

Supplementary Information

A nanobody-based fluorescent reporter reveals human α -synuclein in the cell cytosol

Christoph Gerdes¹, Natalia Waal², Thomas Offner^{3,4}, Eugenio F. Fornasiero¹, Nora Wender¹, Hannes Verbarq¹, Ivan Manzini^{3,4}, Claudia Trenkwalder^{5,6}, Brit Mollenhauer^{6,7}, Timo Strohäker⁸, Markus Zweckstetter^{8,9}, Stefan Becker⁹, Silvio O. Rizzoli¹, Fitnat Buket Basmanav^{1,10,§}, Felipe Opazo^{1,2,§,*}

¹Department of Neuro- and Sensory Physiology, University Medical Center Göttingen, D-37073 Göttingen, Germany

²Center for Biostructural Imaging of Neurodegeneration (BIN), University Medical Center Göttingen, D-37073 Göttingen, Germany

³Institute of Animal Physiology, Department of Animal Physiology and Molecular Biomedicine, Justus-Liebig University Giessen, 35390 Giessen, Germany.

⁴Institute of Neurophysiology and Cellular Biophysics, University of Göttingen, Göttingen, Germany

⁵Department of Neurosurgery, University Medical Center Göttingen, D-37075 Göttingen, Germany

⁶Paracelsus-Elena-Klinik, Klinikstraße 16, 34128 Kassel, Germany

⁷Department of Neurology, University Medical Center Göttingen, D-37075 Göttingen, Germany

⁸German Center for Neurodegenerative Diseases (DZNE), Von-Siebold-Str. 3a, 37075 Göttingen, Germany

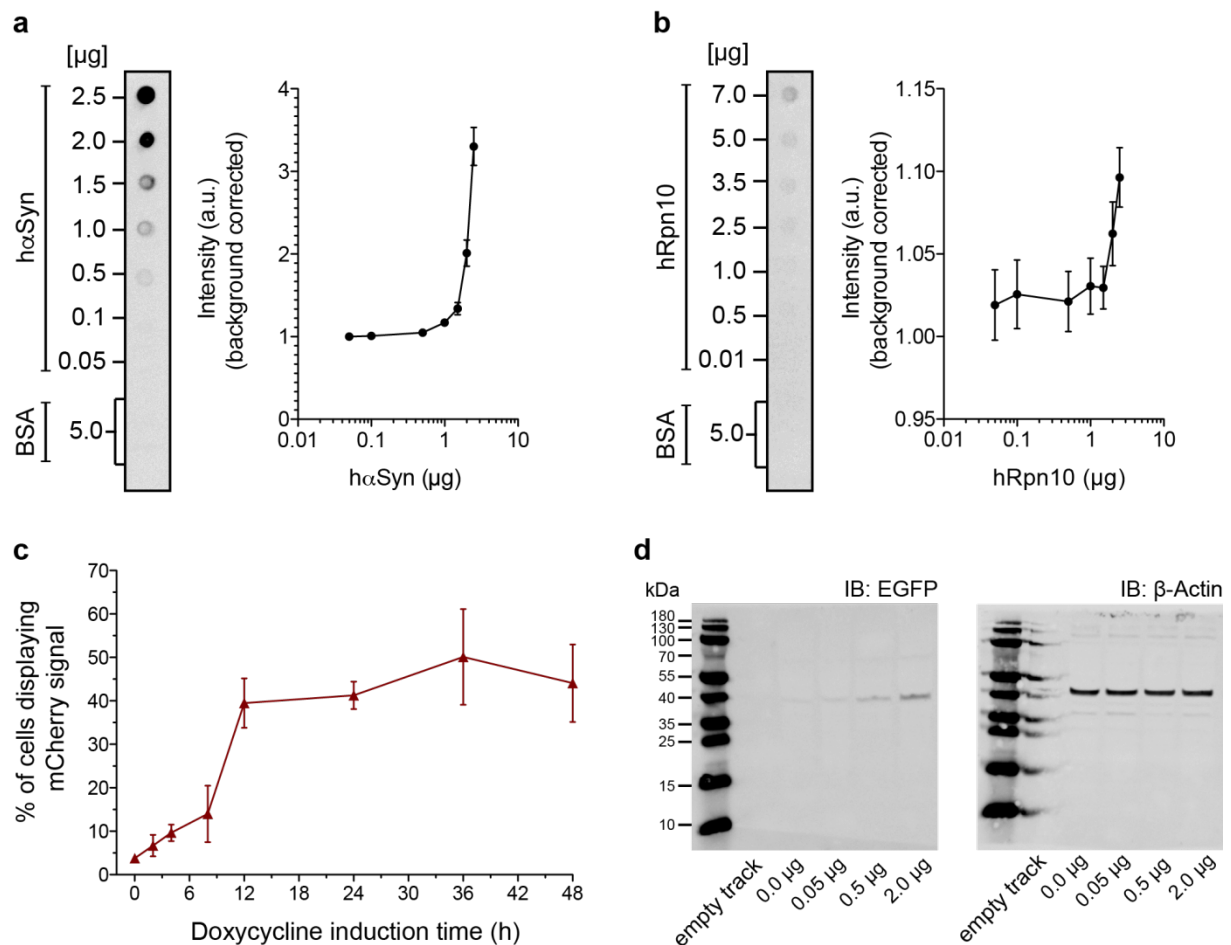
⁹Department for NMR-based Structural Biology, Max Planck Institute for Biophysical Chemistry, Am Faßberg 11, 37077 Göttingen, Germany

¹⁰Campus Laboratory for Advanced Imaging, Microscopy and Spectroscopy, University of Göttingen, D-37073 Göttingen, Germany

