Supplementary Figure Legends

Fig. S1. Optimization of the *in vivo* crosslinking protocol for αSyn. A. Incubation at 37 °C improves αSyn crosslinking efficiency. HEL cells were treated with DMSO alone (-) at 37 °C or with DSG at 37 °C or room temperature (RT) for 30' or 60' (min). TX-100 total protein lysates (centrifuged at 213,000*g*) were blotted with 15G7 mAb. Identical exposures of the same blot are shown. **B.** DSG at 1-2 mM is suitable for crosslinking αSyn in living HEL cells. HEL were treated with DSG at 1, 2, or 5 mM (crosslinking solution volume ~10-20 fold cell pellet volume) at 37 °C, and reactions were quenched after 30 min. High-speed cytosols (213,000*g*) were blotted with αSyn mAb (15G7; right panel) and DJ-1 pAb as a control for crosslinking efficiency (middle panel). Ponceau staining of the blot is also shown (left panel). **C.** Incubation of PVDF membranes in 0.4% paraformaldehyde (PFA) after transfer improves the detection of monomeric αSyn. HEL cells treated *in vivo* with DMSO alone (-) or 1 mM DSG (+) were lysed in PBS/PI and centrifuged at 20,000*g*. After sample transfer, one part of the blot was incubated for 30 min in PBS/0.4% paraformaldehyde (+), while the other part (-) was incubated in just PBS. Blots were then probed with αSyn mAb 15G7.

Fig. S2. Co-immunoprecipitation (co-IP) of differently tagged αSyn molecules after co-expression and crosslinking. αSyn-mycHis and αSyn-V5 were either expressed separately in two different cell populations and mixed before crosslinking (M) or co-expressed in the same cell population and then mixed with mock-transfected cells (C). After *in vivo* crosslinking, lysates were subjected to FLAG-immunoprecipitation. Starting materials ('lysate') and anti-FLAG immunoprecipitations ('IP M2') were analyzed by WB using specific antibodies for the V5-epitope, the FLAG-epitope (M2), and αSyn (15G7) (upper panels). IP purity was demonstrated by the absence of UCH-L1, 14-3-3, or Calmodulin immunoreactivity (lower panels) in the IP lanes. Asterisks mark IgG light and heavy chains.

Fig. S3. Optimization of the *in vitro* crosslinking protocol for αSyn. A. αS-80 is sensitive to vigorous sonication. High-speed (213,000*g*) HEL cytosols were sonicated for the indicated times and then normalized to 2 µg/µL for loading (no significant loss of total protein content was detected by BCA assay). Crosslinking of these pure cytosols was carried out similarly to the protocol for crude cell lysates (see Fig. 5B). Blots for DJ-1 and αSyn (15G7 mAb) are shown. **B.** Pure recombinant human αSyn (rec. αS) can contribute to the αS-80 species when spiked into a HEL cell lysate before *in vitro* crosslinking. High-speed (213,000*g*) HEL cytosols were generated, and total protein was normalized to 1.2 µg/µL. Then, rec. αSyn (lanes marked +) was added at a ratio of 0.7 ng rec. αSyn/µg cytosolic protein. Equal volumes of PBS alone were added to control samples (-). Crosslinking of all these cytosols was then conducted as before (see Fig. 5B). The rec. αSyn contained small amounts of apparent LDS-stable dimers (αS-30). Note that monomer levels (αS-14) are underestimated, as these blots were not treated with 0.4% PFA (see Fig. S1C). Identical exposures of the same blot are shown; film was cut at white lines. **C.** *In vivo* and *in vitro* patterns of αSyn crosslinking are independent of lysis method. DSG crosslinking of HEL cells *in vivo* or crude lysates *in vitro* was performed as before (see Fig. 5B). Vehicle (DMSO) served as control. Cell lysis was carried out by snap-freezing cells in liquid nitrogen (LN_2) or by 15 sec sonication (son.). Lysis buffer was plain PBS (-PI) or PBS plus protease inhibitor mix (+PI). Of note, DMSO-only incubations without PI (first two lanes) caused a reduction in αSyn signal, presumably due to degradation.

