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Supplemental Information

α-Synuclein Translocates to the Nucleus to Activate Retinoic-Acid-Dependent Gene Transcription

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Document S1. Supplemental figures and transparent methods

Figure S1. α-Syn localizes to the nucleus in the presence of retinoic acid (RA).

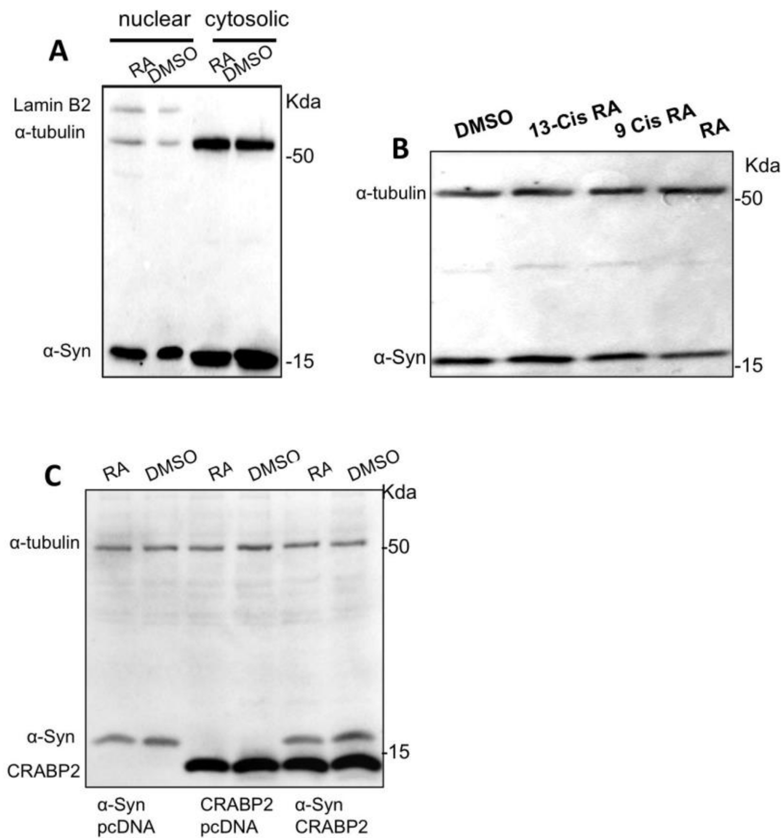


Figure S1. α-Syn localizes to the nucleus in the presence of retinoic acid (RA). Related to Figure 2 and Figure 3.

A. α-Syn expression was induced for 48 hours in SH-SY5Y cells with 1 μg/ml doxycycline. Cells were treated with RA (5 μM) or an equivalent amount of DMSO (vehicle) for 16 hours before harvest. Blot immunoreacted with anti α-Syn antibody, C-20; anti α-tubulin and anti Lamin B antibodies. Representative blot out of n=4.

B. MCF7 cells transfected with human wt α-Syn and treated with either one of the tested retinoids: RA, 9-cis RA, or 13-cis RA (at 1 μM) or DMSO for 3 hours. The cytosolic fractions analysed by Western blotting following immunoreaction with anti α-Syn and anti α-tubulin antibodies. A representative blot out of n=3 experiments.

C. A Western blot analysis of cytosolic fractions as in Fig. 3E.

Figure. S2. α-Syn associations with fatty acids do not enhance its nuclear translocation nor its transcription activation.