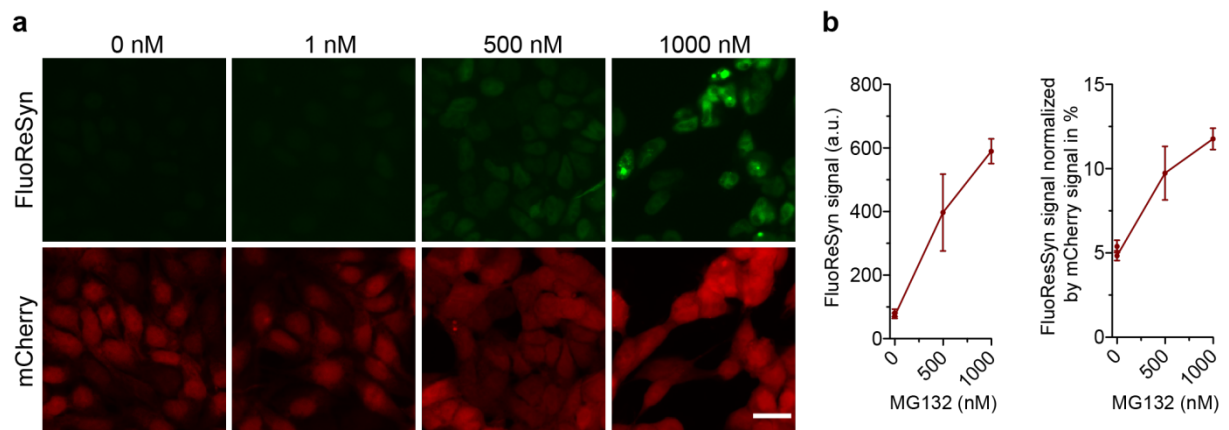
§ These authors contributed equally

*Correspondence should be addressed to:

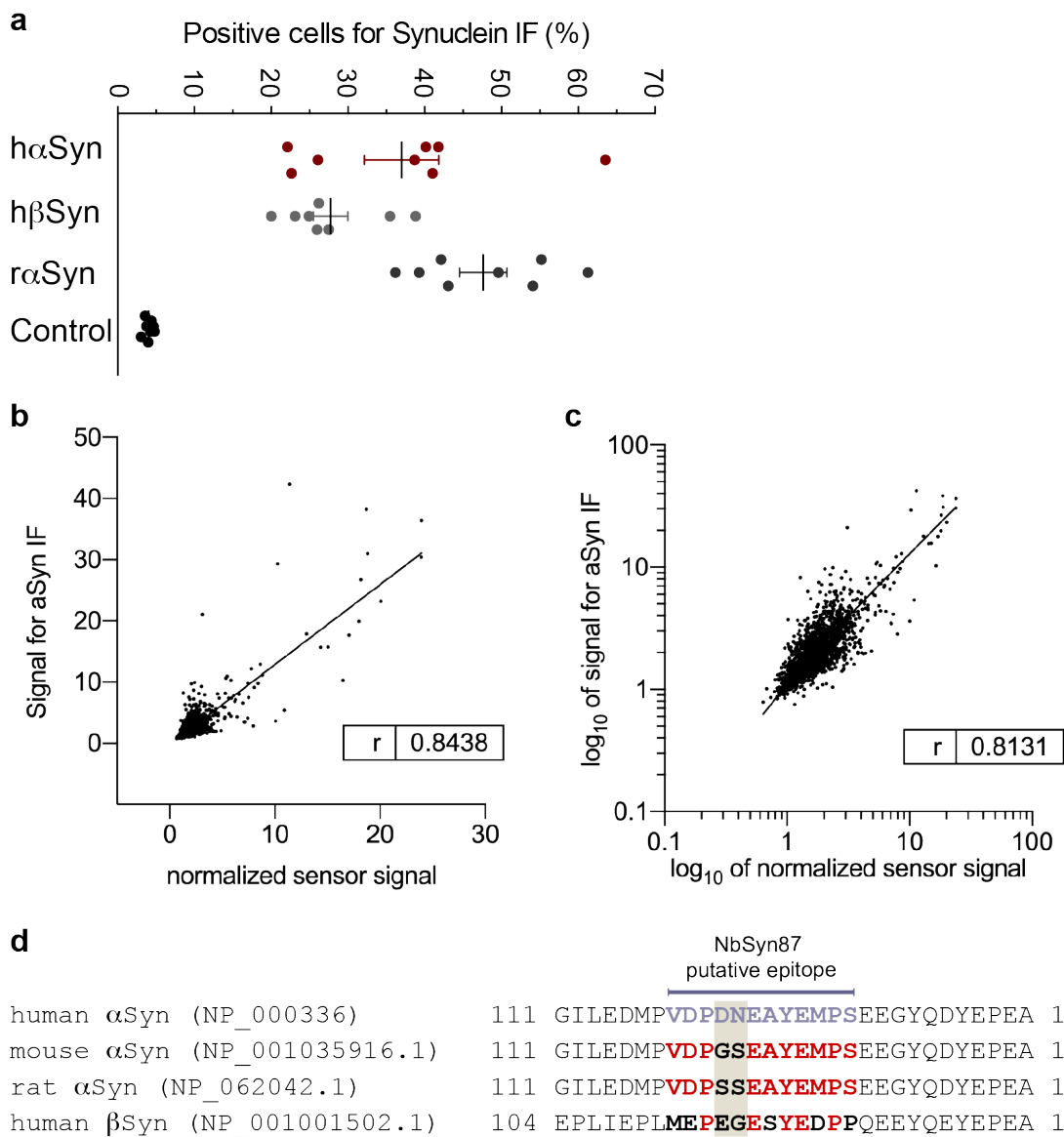
Felipe Opazo (fopazo@gwdg.de)