Supplementary Figures

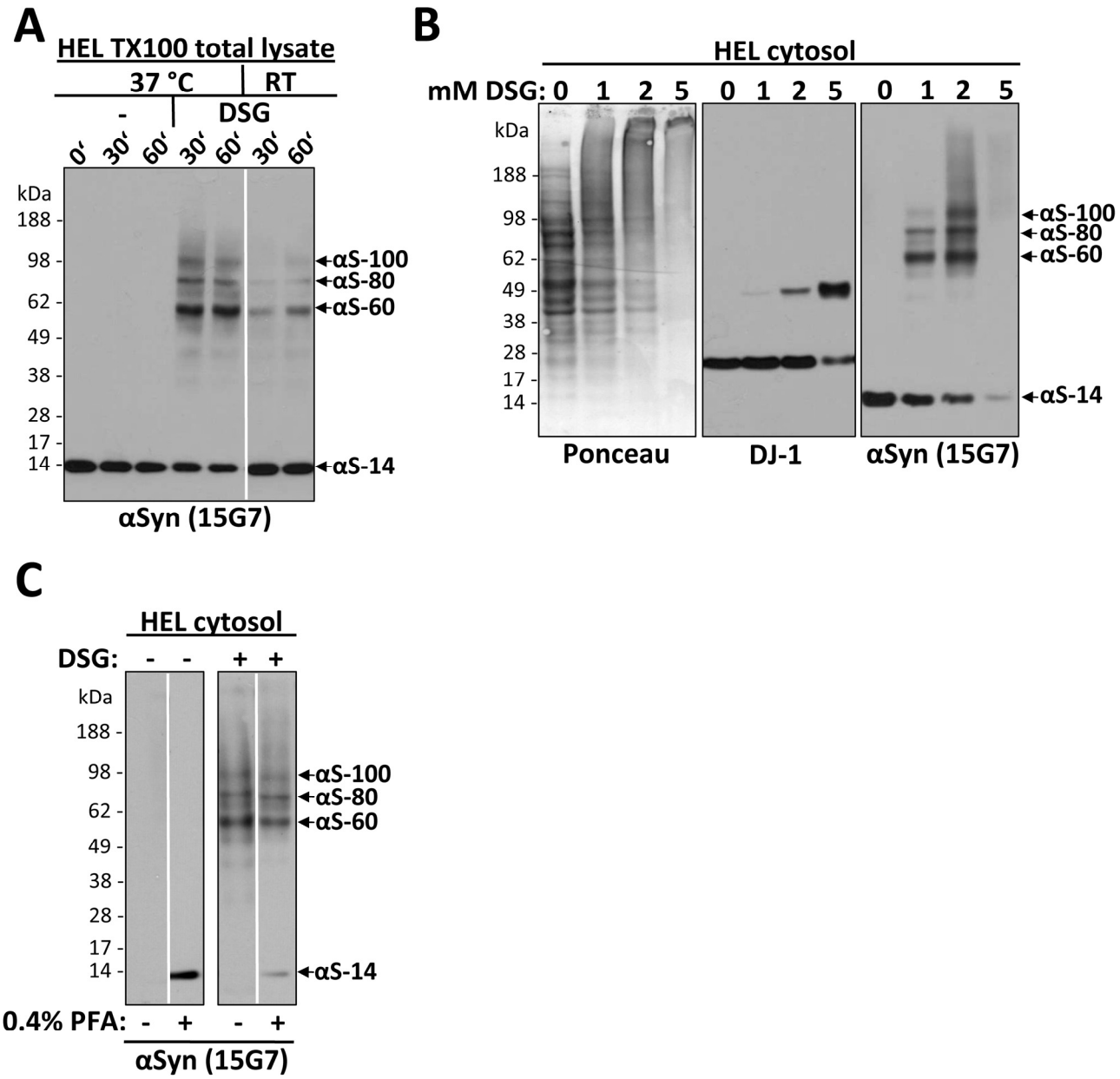


Fig. S1

