Supplementary Information

Synthetic cannabinoid JWH-018 increases α-Synuclein toxicity by ubiquitin-proteasome system impairment

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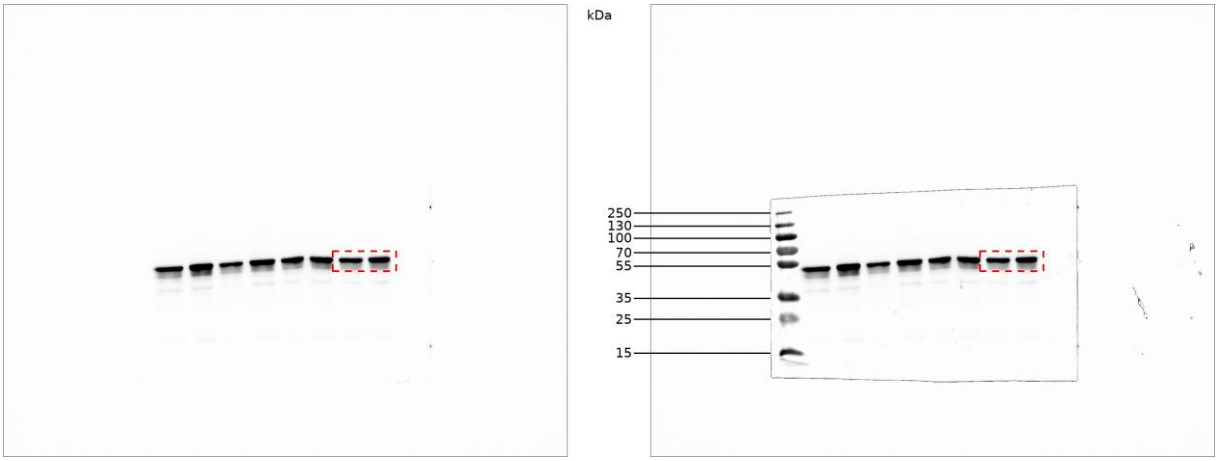
Full-length western blots

This file contains the full-length, uncropped western blot images corresponding to the cropped blots shown in Figs. 2b, 3a, 4a and 4c of the main text (Supplementary Figs. S1–S4). For each membrane, the *left* image is the complete chemiluminescence acquisition (entire field of view of the imaging system, uncropped). The *right* image is the same acquisition overlaid with the colorimetric image of the pre-stained molecular weight marker, acquired from the same membrane in the same position, so that marker bands and membrane borders are visible. The **red dashed box** marks the region displayed in the corresponding main figure. In all cases the lanes shown in the main figures are adjacent lanes of a single membrane; no lanes were removed or rearranged.

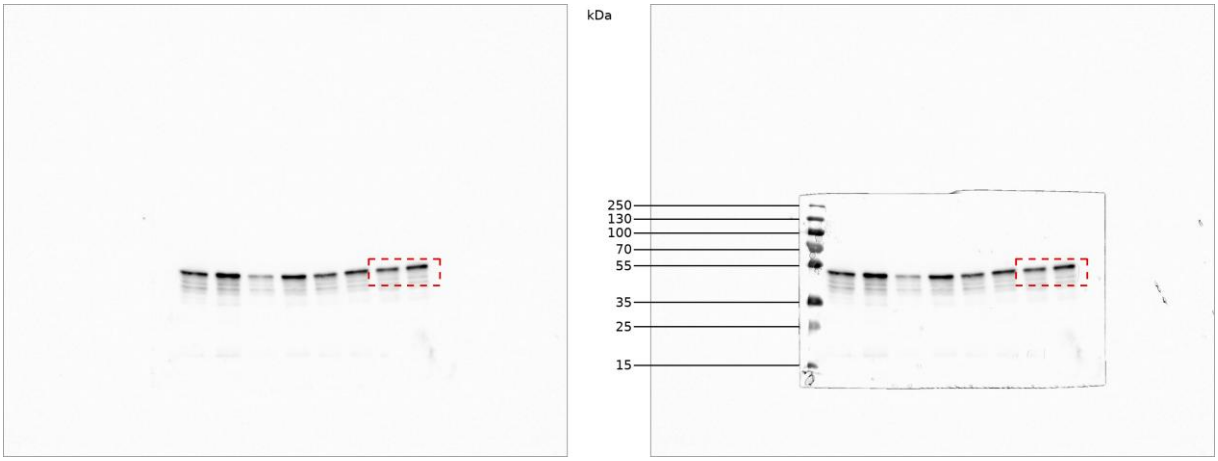
Each antibody (anti-αSyn, anti-pS129-αSyn, anti-GFP and anti-Pgk1) was detected on a separate membrane loaded with the same protein extracts and processed in parallel, as indicated by the different membrane outlines in the marker images. In the main figures, strips originating from different membranes are separated by white space. For display, only linear brightness/contrast adjustments were applied uniformly to each whole image; no non-linear (gamma) adjustments or local edits were made. Molecular weight marker: PageRuler™ Plus Prestained Protein Ladder, 10 to 250 kDa (Thermo Fisher Scientific; bands at 250, 130, 100, 70, 55, 35, 25, 15 and 10 kDa). Marker band sizes (kDa) are indicated to the left of each merged image. In some gels, electrophoresis was deliberately extended so that the 10 kDa band ran off the gel, in order to improve the resolution of the proteins of interest; in these cases the 10 kDa band is absent from the membrane.