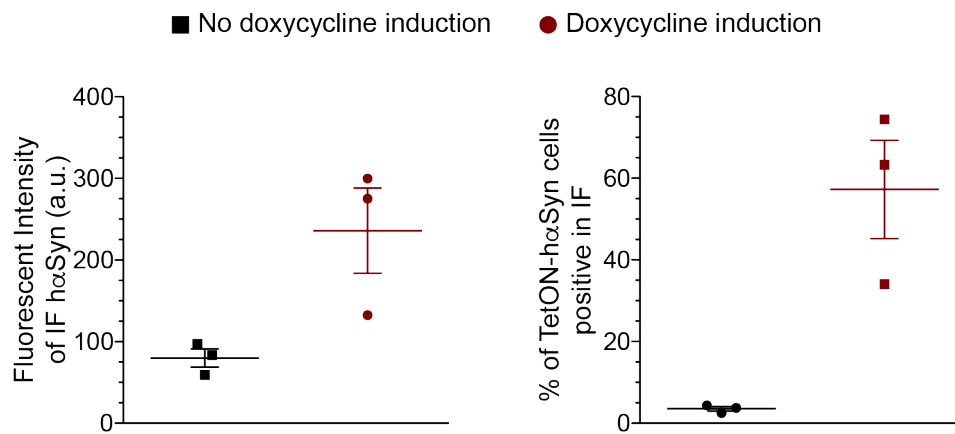
Supplementary Figure 1 | NbSyn87 binding to hαSyn and hRpn10 and the biochemical characterization of Reporter-cells. (a, b) Increasing amounts of hαSyn (a) or hRpn10 (b) were spotted on a nitrocellulose membrane and detected using NbSyn87 directly coupled to Alexa 647. Bovine serum albumin (BSA) was spotted as control protein and its signal was used as background for normalization. Error bars represent the SEM from 3 independent experiments. (c) Induction response curve of the Reporter-cells to determine the optimal duration of doxycycline administration at a concentration of 0.5 μg/ml. Error bars represent the SEM from 4 independent experiments. (d) Full Western blots membranes from Fig. 1f.



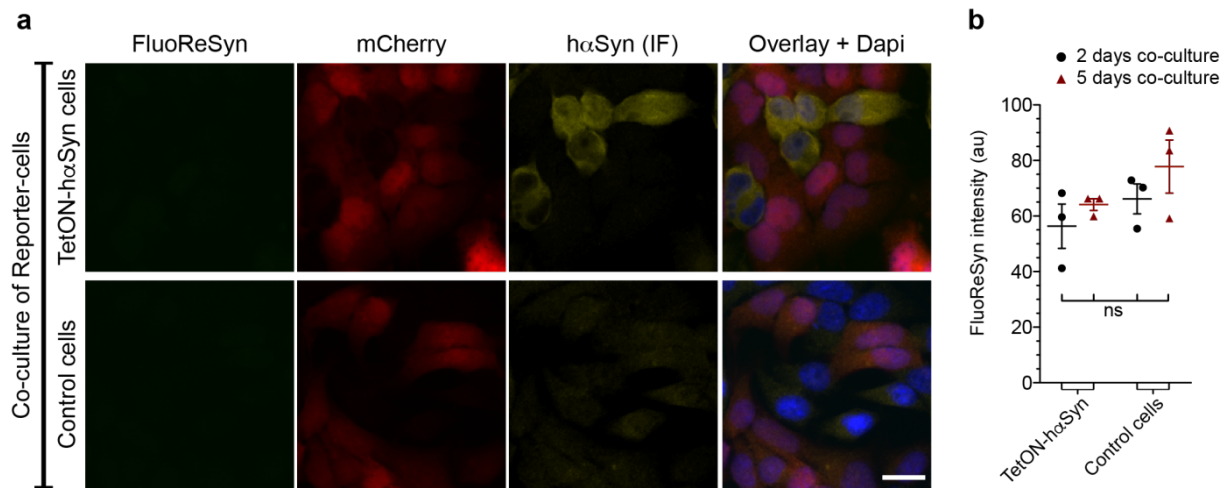
Supplementary Figure 2 | Determination of the optimal concentration for MG132 administration. (a) Fully induced Reporter-cells were exposed to different concentrations of MG132 for 16h. The mCherry signal (red) indicate that the cells are producing FluoReSyn, however, only starting from 500 nM of MG132, the FluoReSyn signal begins to appear (green). (b) Quantification of the FluoReSyn signal (green) in arbitrary units (a.u.) or normalized to the signal of mCherry. Error bars represent the SEM from 3 independent experiments with several hundreds of cells analyzed per experiment.



Supplementary Figure 3 | Specificity of FluoReSyn for hαSyn. (a) Quantification of the signal obtained after immunostaining synucleins with a pan-Synuclein antibody. Lines represent the mean and the error bars the SEM from $n = 8$. (b) Pearson's correlation analysis between the signal from FluoReSyn and immunostaining of αSyn. Signals from αSyn immunofluorescence (IF) and FluoReSyn were obtained by analyzing regions of interest (ROI) determined automatically. The ROIs were obtained by identifying all cell nuclei in the DAPI channel, followed by a morphological dilation of the nucleus ROIs, to account for the rest of the cell surfaces. (b) Raw values, in arbitrary units. (c) Double logarithmic graph of the same values. The Pearson's correlation coefficient (r) was 0.8438 for (b) with a two-tailed $p < 0.0001$ (****), and $r = 0.8131$ for (c) with a two-tailed $p < 0.0001$ (****). (d) Amino acid sequence alignment of the putative epitope recognized by NbSyn87 for hαSyn (letters in light purple), mouse and rat αSyn or human βSyn. (accession numbers for each sequence are on the figure). Red letters show a match amino acid to the putative epitope, black letters represent mismatches. The core difference between the sequences is highlighted by a background box.

