Supplementary Data

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

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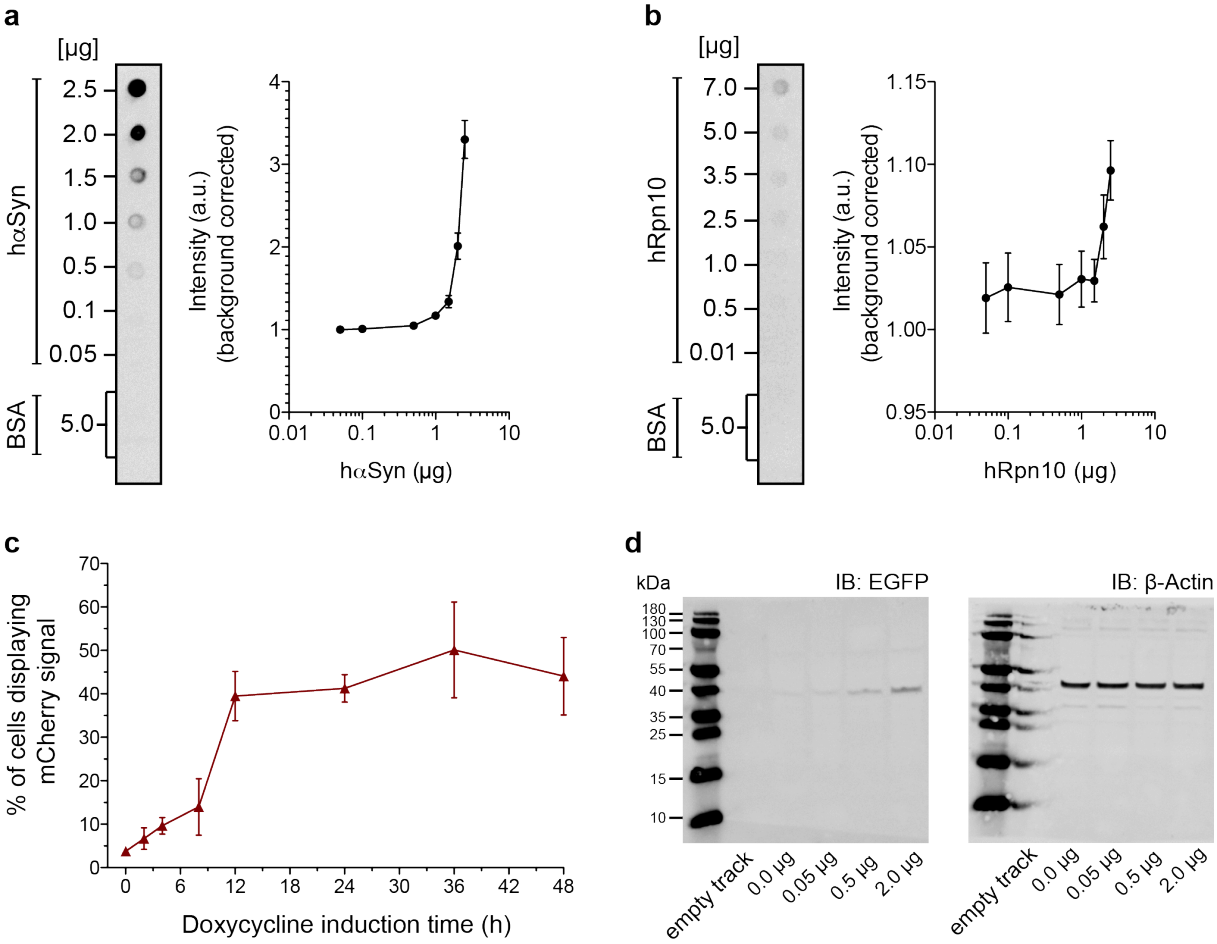
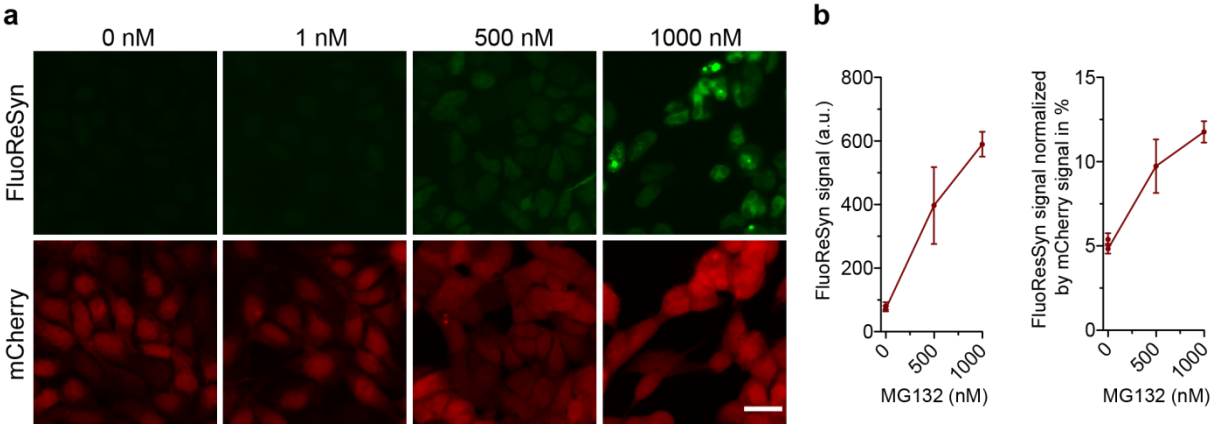
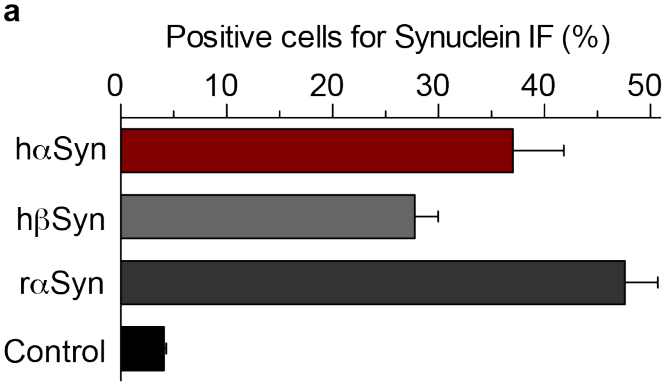