Supplementary Figure S1. Full-length western blots corresponding to Fig. 2b.

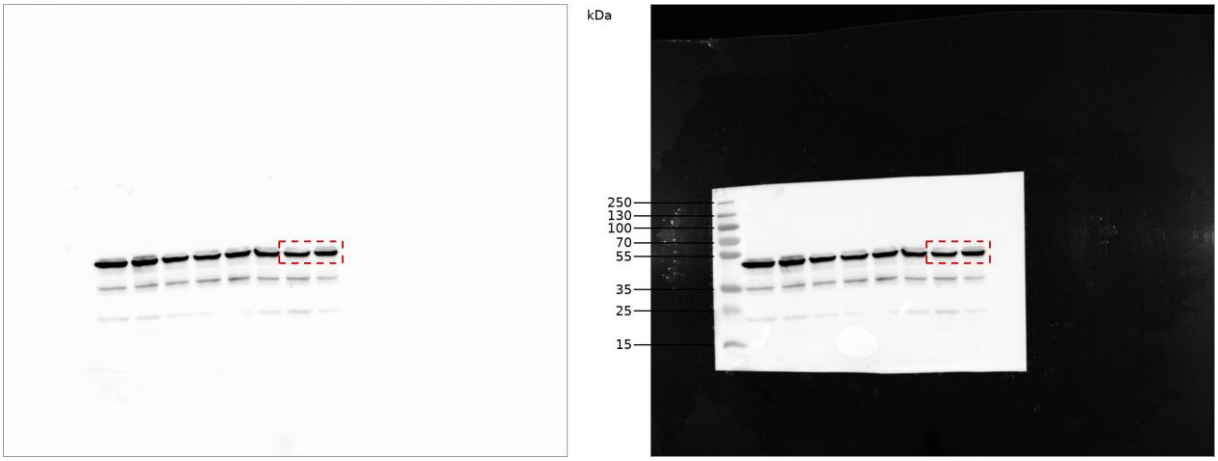
(a) Anti-αSyn (total αSyn).



(b) Anti-pS129-αSyn.