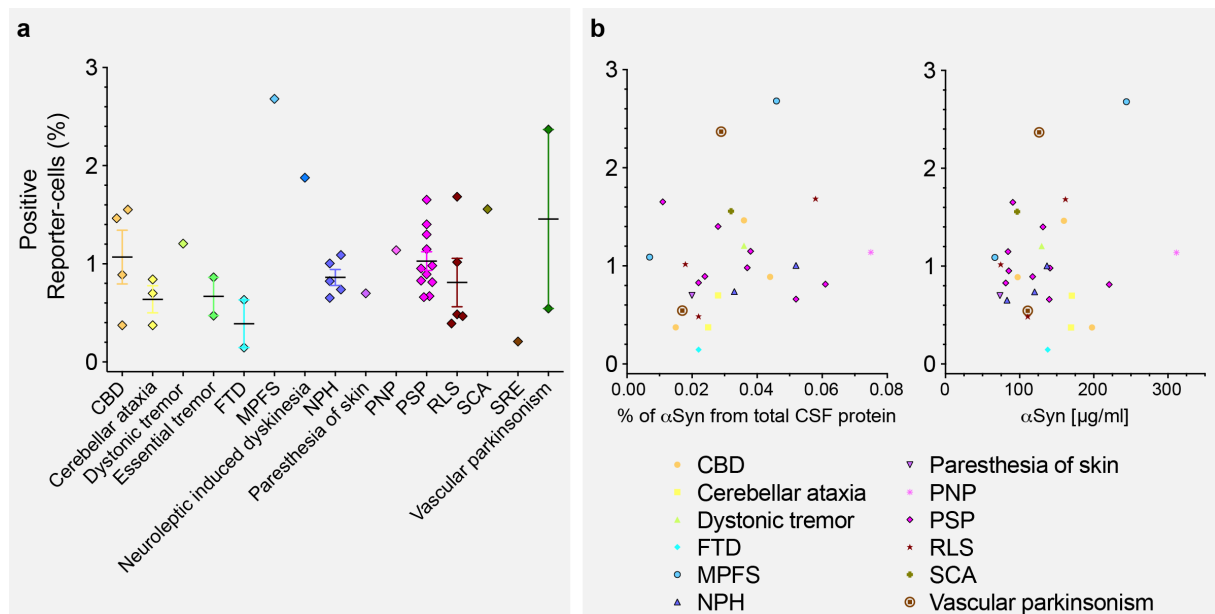


Supplementary Figure 4 | TetON induction of stably-transfected HEK293 cells expressing untagged hαSyn. (left plot) Fluorescence intensity of cells immunoassayed for synuclein after being induced or not with 0.5 µg/ml of doxycycline for 16h. (right plot) Percentage of cells displaying a positive immunofluorescence (IF) signal for synuclein. Error bars represent the SEM from 3 independent experiments (n= 3).



Supplementary Figure 5 | Co-culture of Reporter cells with TetON-haSyn cells.

(a) Reporter-cells co-cultured with either a doxycycline inducible haSyn expressing cell line (TetON-haSyn) or the wildtype HEK293 cell line without endogenous haSyn expression. Induced Reporter-cells can be identified by their mCherry signal while induced TetON-haSyn cells can be identified by the haSyn immunofluorescence (IF, yellow). (b) Quantitative analysis of FluoReSyn signal intensity of cells with mCherry above 300 arbitrary units (a.u.) after 2 or 5 days of co-culturing. Scatter plots show 3 independent experiments ($n = 3$) $\pm$ SEM for all condition. Significance was assessed by One-Way ANOVA and Tukey's Post-hoc test. ns, non-significant. Per replication and condition more than 550 cells were analyzed.



Supplementary Figure 6 | Reporter cell line signals plotted according to the clinical category of the patients and to their haSyn content in CSF. (a) The same data as plotted in Fig 7b, displayed according to the patient clinical description. Note that some categories have only one patient. Each patient is represented as a data point (n), the mean values are depicted with a horizontal lines and error bars represent the SEM for each category. **(b)** The percentage of positive Reporter cells, plotted against (left) the mass percentage of haSyn (within all CSF proteins) or (right) the concentration of haSyn present in the CSF (μg/ml). Note that the concentrations of haSyn or the total protein in CSF were not available for some cases, and are therefore missing in the graphs. CBD, corticobasal dementia; MPFS, muscular pain-fasciculation syndrome; FTD, frontotemporal dementia; NPH, normal pressure hydrocephalus; PNP, peripheral neuropathy; PSP, progressive supranuclear palsy; RLS, restless legs syndrome; SCA, spinocerebellar ataxia; SRE, steroid responsive encephalopathy. Pearson's correlation (r) was 0.6778 when haSyn concentrations were normalized by total protein in the CSF (two-tailed $p = 0.5260$, not significant), and $r = -0.3424$ when using the direct haSyn concentrations (two-tailed $p = 0.7775$, not-significant).

Supplementary Table 1. Demographic and clinical characteristics of the individuals in the CSF study

ID	Gender	Age	Diagnosis
1	f	65	Muscular pain-fasciculation syndrome (MPFS)
2	f	73	RLS
3	m	77	FTD
4	f	60	RLS
5	m	73	CBD
6	m	74	Cerebellar ataxia
7	m	68	PSP
8	f	82	PSP
9	f	71	PSP
10	f	64	FTD
11	m	87	Vascular parkinsonism
12	m	88	Essential tremor
13	m	76	Vascular parkinsonism
14	f	81	PSP
15	m	70	PSP
16	f	81	NPH without evidence for other neurodegenerative disorders
17	f	73	CBD
18	m	76	RLS
19	m	73	NPH without evidence for other neurodegenerative disorders
20	f	69	NPH without evidence for other neurodegenerative disorders
21	f	77	NPH without evidence for other neurodegenerative disorders
22	m	75	PSP
23	f	73	CBD
24	f	64	Cerebellar ataxia
25	m	60	PSP
26	f	75	SCA
27	f	77	Steroid responsive encephalopathy associated with athyroiditis
28	f	71	Essential tremor
29	m	69	PSP
30	f	75	Dystonic tremor
31	m	84	PNP
32	f	35	Paraesthesia of skin
33	f	65	Neuroleptic-induced dyskinesia
34	m	48	RLS
35	f	59	PSP
36	m	76	PSP
37	m	65	NPH without evidence for other neurodegenerative disorders
38	m	60	Cerebellar ataxia
39	f	71	CBD
40	f	80	Benign paroxysmal positional vertigo
41	f	73	RLS
42	m	67	PSP

m, male; f, female; CBD, corticobasal dementia; FTD, frontotemporal dementia; NPH, normal pressure hydrocephalus; PNP, peripheral neuropathy; PSP, progressive supranuclear palsy; RLS, restless legs syndrome; SCA, spinocerebellar ataxia.