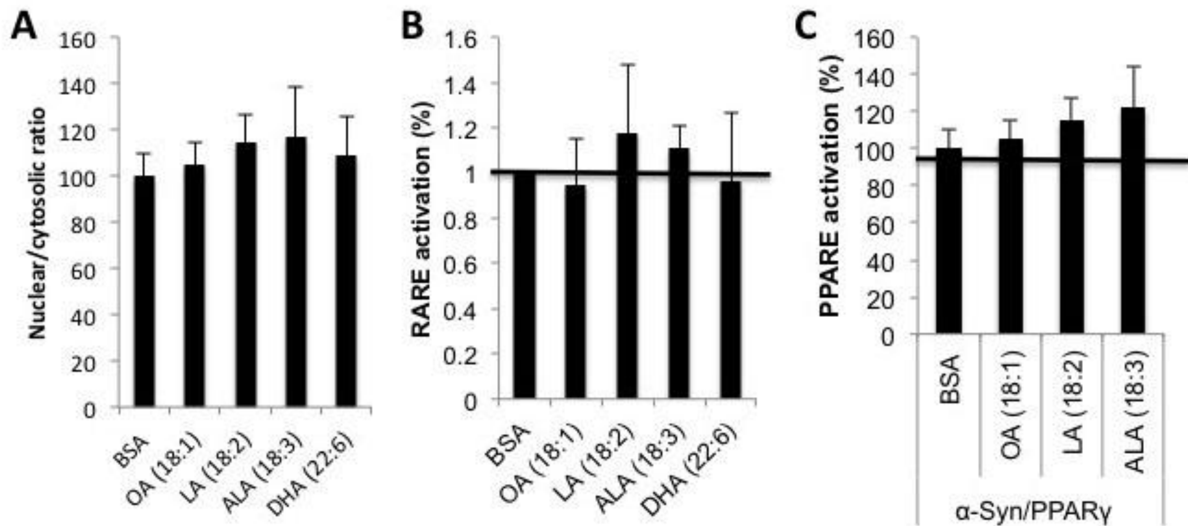


Figure. S2. α-Syn associations with fatty acids do not enhance its nuclear translocation nor its transcription activation. **Related to Figure 3.**

A. Bar graph showing the results of quantitative values, representing the nuclear to cytosolic ratio of α-Syn signal obtained by Western blotting. MCF7 cells transiently transfected to express α-Syn for 48 hours. Cells were conditioned in DMEM supplemented with 0.1% serum together either with the indicated fatty acids (0.25 mM fatty acid, 0.05 mM BSA) or BSA (0.05 mM) for 16 hours before harvest. Nuclear and cytosolic fractions were analyzed by Western blotting and immunoreacted with anti α-Syn antibody, C-20. Mean ± SD of n= 2 independent experiments.

B. MCF7 cells were co-transfected to express α-Syn, RAR response element (RARE)-driven luciferase reporter gene and β-galactosidase (β-gal) plasmids. Cells were treated with the indicated fatty acids (0.25 mM and 0.05 mM BSA) for 16 hours. Control cells were treated in parallel with BSA only (0.05 mM). Luciferase activity was determined and normalised to β-gal activity and to the protein level in the sample. Vertical bar represents control cells, transfected with a mock plasmid (set at 100%), showing no significant differences. Oleic acid (OA; 18:1), Linoleic acid (LA; 18:2), alpha linolenic acid (ALA; 18:3) and docosahexaenoic acid (DHA; 22:6).

C. MCF7 cells were co-transfected to express α-Syn, PPAR response element (PPARE)-driven luciferase reporter gene, PPARγ2 and β-gal plasmids. Cells were treated with the indicated fatty

acids (0.25 mM with 0.05 mM BSA) or with BSA for 16 hours. Normalized luciferase activity is presented as percent of control cells, that express a mock plasmid and were analysed in parallel (represented by the vertical bar; mean ± SD of n=3-4 experiments).

Figure. S3. α-Syn binds RA.

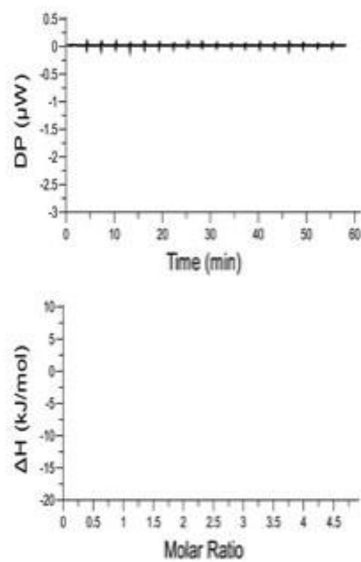


Figure. S3. α-Syn binds RA. Related to Figure 5.