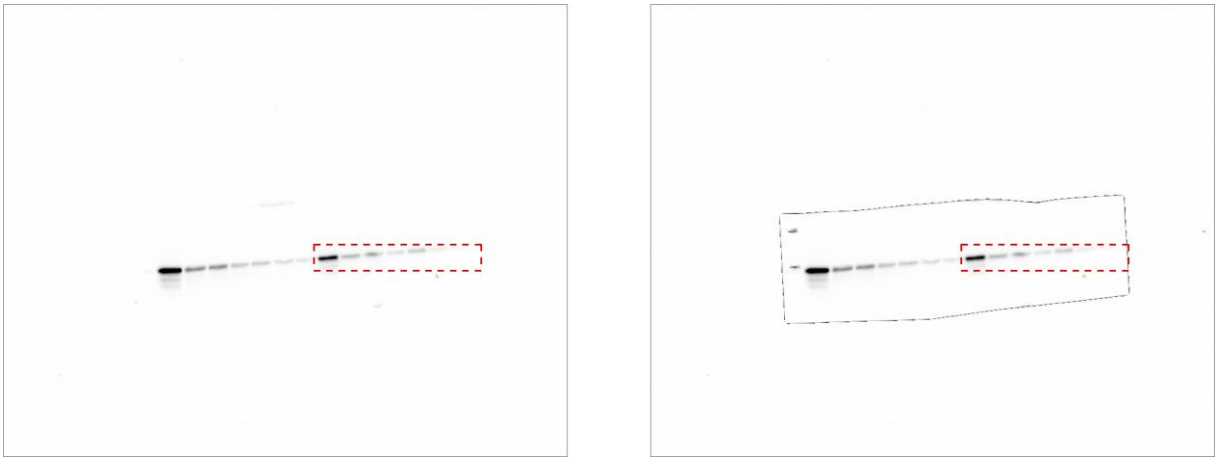
(c) Anti-Pgk1 (loading control).



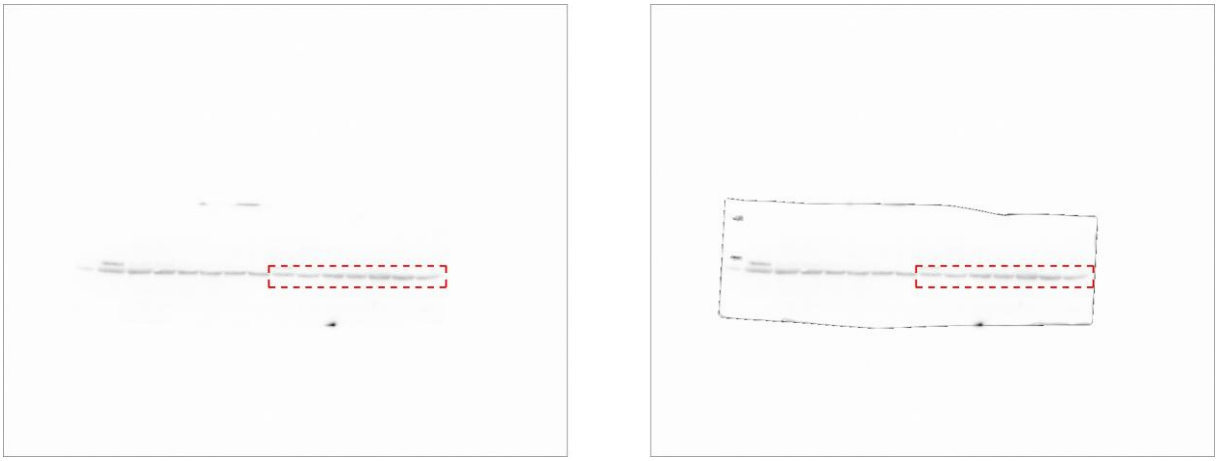
Total αSyn, pS129-αSyn and Pgk1 levels in αSyn-GFP-expressing BY4741 cells after 14 h of expression, untreated (0 μM) or exposed to 100 μM JWH-018. The boxed region (the two right-most lanes of each membrane; 0 μM and 100 μM JWH-018, left to right) is shown in Fig. 2b. In panel c, the right-hand image is the merged chemiluminescence/marker image exported by the imaging system (marker image shown on a dark background). Remaining lanes: independent experiments included in the densitometric analysis.

Supplementary Figure S2. Full-length western blots corresponding to Fig. 3a.

(a) Anti-αSyn.