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors show that the nanobody with specificity towards α -synuclein has a cross reactivity to an amino acid stretch that also occurs in Rpn10, a protein at the entry of the proteasome. As a consequence this nanobody tagged with fluorescent protein (FP) is only short lived in cells (rapidly degraded by the proteasome) unless α -syn is also present in these cells (or if proteasomal activity is blocked with MG132).

This finding is exploited to generate a sensor for scoring the intracellular presence of human α -syn either produced inside the cells or transmitted inside the cell, by various pathways. The sensor (reporter cell) is specific for the α -syn (rat or human) as the epitope seems to be conserved as well as the conserved epitope of Rpn10 that cross reacts with this nanobody. These reporter cells also detect any transmittable form of the human α -syn, such as that in human CSF. A minor criticism is at its place: the correlation among the amount of α -syn in the CSF and the signal in the reporter cells is not obvious from the manuscript.

Furthermore, although the findings described in these report are of general interest, it is not clear whether this reporter or screening system could be broadened to other targets beside α -syn (by making use of bispecific nanobody constructs). Probably other monomeric nanobodies with other epitope specificity will not work as they will not cross react to the rpn10 or any other proteasome epitope.

Finally, a discussion is missing on the general sensitivity of intracellular nanobodies to degradation if their target is absent. It was indeed shown by Keller BM (group of Rothbauer U; papers in Mol Cell Proteomics and Antibodies) that intracellular nanobodies are rapidly degraded in the cytosol (probably independent on targeting the Rpn10 peptide).

Reviewer #2 (Remarks to the Author):

Gerdes et al describe a nanobody-based reporter that they have developed for the detection of monomeric forms of alpha-synuclein. They show that NbSyn87 may be stabilized by binding to human aSyn, preventing its degradation in the proteasome, to which it is otherwise recruited by weak binding to Rpn10.

I have two major comments, one related to the use cases for this reporter, and one related to the experiments reported in this paper.

1. As shown in Figure 6, FluoReSyn detects primarily monomeric forms of human aSyn (no signal with introduction of haSyn fibrils). It is not clear exactly what the use case of such a reporter might be – in most disease contexts, it is the detection of pathological forms of haSyn that is most valuable.

2. With respect to the experiments reported here, the only context in which any type of dose curve is shown is in Figure 1, where variable amounts of haSyn plasmid are transfected into HEK293 cells, and higher levels appear to result in a higher % of positive reporter cells. However, as shown in Figure 6c, variability under the same conditions is pretty large – replicates treated with haSyn and iMAX show anywhere from 10% to 35% FluoReSyn+ neurons. This assay is really only useful if there is some ability to quantify aSyn, which is minimally shown by the authors (with some data suggesting that in most use contexts, variability is too high for meaningful quantification).

Reviewer #3 (Remarks to the Author):

This is a very interesting paper, with a significant number of strong experiments to support the conclusion that a fluorescent fusion of the NbSyn87 nanobody can serve as a reporter for the presence of human α -Syn in cellular cytoplasm. The range of assays used to measure both target that is over-produced in transfections, as well as penetration of exogenously introduced protein, form a convincing series. Methods descriptions seem very detailed.

The authors have acknowledged in the discussion that there are multiple variables of cell lines, culture conditions, and protocols used to produce α -Syn for the extracellular experiments. It might be worthwhile to explore these questions in a bit more detail, since they are very critical to the interpretation of the results. Not all readers will have the time or access to look up the cited papers. The 7-line final sentence makes it clear that the authors plan to do major optimizations, presumably including much more neuronal cell lines as more faithful reporters. Their more explicit expert opinion on which directions to prioritize could be a useful addition to the field.

The only experiment that seems to have been incompletely reported is the one testing the human CSF from a range of disorders. While it is clear that the HEK293-derived reporter line is more proof of general concept than definitive, it would still be useful to see the actual distribution of the different diseases in this assay. Since the data are presented individually, it would seem possible to do this, in which case the scatter plot would become a regular, rather than a supplemental, figure.

Reply to the reviewer comments

Reviewer #1:

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This finding is exploited to generate a sensor for scoring the intracellular presence of human α -syn either produced inside the cells or transmitted inside the cell, by various pathways. The sensor (reporter cell) is specific for the α -syn (rat or human) as the epitope seems to be conserved as well as the conserved epitope of Rpn10 that cross reacts with this nanobody. These reporter cells also detect any transmittable form of the human α -syn, such as that in human CSF.

We thank the reviewer for these comments. To avoid misunderstandings, we would like to clarify that our sensor is specific for human α Syn. The epitope is not perfectly conserved between human, rat or mouse (as shown in Supp. Fig. 3). The sensor is therefore unable to detect rat α Syn (Figure 4), and only responds to human α Syn.

A minor criticism is at its place: the correlation among the amount of α -syn in the CSF and the signal in the reporter cells is not obvious from the manuscript.

We thank the reviewer for this suggestion. We have now added a series of graphs (the new Supp. Fig. 6) displaying the Reporter-cell signal in relation to the clinical report of the patients, as well as correlating it with the α Syn concentrations in CSF samples. We want to remark here the point that the sensor will only detect h α Syn that enters into the cytoplasm of a Reporter cell, thus it is not necessarily correlating to total α Syn present in the patient CSF.

Due to the limited number of patient samples we had access to, with different pathologies, we were unable to find conclusive correlations between the clinical phenotypes and the Reporter-cell responses. Future studies, with larger patient cohorts, should test whether the FluoReSyn system can be used as a diagnostic tool in a large scale.

Along these lines we also modified the text in Figure 7 and the Discussion of the manuscript to make this clearer.

Furthermore, although the findings described in these report are of general interest, it is not clear whether this reporter or screening system could be broadened to other targets beside α -syn (by making use of bispecific nanobody constructs). Probably other monomeric nanobodies with other epitope specificity will not work as they will not cross react to the rpn10 or any other proteasome epitope.