Isothermal titration calorimetry (ITC) measurements showing titration of 100 microM RA in the absence of α-Syn protein.

Figure. S4. Alterations in nuclear receptor activation with RA and α-Syn

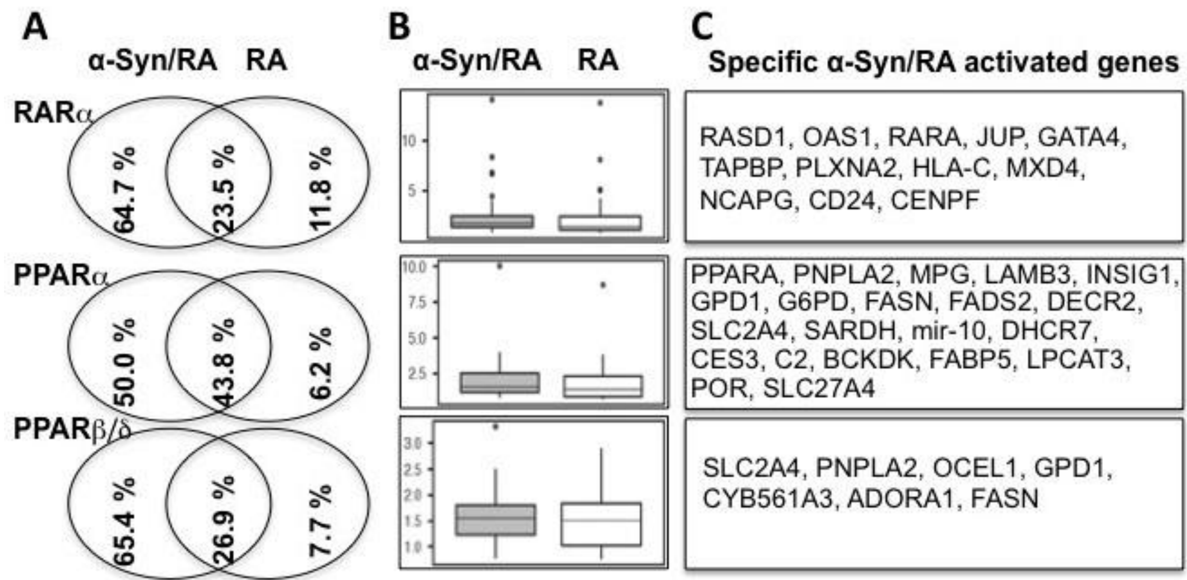


Figure. S4. Alterations in nuclear receptor activation with RA and α-Syn. Related to Figure 6.

A. Venn diagrams showing the distribution of the differentially expressed genes (as in Fig. 6B), presented in percent of the altered genes, down stream of each nuclear receptor.

B. Box plots showing the fold changes in expression of the shared differentially expressed genes in both treatments, RA vs. α-Syn/RA. Presented as log (2) fold change.

C. List of differentially expressed genes specifically altered by α-Syn associations with RA downstream to the respected nuclear factor.

