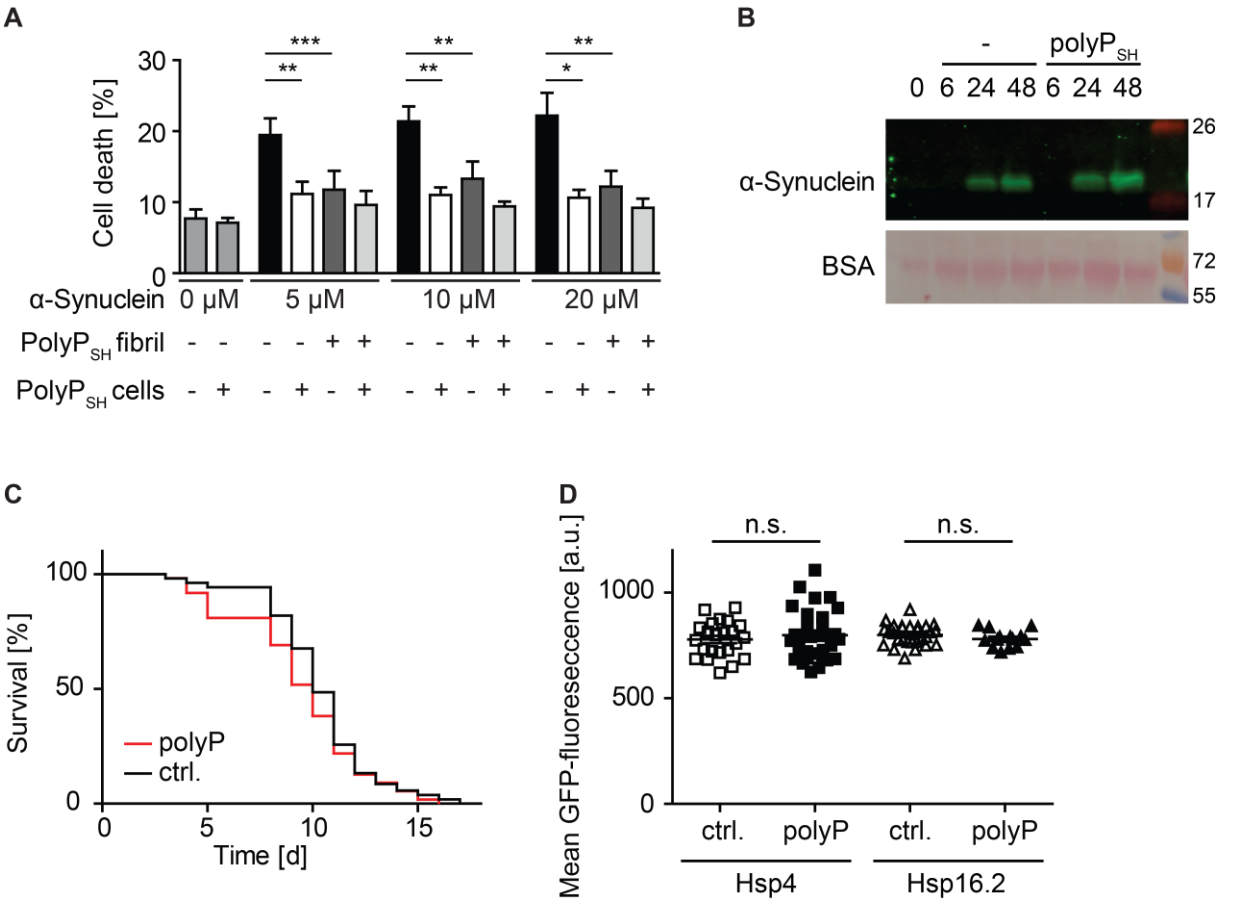

Fig. S5. Effects of PolyP on Amyloid Toxicity (related to Figure 5).
(A) α-Synuclein (150 µM) was incubated in the absence or presence of 50 µM polyP$_{SH}$ for 24 h (indicated with "-" and "+" polyP$_{SH}$ fibrils). Then, increasing amounts of the fibril-containing samples were added to PC-12 cells, which were either left untreated or pretreated with 20 µM polyP$_{SH}$ for 1 h before fibril addition (indicated by "-" and "+" polyP$_{SH}$ cells). After 40 hr of incubation, cytotoxicity was determined using live/dead staining and cell counting. Mean ± SEM are shown (n = 3–5 independent experiments), with significance determined by a paired two-tailed Student's t-test; *p<0.05, **p<0.005, ***p<0.0005. (B) Secretion of α-Synuclein from α-Synuclein-expressing HeLa cells in the absence and presence of 20 µM polyP$_{SH}$ in the media was analyzed by western blot. Samples of the spent media were taken after 0, 6, 24, and 48 h, separated by SDS-PAGE, blotted onto nitrocellulose membrane and detected using anti-α-Synuclein antibody. Ponceau S red staining was conducted as loading control. (C) Lifespan of *C. elegans* N2 wild type after treatment of L4 larvae with or without 1 mM polyP$_{SH}$ in liquid NGM buffer for 12 h at 20°C (n = 105 - 110 worms per group). After treatment, worms were transferred onto NGM-plates and life span was observed at 20°C in the presence of FUdR. Analysis was performed by a Log-Rank (Mantel-Cox) test. (D) *C. elegans* L3-L4 larvae of the zcIs4 (Hsp4::Gfp) or adult dvls70 (Hsp16.2::Gfp) reporter strain were incubated in NGM-buffer with or without 5 mM polyP$_{SH}$-chains for 5 h at 20°C. After the incubation, worms were imaged using an Olympus fluorescence microscope. The mean GFP-fluorescence of the whole worm was analyzed.