The reviewer is right that other monomeric nanobodies would not work in this fashion unless they also by chance have an affinity towards Rpn10. We also agree with the reviewer that it will be of significant interest to be able to apply this tool to a large number of other targets. The NbSyn87 nanobody (used for FluoReSyn) could be used in a general manner by introducing the human α Syn epitope on the protein of interest. This strategy could work as an example by introducing the α Syn epitope at the genomic level with CRISPR/Cas9 to tag endogenous proteins of interest. By doing this, the sensor could be used for nearly every

protein with the only disadvantage of having altered the endogenous protein by adding ~12 amino acids to it. Obviously, this can only be envisioned in cells where no human α Syn is endogenously present, to avoid cross-reactivity.

Following on the lead of the reviewer, a similar strategy could be developed by using a bispecific nanobody construct where NbSyn87 nanobody would be combined with a second nanobody that is specific for another protein. In principle, the binding of NbSyn87 to the conserved epitope of Rpn10 would direct the whole construct to degradation, unless the second nanobody finds its target, and brings the whole construct to its cellular location, thus preventing degradation. However, depending on the concentrations of all players involved, and on the affinity of the second nanobody, it is possible that the bispecific construct actually shuttles the target protein to degradation, which would prevent this construct from working as a general sensor system. Such constructs would therefore need to be carefully designed, tested and optimized.

Finally, a discussion is missing on the general sensitivity of intracellular nanobodies to degradation if their target is absent. It was indeed shown by Keller BM (group of Rothbauer U; papers in Mol Cell Proteomics and Antibodies) that intracellular nanobodies are rapidly degraded in the cytosol (probably independent on targeting the Rpn10 peptide).

This is an important point that has been difficult to tackle in the field of nanobodies/intrabodies. The affinities and thermostabilities of nanobodies are always measured *in vitro*, and we are not aware that it has been possible to measure them inside living cells, so in practice it is difficult to estimate the fraction of the nanobodies that are bound to their targets in the cytosol. The main problem here is that some nanobodies are intrinsically unstable in the cytosol, and tend to be incorrectly folded, which renders them impractical for use as intrabodies. Binding to the target may stabilize the nanobodies, but not in all cases, as some nanobodies will misfold irrespective of the target presence. In such cases the nanobodies will tend to form large aggregates in the perinuclear area, or will be removed by the proteasome system. The opposite example comes from our recently published nanobody anti-ALFA-tag (PMID: 31562305). This nanobody works as an intrabody, and we do not observe that it is being degraded rapidly in the cytosol.

Until recently, there were no general guidelines to predict which nanobodies would (or wouldn't) work as an intrabody. One hint is the presence of 2 or more disulfide bonds, since nanobodies containing several disulfide bonds have low chances of working as intrabodies. A very interesting study from Tang et al. (PDB: 27205882) shows that by mutating some specific amino acids on nanobodies it is possible to destabilize them in the cell cytosol. Tang and colleagues used the anti-GFP nanobody from Rothbauer (which is stable in the cytosol), and they found 6 amino acid positions that can destabilize nanobodies. Three are mild destabilizers, and 3 are major destabilizers. The SynNb87 nanobody used here has indeed only one of the minor destabilization mutations. All other 5 positions contain amino acids considered as providing stability to the nanobody scaffold, which suggests that this nanobody would normally be stable in the cytosol.

Reviewer #2:

Gerdes et al describe a nanobody-based reporter that they have developed for the detection of monomeric forms of alpha-synuclein. They show that NbSyn87 may be stabilized by binding to human α Syn, preventing its degradation in the proteasome, to which it is otherwise recruited by weak binding to Rpn10.

I have two major comments, one related to the use cases for this reporter, and one related to the experiments reported in this paper.

We thank the reviewer for the comments.

1. As shown in Figure 6, FluoReSyn detects primarily monomeric forms of human α Syn (no signal with introduction of h α Syn fibrils). It is not clear exactly what the use case of such a reporter might be – in most disease contexts, it is the detection of pathological forms of h α Syn that is most valuable.

We apologize for this confusion. We recognized after the reviewer's comment that the way we had described the fibril experiments in the initial manuscript was not optimal and was therefore prone to misunderstandings.

The nanobody on which FluoReSyn was derived is fully able to detect fibrillar forms of α Syn. The nanobody has been characterized thoroughly *in vitro* by the group of Christopher Dobson (PMID: 23557833). They demonstrated that the nanobody can bind equally well to monomeric α Syn and to fibrils. We have also replicated this finding, by showing that the nanobody was capable of binding to our *in vitro*-generated fibrils (Fig. 6d).

Fibrils from the same batch used for *in vitro* detection were employed later by adding them to the culture medium of neurons. There the fibrils were not detected by the sensor (Fig. 6). This is not due to the inability of the sensor to bind them, but rather only an indication that such fibrils did not penetrate into the cells, and therefore could not be detected. α Syn fibrils are objects of several micrometers (see for example PMID: 23557833), and therefore we do not expect to find such structures entering the cytosol of living cells. We have now clearly addressed this argumentation in the Results and Discussion sections of the revised manuscript. We have also modified the wording of other discussion points about the interpretations and implications of experiments with fibrillary α Syn for enhancing clarity.

Our observations are in agreement with several theories that advocate for oligomers and/or pre-formed fibrils (pffs) as the toxic species, but typically not full fibrils (PMID:19888725). A general hypothesis is that oligomers or α Syn mutants (PDML: 19745811) might be able to enter the cytosol and act as nucleator elements, triggering the formation of oligomers or fibrils that eventually cause cellular dysfunction. In spite of current debates in the field, few works indicate that large fibrils might penetrate from the outside into the cell cytoplasm. We therefore see these results as simply in line with current hypotheses on α Syn behavior: the sensor could not respond to the fibrils, because the fibrils could not penetrate into the cytosol. Even small pre-formed fibrils have major difficulties in entering the cell cytosol (PMID: 28611062).

We have now explained the experiments and their results in more detail, to increase clarity on this matter.

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The first figure of the manuscript was performed to characterize FluoReSyn. For this, we used Reporter-cells that were expressing FluoReSyn in a stable fashion. These cells were then transiently transfected with well-defined amounts of α Syn expression constructs. Here we could measure easily a dose-response curve for the homogeneously expressed FluoReSyn in the presence of variable amounts of plasmids coding for human α Syn (Fig 1e).