Figure. S5. Nuclear localization of α-Syn is calreticulin-dependent

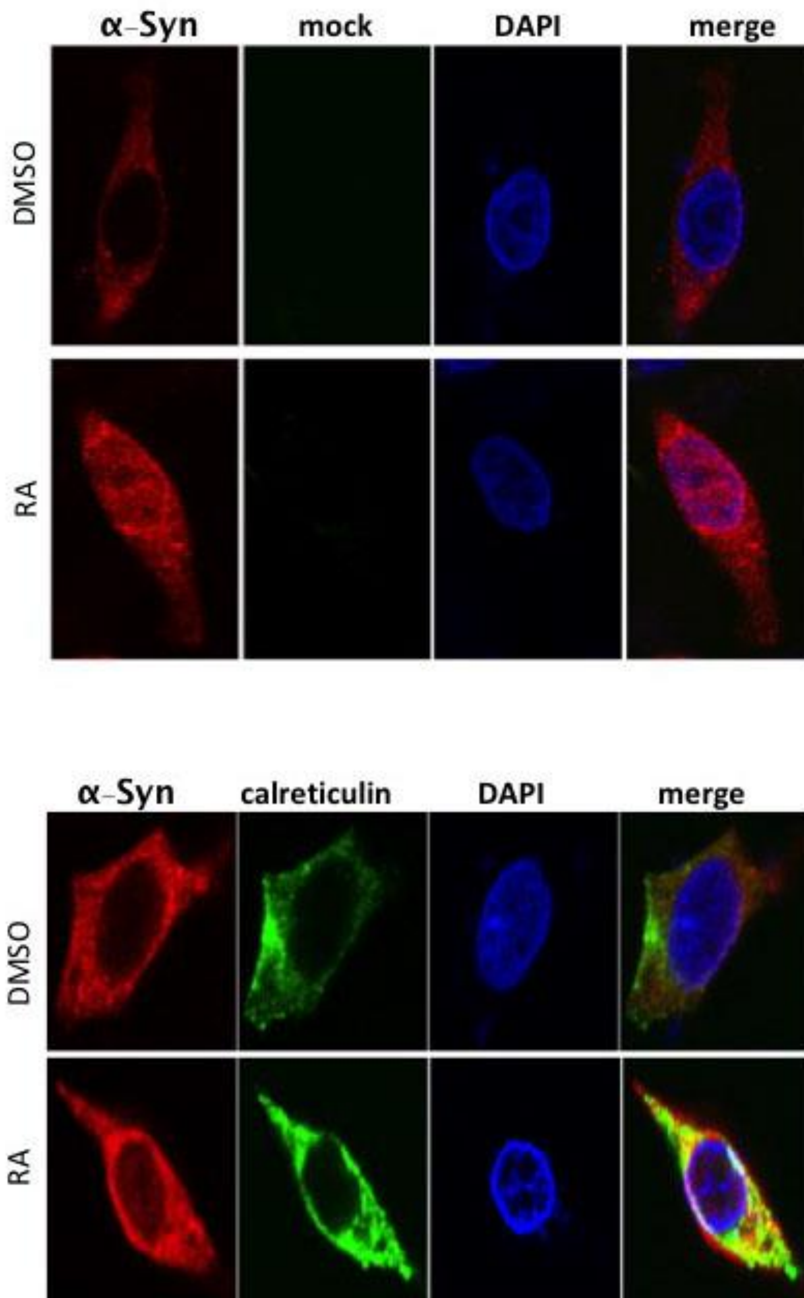
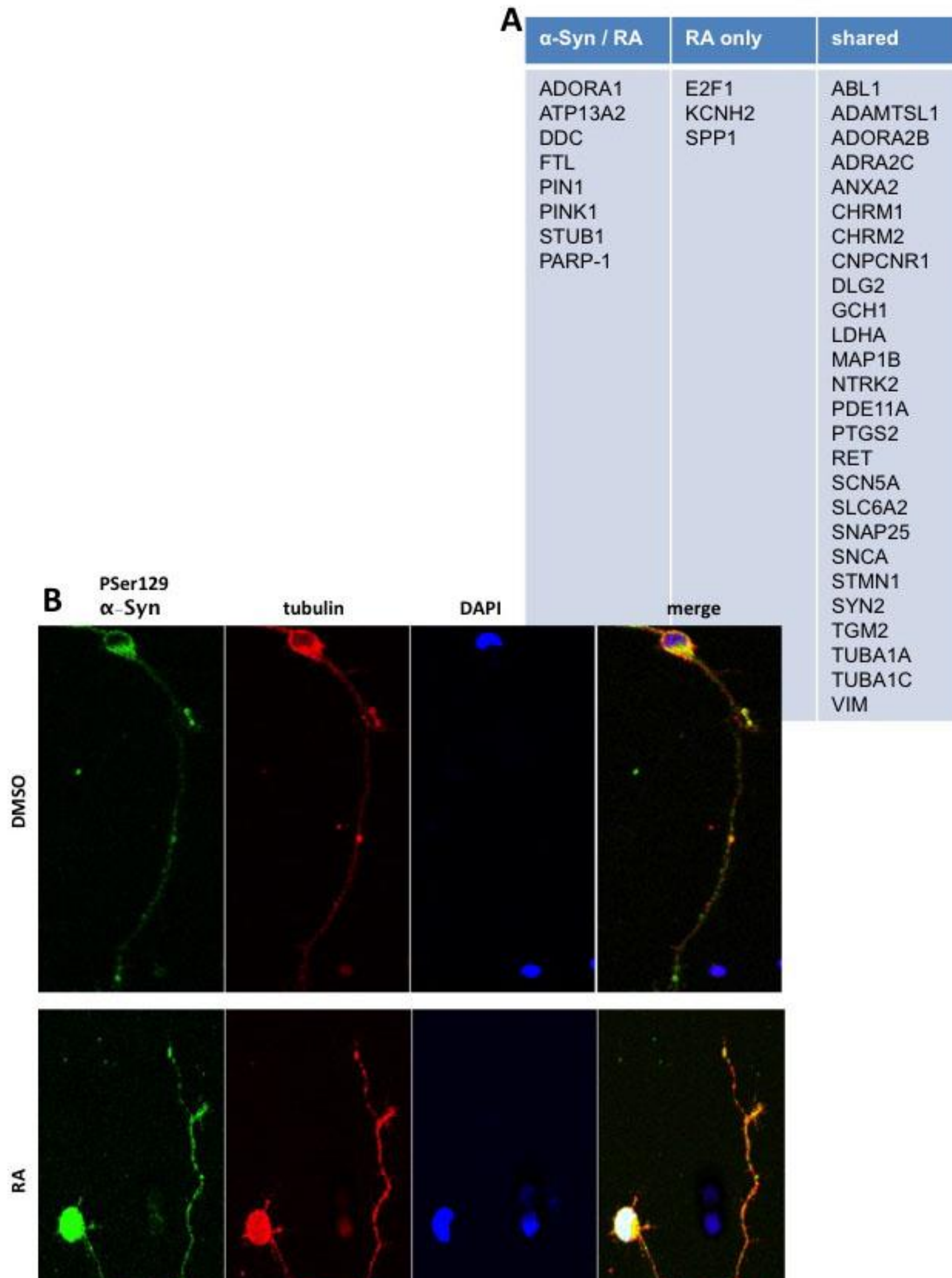