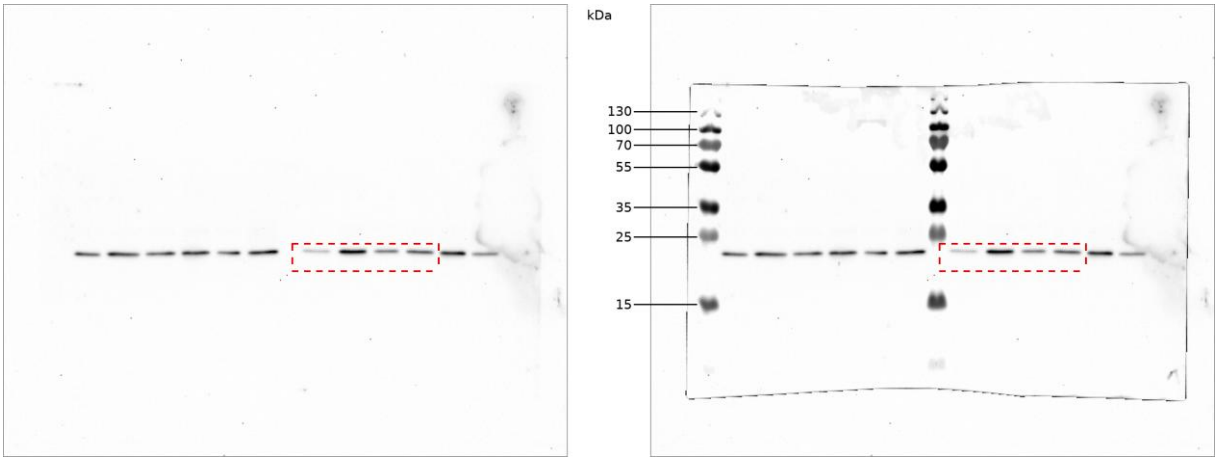
(b) Anti-Pgk1 (loading control).



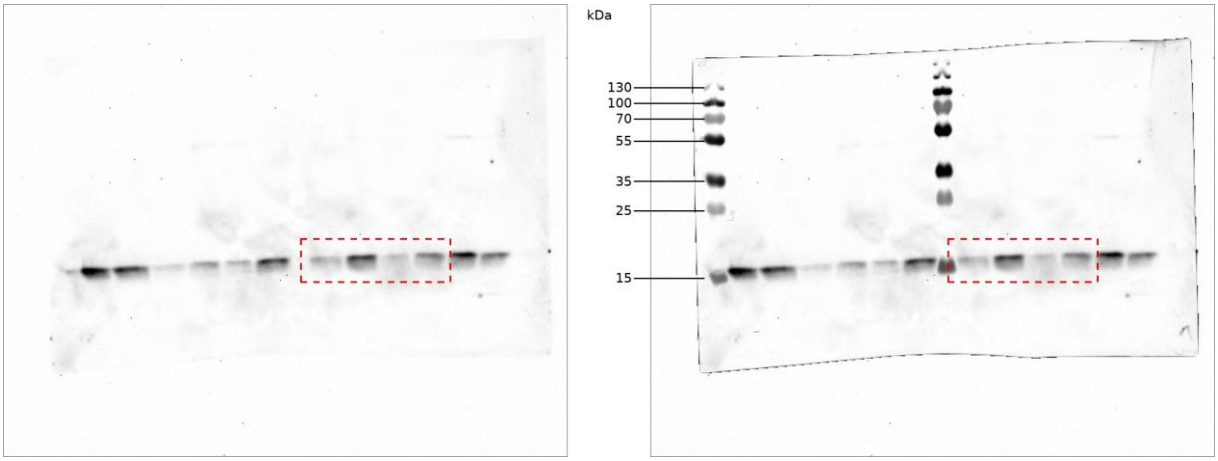
αSyn-GFP clearance assay: αSyn levels at 0 h and at 6, 9 and 12 h after promoter shut-off, in the absence (0 μM) or presence of 100 μM JWH-018. The boxed region (seven adjacent lanes: 0 h; 6 h 0/100 μM; 9 h 0/100 μM; 12 h 0/100 μM, left to right) is shown in Fig. 3a. The membranes were cut horizontally before immunodetection, so only the marker bands closest to the proteins of interest are retained on each strip. Remaining lanes: Remaining lanes: independent experiments included in the densitometric analysis.

Supplementary Figure S3. Full-length western blots corresponding to Fig. 4a.