Following the reviewer's comment, we have now also analyzed the correlation between the sensor signal and the immunostaining of α Syn on Reporter-cells (please see Figure 1, below). As expected from our observations and the Western-blot in Fig. 1 of the manuscript, a high correlation between the sensor signal and α Syn amounts was observed.

However, this type of analysis is less trivial to perform in primary hippocampal neurons, as in Figure 6, where we used FluoReSyn to determine if recombinant α Syn is able to enter into the cytoplasm of neurons. These neuronal cultures contain varying levels of the FluoReSyn-expressing plasmid, unlike in Figure 1 of the manuscript, where a homogenous population of stably transfected cells was employed. Additionally, primary hippocampal cultures contain pyramidal neurons of different morphologies, as well as different inhibitory neurons, which enhances the variability of the experimental setup. This is why we did not characterize the system in primary neurons by dose-response curves. This experiment was only performed to determine whether neurons respond to the extracellular application of α Syn. In our opinion, it is therefore expected to encounter a relatively large variation in such an experiment.

This work is the first proof-of-principle of the FluoReSyn sensor, and we are aware that FluoReSyn is not yet working in a straightforward quantitative manner in every cell type, especially if transient transfection is employed. However, it is a tool that can indicate, for example, whether transmittable α Syn exists in human CSF. This is an important type of information, which currently no other technique is able to provide.

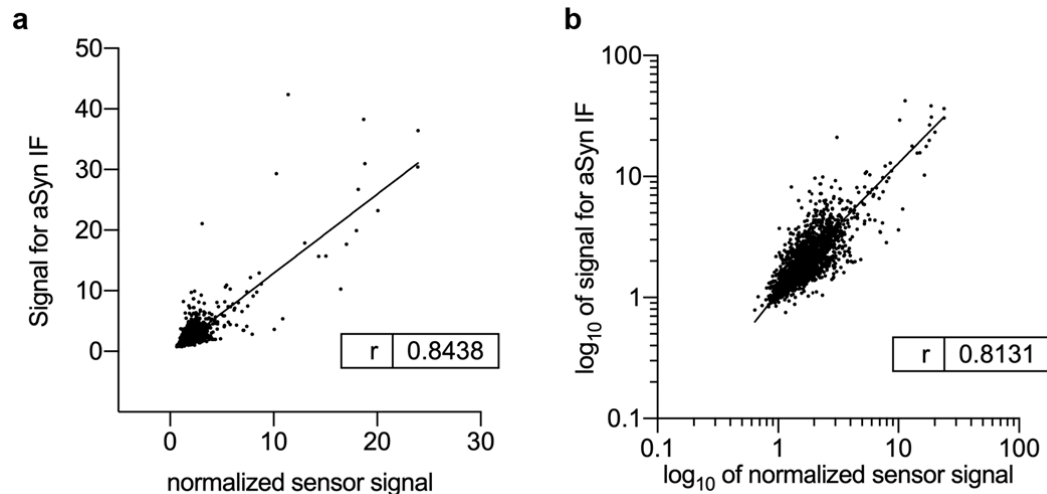


Figure 1. Pearson's correlation analysis between the signal from FluoReSyn and immunostaining of α Syn. The experiment was performed using the Reporter cell line transiently transfected with a plasmid encoding for human α Syn. Signals from α Syn immunofluorescence (IF) and FluoReSyn were obtained by analyzing regions of interest (ROI) determined automatically. The ROIs were obtained by identifying all cell nuclei in the DAPI channel, followed by a morphological dilation of the nucleus ROIs, to account for the rest of the cell surfaces. (a) Raw values, in arbitrary units. (b) Double logarithmic graph of the same values. The Pearson's correlation coefficient (r) is indicated in each case.

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The authors have acknowledged in the discussion that there are multiple variables of cell lines, culture conditions, and protocols used to produce α -Syn for the extracellular experiments. It might be worthwhile to explore these questions in a bit more detail, since they are very critical to the interpretation of the results. Not all readers will have the time or access to look up the cited papers. The 7-line final sentence makes it clear that the authors plan to do major optimizations, presumably including much more neuronal cell lines as more faithful reporters. Their more explicit expert opinion on which directions to prioritize could be a useful addition to the field.

We thank the reviewer for this suggestion. We have significantly expanded the explanation on the discussion about the options to produce α Syn *in vitro*, and their implication on the potential variability of the results in the α Syn field. Similarly, we have extended the paragraph discussing potential optimizations of our system and addressed what could be considered relevant as the next steps.

The only experiment that seems to have been incompletely reported is the one testing the human CSF from a range of disorders. While it is clear that the HEK293-derived reporter line is more proof of general concept than definitive, it would still be useful to see the actual distribution of the different diseases in this assay. Since the data are presented individually, it would seem possible to do this, in which case the scatter plot would become a regular, rather than a supplemental, figure.

We did not initially consider to display the details of the patient cohort because this set of patients has not been diagnosed with α Syn-associated diseases. Thus, we probably detect a basal level of transmittable α Syn. Unfortunately, it is even more challenging to obtain CSF from healthy controls, since in Germany this type of sample can typically only be collected from disease cases, so that we cannot state whether such basal levels of transmittable α Syn are also present in healthy donors or young adults.

We have now added the missing details to our graphs (the new Supp. Fig 6). We display the Sensor activity in relation to the clinical diagnoses, as well as to the levels of α Syn present in the CSF of each patient, separated by disease categories. Unfortunately, the small number of patients and the large variability in clinical diagnoses makes it difficult to provide any significant differences between diseases (at least using our cohort). Future work, using larger cohorts and including healthy controls, should test whether this sensor would help in the diagnosis of α Syn-associated diseases.

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

In this revised version and/or in their rebuttal letter, authors meet all comments and critics raised by reviewers.

Reviewer #2 (Remarks to the Author):

Thank you for the clarification re what forms of aSyn the reporter recognizes. This is an important point for use cases of this tool.