Figure. S5. Nuclear localization of α-Syn is calreticulin-dependent. Related to Figure 7. MCF7 cells transfected to express α-Syn and calreticulin-m-Cherry. Control cells transfected in parallel with α-Syn and a mock plasmid. Cells were treated with RA (1 μM) or DMSO for 3 hours and processed for immunocytochemistry with the anti α-Syn ab, MJF-1. Calreticulin observed directly through its m-Cherry tag (580 nm).

Figure. S6. Nuclear localization of α-Syn is linked to Parkinson's disease



α-Syn / RA	RA only	shared
ADORA1	E2F1	ABL1
ATP13A2	KCNH2	ADAMTSL1
DDC	SPP1	ADORA2B
FTL		ADRA2C
PIN1		ANXA2
PINK1		CHRM1
STUB1		CHRM2
PARP-1		CNPCNR1
		DLG2
		GCH1
		LDHA
		MAP1B
		NTRK2
		PDE11A
		PTGS2
		RET
		SCN5A
		SLC6A2
		SNAP25
		SNCA
		STMN1
		SYN2
		TGM2
		TUBA1A
		TUBA1C
		VIM

Figure. S6. Nuclear localization of α-Syn is linked to Parkinson's disease. Related to Figure 8.

A. Differentially expressed PD- associated genes identified with IPA analysis in α-Syn/RA or RA-only treated cells, or in both treatments.

B. Primary cortical neurons from A53T α-Syn tg mouse brain at 14 DIV. Neurons treated and immunoreacted with anti PSer129 α-Syn antibody as in Fig. 8H, demonstrating the entire neuronal

cell.