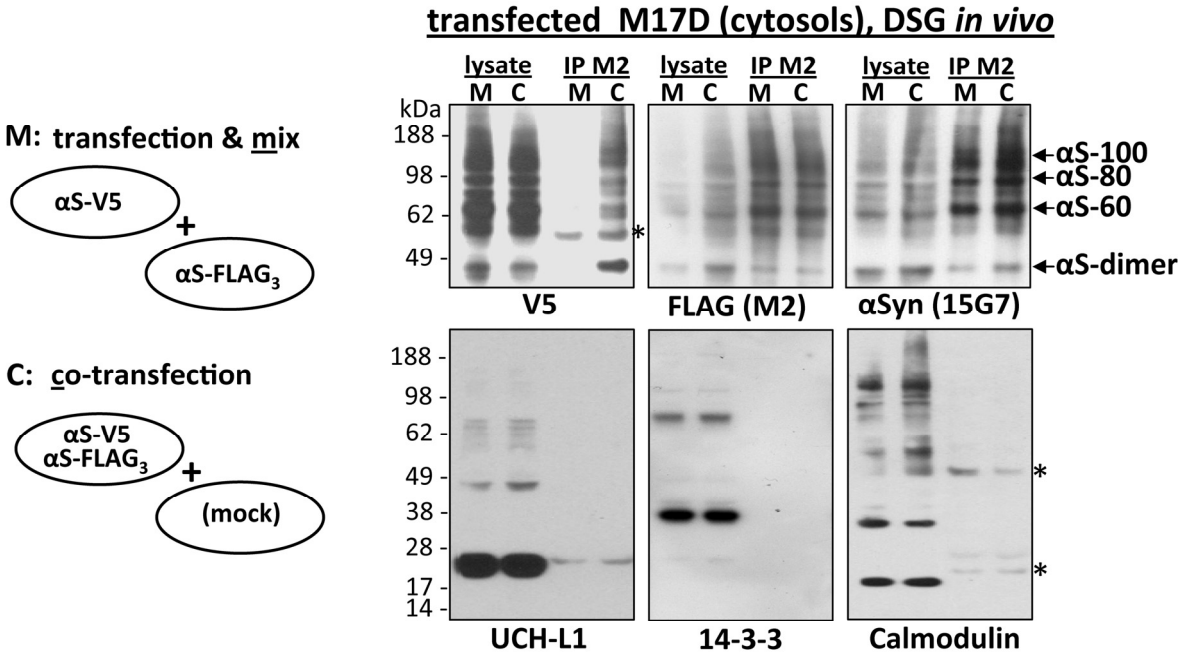
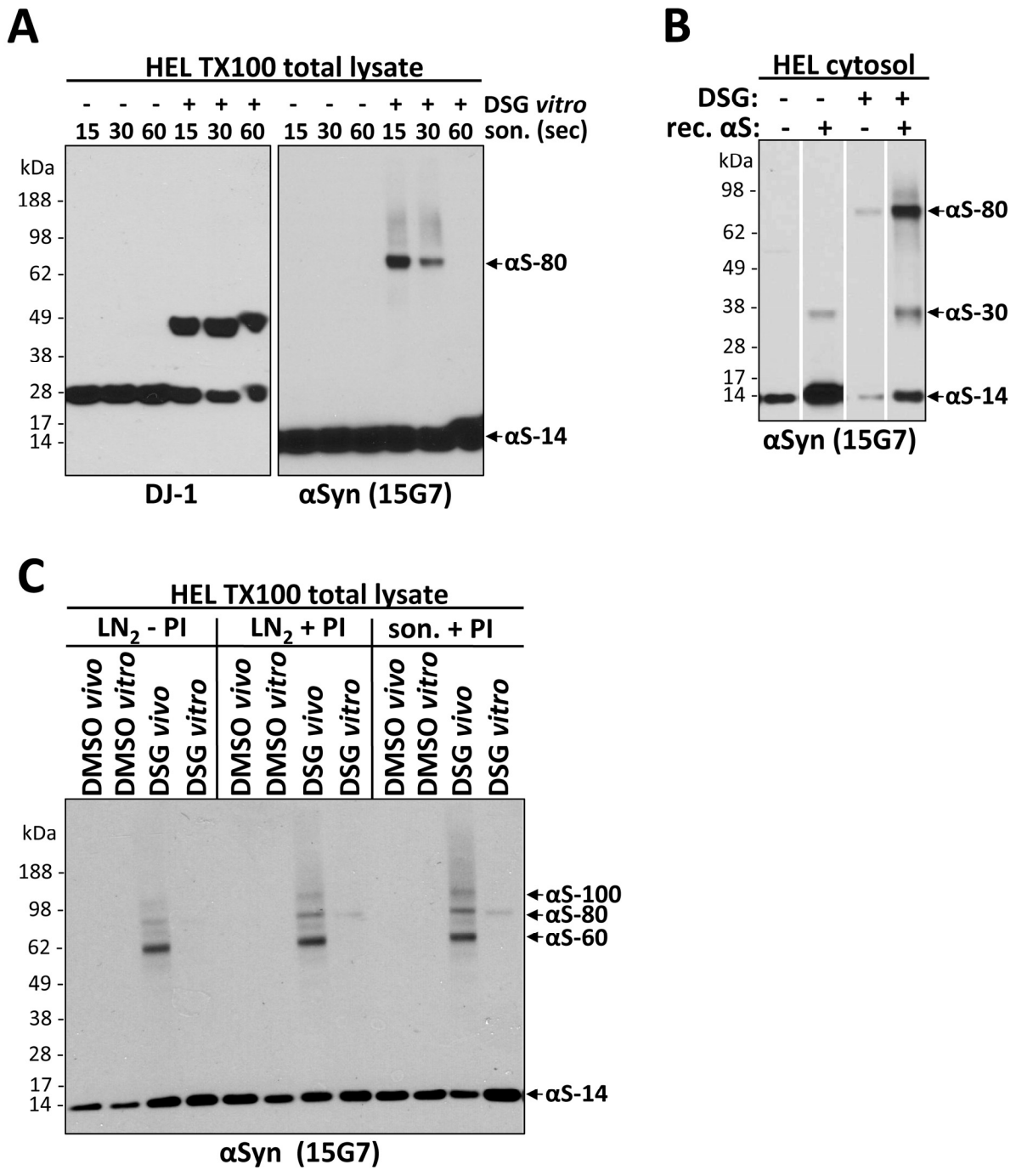