I suggest adding the figure provided in the rebuttal letter (relating Fluoresyn readout to IF for aSyn) as a supplemental figure; I found the information valuable, as may others thinking of adopting this tool.

The addition of Supplemental Fig 6 is valuable in interpreting results, even though the cases do not have PD. For both the figure suggested above (relating Fluoresyn readout to IF for aSyn) and Supplemental Fig 6b, please provide both p-value and correlation coefficients for the relationships shown.

Alice Chen-Plotkin

Reviewer #3 (Remarks to the Author):

The changes that were made have clarified several issues, and expanded on others. This increases the value of this paper.

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[This figure is now included in the Supplementary file.](#)

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Alice Chen-Plotkin

[The requested statistics parameters are now included for both Figures in the Supplementary file.](#)

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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- | | |
|-------------------------------------|--|
| n/a | Confirmed |
| ☐ | ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| ☐ | ☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☐ | ☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| ☒ | ☐ A description of all covariates tested |
| ☐ | ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| ☐ | ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals) |
| ☐ | ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i> |
| ☒ | ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| ☒ | ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| ☐ | ☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Epifluorescence microscopy: Olympus IX71 microscope equipped with a CellSens Dimension 2.3 Software
Nikon Eclipse microscope
Nikon A1R MP (2 photon)
Western Blot imaging: LiCor Sytem Odyssey Clx
AKTA chromatography: UNICORN™ 7.0.2 software (GE Healthcare Life Sciences)

Data analysis

GraphPad Prism 5, 2012 GraphPad Software Inc.;
Matlab Version 2014b, 2007, Mathworks Inc;
Sigma Plot 11 (Systat Software, San Jose, CA, USA);
ImageJ, Fiji, version: 2.0.0-rc-69/1.52p; Build 269a0ad53f
Phyton Seaborn package, python.org; version 0.9; 10.5281/zenodo.1313201

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data Availability

The data that supports the finding of this study is readily available within this paper, its Supplementary file and in the Source Data file. The data set for Figure 1e, 2b,

2c, 3i, 4b, 5b, 6b, 6c, 7b and Supplementary Figures 1a, 1b, 1c, 3a, 3b, 5b and 6 are provided in the Source Data file. All data sets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size calculation were not performed a priori. In quantitative data, the aim was to collect as much data as possible, the sample size provided a sufficient accuracy to distinguish statistically significant differences between conditions. In descriptive experiments we chose a sample size that was large enough to show difference between the conditions.
Data exclusions	No data were excluded from the analysis, except raw images which were out of focus.
Replication	Replicates and independent experiments were carefully kept that way. Most of our Experiments were replicated independently at least 3 times. Specific n can be found in the each Figure legend.
Randomization	Randomization was not performed for this study.
Blinding	Blinding was not performed in this study.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	rabbit polyclonal anti- α / β -Synuclein antibody; dilution used 1:500; Cat. No. 128002; Synaptic System GmbH guinea pig polyclonal anti-synaptophysin antibody; dilution 1:100; Cat. No. 101004; Synaptic System GmbH donkey anti-guinea pig labeled with ATTO 647N; dilution 1:500; Cat. No. N0602-At647N-S; Synaptic System GmbH donkey anti-rabbit; dilution 1:500; Cat. No. 711-175-152, Dianova GmbH mouse monoclonal (clone 3E6) anti EGFP; dilution 1:500; Cat. No. A11120, Thermo-Scientific rabbit polyclonal (affinity purified) anti beta-Actin directly labeled with Cy5; dilution 1:1000; Cat. No. 251003, Synaptic System GmbH
Validation	anti- α / β -Synuclein: antiserum, Synthetic peptide corresponding to AA 2 to 25 from human α -Synuclein; Reacts with: rat, mouse, zebrafish and human α synuclein and β synuclein. Validated for IF, IHC. anti-synaptophysin antibody: Synthetic peptide corresponding to AA 301 to 313 from human Synaptophysin1, Reacts with: human, rat, mouse, hamster, cow, chicken, frog. Validated for IF and IHC. Specific for synaptophysin 1, no cross-reactivity to other synaptophysins. Mouse monoclonal anti EGFP (clone 3E6) validated in IF, IHC, WB and more applications source Thermo-fisher. Rabbit polyclonal anti Beta actin, antigen is the AA2 to AA16 of mouse Beta Actin, Externally validated for WB stated in suppliers webpage: Synaptic System GmbH

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	Wildtype HEK293 were purchased from the German Collection for Microorganisms and Cell Cultures (DSMZ), Braunschweig, Germany. Stably transfected lines like the Report cell line and the aSyn cell line were produced by Sirion Biotech GmbH (Martinsried, Germany). A full report on their generation was provided using DSMZ HEK-293 cell lines.
Authentication	We relied on the authentication from DSMZ and Sirion Biotechnology GmbH
Mycoplasma contamination	Cells were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	HEK-293 were not reported by the ICLAC to be misidentified.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Xenopus laevis tadpoles (albinos)
Wild animals	<i>Provide details on animals observed in or captured in the field; report species, sex and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.</i>
Field-collected samples	<i>For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.</i>
Ethics oversight	All procedures for animal handling were approved by the governmental animal care and use office (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit, Oldenburg, Germany, Az.12/0779) and were in accordance with the German Animal Welfare Act as well as with the guidelines of the Göttingen University Committee for Ethics in Animal Experimentation

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics	Study participants consisted of individuals who were in treatment at the Paracelsus Elena Klinik, Kassel, Germany and had been diagnosed with a variety of neurological disorders non-related to α Syn aggregation disorders.
Recruitment	CSF samples from all individuals were collected after the informed consent of the participant at the Paracelsus Elena Klinik (Kassel, Germany) in accordance with the principles of Declaration of Helsinki and following identical standard operating procedures.
Ethics oversight	The use of the CSF samples in this study was approved by the ethical committee of the Medical Center Göttingen with the approval numbers 36/7/02 and 9/7/04

Note that full information on the approval of the study protocol must also be provided in the manuscript.