Transparent Methods

Plasmids. RARE-luc plasmid (pGL3-RARE-luciferase) from T. M. Underhill (Addgene plasmid # 13458(Hoffman et al., 2006)); PPARE-luc and PPARγ plasmids from Prof. Yaakov Nahmias (Hebrew University); HNF4RE generated by cloning the sequence of the response element: GGCAAAGGTCATGGCAAAGGTCATGGCAAAGGTCATGGCAAAGGTCATGGCAAAGGTCAT (Sladek et al., 1990) into PGL2 promoter vector (Promega, WI, USA); HNF4α plasmid form Dr. Rachel Hertz (Hebrew University). mCherry-calreticulin from Michael Davidson (Addgene plasmid # #55006). shRNA calreticulin (Mission shRNAi, SHCLNG-NG-004343 Sigma Aldrich).

Inducible α-Syn expression in SH-SY5Y cells. A Tet-On 3G system Tetracycline-Inducible Expression Systems (Clontech Laboratories, CA, USA) was used. Stable clones, expressing either human wt α-Syn or a mock plasmid, downstream of pCMV-Tet 3G plasmid, were generated according to manufacturer instructions. α-Syn expression induced with 1 μg/ml doxycycline (Clontech Laboratories, CA, USA).

RNAseq and analysis for upstream regulators. Total RNA was extracted from confluent 10 cm dishes following 0, 24 and 72 hours (in duplicate) of conditioning the cells in the presence of doxycyline (1 μg/ml, in standard serum-supplemented DMEM), using RNeasy Plus mini kit (Qiagen, Eldan Petach-Tikva, Israel). The quality of the RNA was tested using RNA Analysis Screen-Tape, Agilent 2200 Tape-Station system (Agilent, Eldan, Petach-Tikva, Israel). Library preparations were performed with Truseq RNA kit (Illumina, Danyel Biotech, Rehovot, Israel) from 300 ng of total RNA and analyzed by next generation whole transcriptome sequencing Hiseq, single end sequencing method (Illumina, CA, USA; performed at the Technion Genome Center, Haifa, Israel). Data were analyzed by Fast-QC, for the quality of the raw sequence data and ENSEMBL, for reference genome and annotations. The following analyses were performed: STAR-2.3.0, to align reads to reference genome; Htseq count, for counting number of mapped reads per gene; and DESeq2 package v1.6.3, for normalization and statistical analysis.

RA treatment:

SH-SY5Y cells: Cells were conditioned in DMEM containing 0.1 % serum and RA (1 or 5 μM) or an equivalent amount of the DMSO (0.1%) solvent for 16, 24 or 48 hours before harvest.

MCF7 cells: Cells were conditioned in DMEM containing 0.1% serum (RA starvation) for 16 hours. RA (1μM or 50 nM, as indicated) or 0.1% DMSO, were added to the conditioning medium and cells were conditioned for additional 3 hours. In experiments in which RA treatment was longer than 3 hours we did not starve the cells prior to the treatment.

Primary neurons: 24 hours before harvest, the conditioning medium was replaced to contain medium supplemented with vitamin A-deficient, B27 serum (Biological Industries, Beit Haemek, Israel). RA (1 μM or 50 nM) or DMSO (0.1%) were added to the cells for 6 or 24 hours.

Differential expression following RA or fatty acid treatments

RNA extraction, quality and library preparation was as described above. Illumina HiSeq3000 was used to generate paired-end sequencing with a read length of 150 bp. Minimum 40 million reads were generated per sample. Quality control assessment was done using Illumina RNA-seq pipeline to estimate genomic coverage, percent alignment and nucleotide quality. Raw sequencing data were transformed to fastq format.