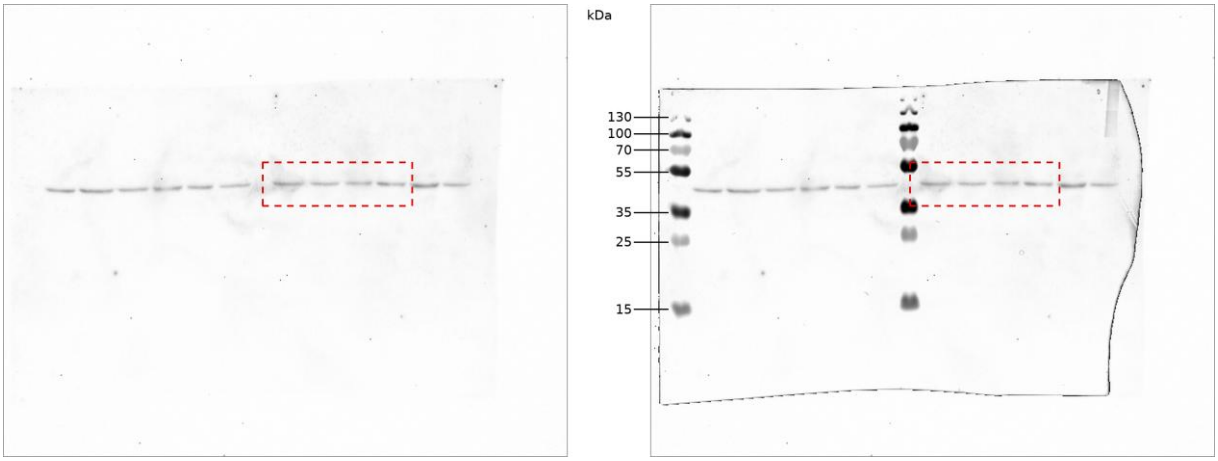
(a) Anti-GFP (GFPu).



(b) Anti-αSyn.



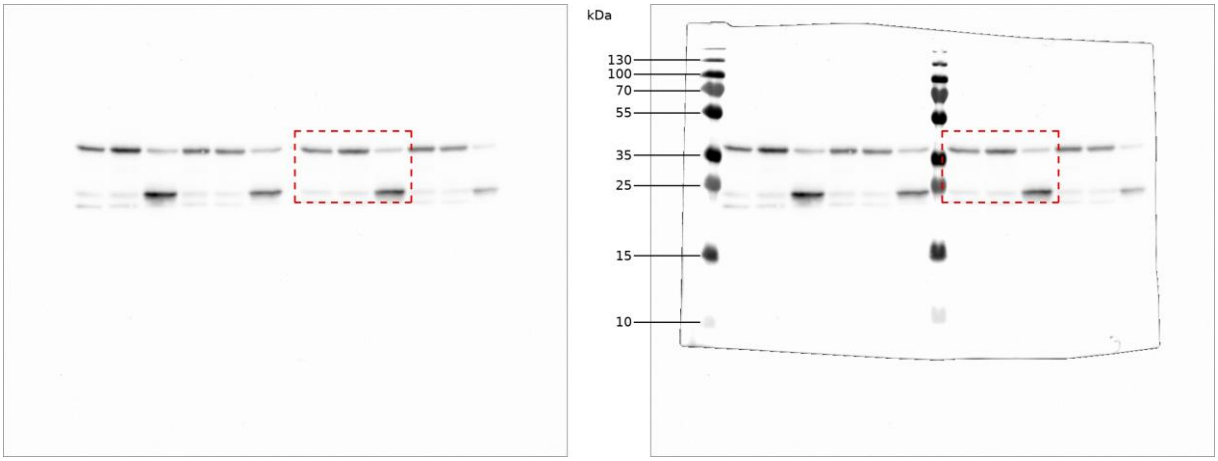
(c) Anti-Pgk1 (loading control).