Supp. Figure 1| NbSyn87 binding to hαSyn and hRpn10 and the biochemical characterization of Reporter-cells. (**a, b**) Increasing amounts of hαSyn (**a**) or hRpn10 (**b**) were spotted on a nitrocellulose membrane and detected using NbSyn87 directly coupled to Alexa 647. BSA was spotted as control protein and its signal was used as background for normalization. Error bars represent the s.e.m 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 s.e.m from 4 independent experiments. (**d**) Full Western blots membranes from Fig. 1f.

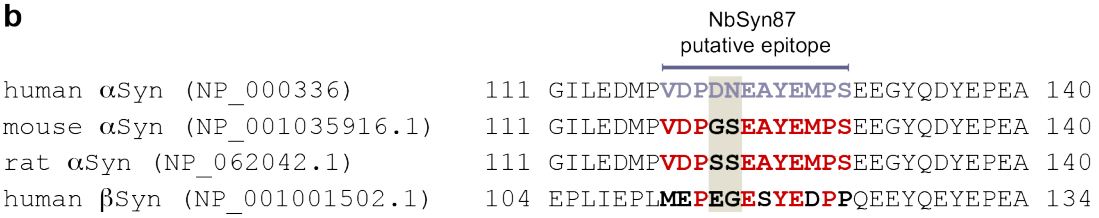


Supp. Figure 2 | Determination of the optimal concentration for MG132 administration. (**a**) Fully induced Reporter-cells were exposed to different concentrations of MG132 for 16h. The mCherry signal (red) indicate that the cells are producing FluoReSyn, however, only starting from 500 nM of MG132, 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 s.e.m from 3 independent experiments with several hundreds of cells analyzed per experiment.