transfected M17D (cytosols), DSG in vivo
M: transfection & mix
αS-V5
+
αS-FLAG3
C: co-transfection
αS-V5
αS-FLAG3
+
(mock)
lysate
IP M2
M C M C
kDa
188
98
62
49
38
28
17
14
*
αS-100
αS-80
αS-60
αS-dimer
V5
FLAG (M2)
αSyn (15G7)
UCH-L1
14-3-3
Calmodulin

Fig. S2


A
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DMSO vitro
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αS-100
αS-80
αS-60
αS-14
αSyn (15G7)

Fig. S3

Supplementary Table 1 (Table S1)

Suggested αSyn interactor	kDa	Reference	Presence in crosslinked αSyn oligomers not supported by
HSP-70	70	Zhou et al, 2004 (1)	WB (Fig. 5A); size
β-tubulin	50	Payton et al, 2001 (2)	WB (Fig. 1B)
Parkin	50	Shimura et al, 2001 (3)	WB (Fig. 6A)
Synaptobrevin-2	20	Burré et al, 2011 (4)	WB (Fig. 6A, B); membr. loc.
Calmodulin	17	Lee et al, 2002 (5)	WB (Fig. S2)
UCH-L1	25	Liu, et al 2002 (6)	WB (Fig. S2)
14-3-3	30-33	Ostrerova et al, 1999 (7)	WB (Fig. S2)
Synphilin	130	Engelender et al, 1999(8)	size
MAP1B	> 250	Jensen et al, 2000 (9)	size
PLD2	> 100	Jenco et al, 1998 (10)0	size, membrane localization
Dopamine transporter	80	Torres et al, 2001 (11)	size, membrane localization
PKC	> 70	Ostrerova et al, 1999 (7)	size
p62	62	Kuusito et al, 2001 (12)	size
Tyrosine hydroxylase	59	Nakashima and Ikuta, 1984 (13)	size, tissue expression
ERKs		Iwata et al, 2001 (14)	membrane localization
Rab5A	26	Sung et al, 2001 (15)	membrane localization
Cytochrome-oxidase		Elkon et al, 2002 (16)	membrane localization

Table S1: Candidate-based exclusion of published αSyn interactors as components of a potential 60 kDa cytosolic hetero-oligomer; not all proteins shown have actually been proven to physically interact with αSyn, and some have not been confirmed independently. Among the suggested interactors, cytosolic proteins smaller than ~60 kDa and expected to be expressed in both HEL cells and neurons were analyzed by Western blotting (see Figures 1, 5, 6) or IP/Western blotting (see Fig. S2) for their possible presence in αS-60 (as well as αS-80 and αS-100); others were excluded because of their size, membrane-localization (αS-60, -80, -100 are cytosolic), or tissue expression. Because of their relative importance in the literature Synaptobrevin-2 and HSP-70 were analyzed despite their membrane-localization (Synaptobrevin-2) or size > 60 kDa (HSP-70), respectively. Abbreviations used: HSP-70, heat-shock 70 kDa protein; MAP1B, microtubule-associated protein 1B; PLD2, phospholipase D2; PKC, protein kinase C; p62, nucleoporin p62 protein; ERKs, extracellular signal-regulated kinases; Rab5a, Ras-related in brain 5a; UCH-L1, Ubiquitin carboxy-terminal hydrolase L1; membr. loc., membrane localization.

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