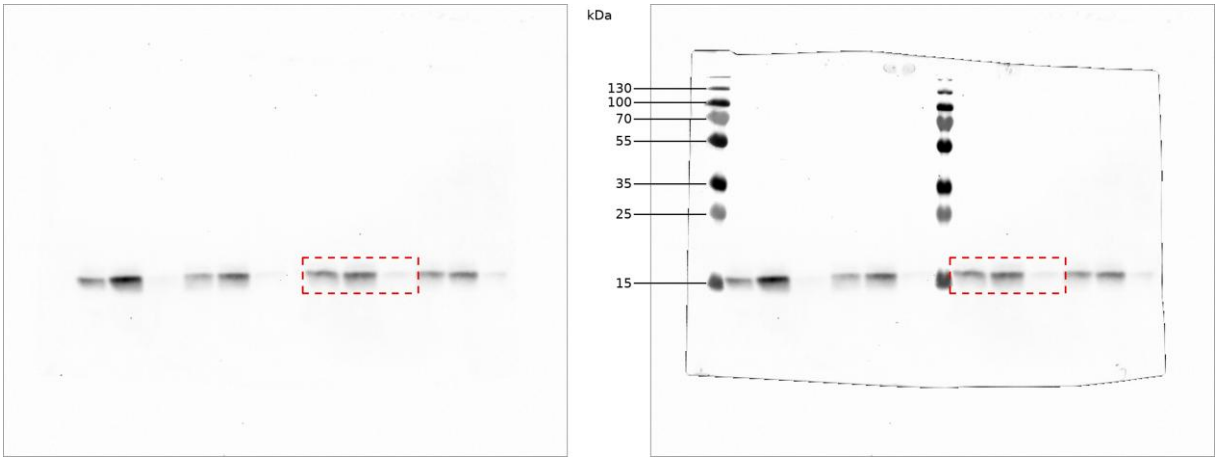
GFPu and αSyn levels in cells co-expressing αSyn and GFPu at 14 h and 18 h, untreated (0 μM) or exposed to 100 μM JWH-018. The boxed region (four adjacent lanes immediately to the right of the central marker lane: 14 h 0 μM, 14 h 100 μM, 18 h 0 μM, 18 h 100 μM, left to right) is shown in Fig. 4a. Remaining lanes: Remaining lanes: independent experiments included in the densitometric analysis.

Supplementary Figure S4. Full-length western blots corresponding to Fig. 4c.

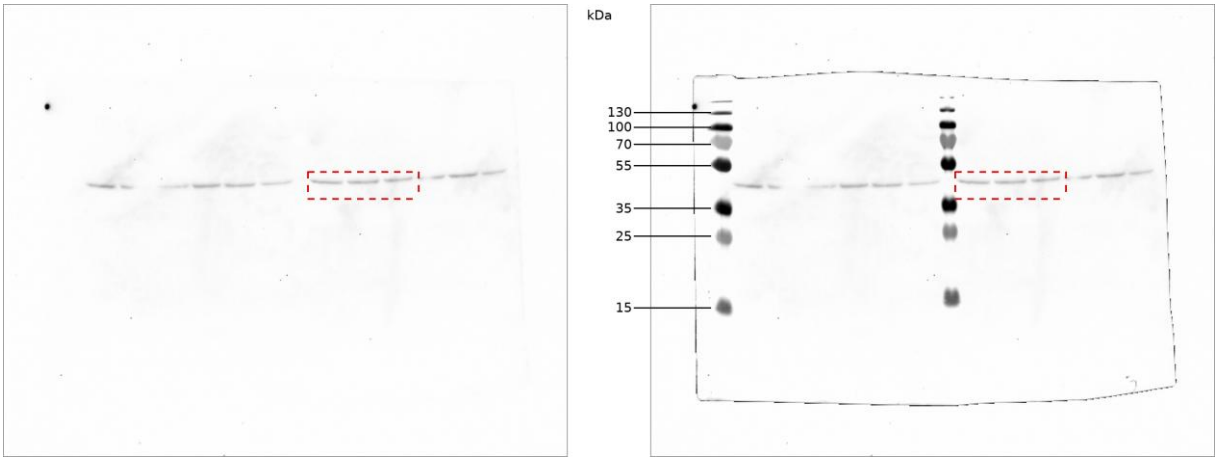
(a) Anti-GFP (upper band: GFP-Atg8; lower band: free GFP released by vacuolar degradation).



(b) Anti-αSyn.



(c) Anti-Pgk1 (loading control).



Autophagy assessment in cells co-expressing αSyn and GFP-Atg8 after 14 h, untreated (0 μM), exposed to 100 μM JWH-018, or subjected to starvation (C+, positive control of autophagy induction). The boxed region (three adjacent lanes immediately to the right of the central marker lane: 0 μM, 100 μM, C+, left to right) is shown in Fig. 4c. GFP-Atg8 and free GFP shown in Fig. 4c were detected on the same membrane (panel a). Remaining lanes: Remaining lanes: independent experiments included in the densitometric analysis.