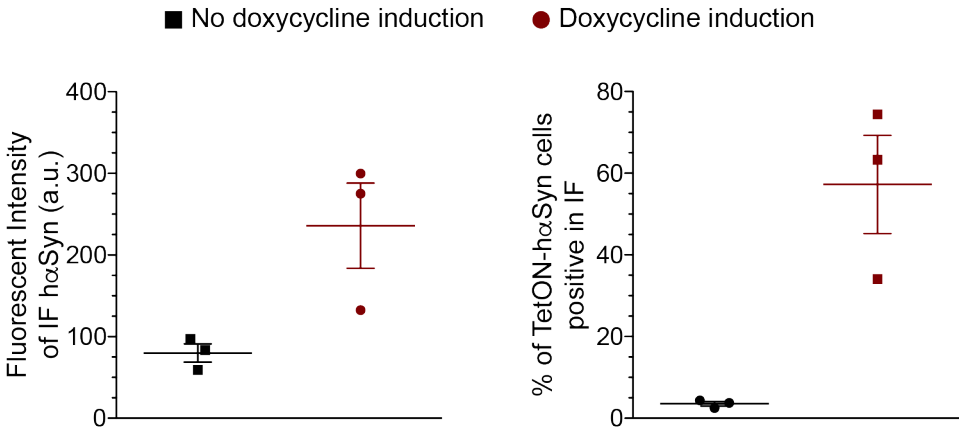
a



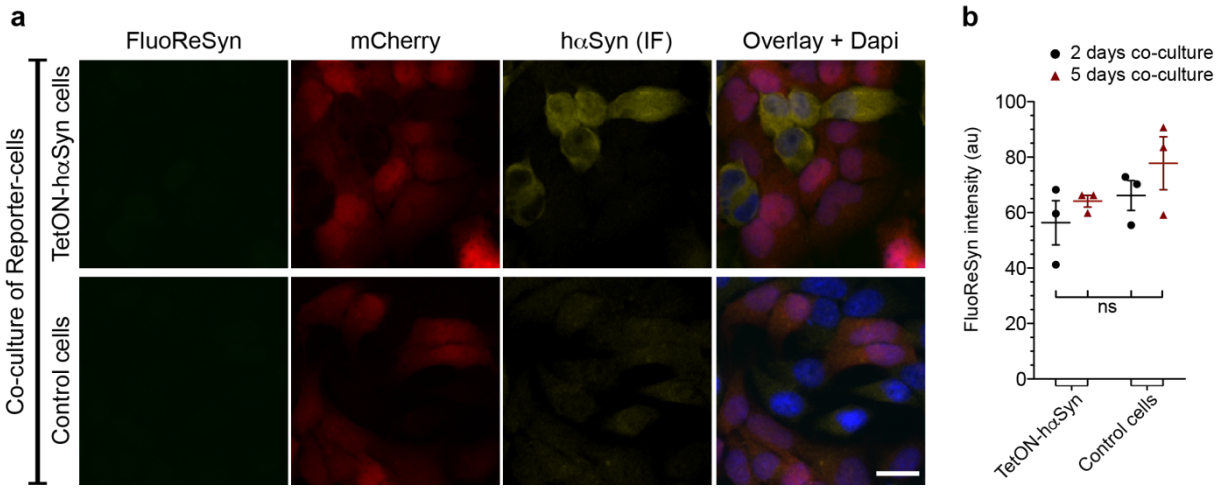
b



Supp. Figure 3 | Specificity of FluoReSyn for hαSyn. (**a**) Quantification of the signal obtained after immunostaining synucleins with a pan-Synuclein antibody. Error bars represent the s.e.m from 3 independent experiments. (**b**) 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.



Supp. Figure 4 | TetON induction of stably-transfected HEK293 cells expressing untagged hαSyn. (left plot) Fluorescence intensity of cells immunostained for synuclein after being induced or not with 0.5 µg/ml of doxycycline for 16h. (right plot) Percentage of cells displaying a positive immunofluorescense signal for synuclein. Error bars represent the s.e.m from 3 independent experiments.



Supp. Figure 5 | Co-culture of Reporter cells with TetON-hαSyn cells. (**a**) Reporter-cells co-cultured with either a doxycycline inducible hαSyn expressing cell line (TetON-hαSyn) or the wildtype HEK293 cell line without endogenous hαSyn expression. Induced Reporter-cells can be identified by their mCherry signal while induced TetON-hαSyn cells can be identified by the hαSyn immunofluorescence (IF, yellow). (**b**) Quantitative analysis of FluoReSyn signal intensity of cells with mCherry above 300 AU after 2 or 5 days of co-culturing. Scatter plots show 3 independent experiments ± 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.

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

ID	Gender	Age	Diagnosis
1	f	64	Muscular pain-fasciculation syndrome
2	f	73	RLS
3	m	77	FTD
4	f	60	RLS
5	m	73	CBD
6	m	74	SCA
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 dementia
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	SCA
25	m	60	PSP
26	f	75	SCA
27	f	77	Steroid responsive encephalopathy associated with autoimmune thyroiditis
28	f	71	Essential tremor
29	m	69	PSP
30	f	75	Dystonic tremor
31	m	84	PNP
32	f	35	PNP
33	f	65	Neuroleptic-induced dyskinesia
34	m	48	RLS
35	f	59	PSP
36	m	76	PNP
37	m	65	NPH without evidence for other neurodegenerative disorders
38	m	60	SCA
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.