Normalization and differential expression were done with the DESeq2 package (version 1.14.1) (Love et al., 2014). The following samples were analyzed: SH-SY5Y cells induced to express α-Syn (with 1 μg/ml doxycycline) and treated for 16 hours in DMEM supplemented with 0.1% serum and either one of the following, RA (5 μM); DMSO (0.1%); ALA (18:3, at 0.25 mM together with 0.05 mM BSA); BSA (0.05 mM) or no treatment (i.e., standard serum-supplemented medium). Cells were collected and analyzed following 72 hours from induction of α-Syn expression. Control samples included an identical set up but without induced α-Syn expression. In addition, the effect of doxycycline on gene expression was tested in induced SH-SY5Y cells expressing a mock plasmid. Total of 12 different treatments, each treatment was tested in triplicate dishes. Normalization with all samples showed that the different treatments had distinct patterns of expression, thus measuring the effect of each treatment was done only with the samples of the relevant treatment. Quality control assays, such as count distributions and principal component analysis, were calculated and visualized in R (version 3.4.1, with packages 'RColorBrewer_1.1-2', 'pheatmap_1.0.8', 'ggplot2_2.2.1' and ggrepel_0.7.0).

The following differential comparisons were performed: A. RA versus DMSO treated cells with induced α-Syn expression; B. RA versus DMSO treated cells without induced α-Syn expression.

Similarly, the effect of fatty acids associations with α-Syn were analyzed by comparing: C. Fatty acids versus BSA treated cells with α-Syn expression; and D. Fatty acids versus BSA without induced α-Syn expression. Then, differential expression was calculated for each system (either RA or fatty acid) with a design of two factors and their interaction as the full model, and omitting the interaction in the reduced model, using the LRT test. The two factors were α-Syn expression (induced or not induced) and the respective treatment (ligand or vehicle). Results were obtained with default parameters, except not using the independentFiltering algorithm, and setting the significance threshold to padj<0.05. The results showed that for many genes, even minor changes were considered significant (probably due to a very high reproducibility between replicates). In order to somewhat filter the results and consider only genes with a stronger effect (fold-change), additional filtering was applied according to the following criterion: significant genes were considered only if their baseMean expression was above 5, and their absolute log2FoldChange was above 5/baseMean^0.5 + 0.6, for the RA system, or 5/baseMean^0.25 + 0.6, for the FA system. This criterion filters genes in an expression-dependent manner, allowing for a fold-change of ~1.5 at very high expression levels, and requiring a fold-change above 7 at the very low expression levels. Only genes that were significant and passed the additional criterion were used in further advanced analysis with IPA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis) in order to find enriched pathways, molecular functions and up-stream regulators. IPA® Z-scores greater than 2 or smaller than -2 is considered to be significantly predicted to be up or down- regulated, respectively. P- value of overlap of 0.01 or less is considered significant. Box plots of common downstream genes of each nuclear receptor were generated by ggplot2 R package and its geom boxplot function.

The accession number for the data reported in this paper is GEO: GSE145804

Total RNA extraction and quantitative (q)PCR. Mice (equal numbers of males and females) were anesthetized with isoflurane (2.5%; Piramal Healthcare Limited, Digwal, India). Brains were removed, divided in hemispheres and kept frozen. Total RNA was isolated with TRI-Reagent (MRC, Cincinnati, OH, USA) from one hemisphere of C57BL⁄6 wt or homozygous PrP-A53T α-

Syn tg mouse brains (Giasson et al., 2002) (Jackson laboratory, Bar Harbor, ME, USA); or from cultured cells, transfected to express human wt α-Syn or a mock plasmid using ICAFectin 411(Incellart, Nantes, France). The RNA was converted to cDNA using High Capacity cDNA, Reverse Transcription Kit (Applied Biosystems, Foster City, CA USA). Primer pairs designed to exon-exon boundaries by Primer3 (v3.0.1 software). Primers sequence is listed in supplementary Table 1. Results were normalized against the expression levels of G6PD (cultured cells of human origin) and 18S for (mouse brains and primary neurons). In experiments in which cells were transduced with lenti virus, results were normalized to the WPRE gene. Analyses were performed using Applied Biosystems Step One Software v2.2.2 with CYBR Green Master Mix (Applied Biosystems Foster City, CA USA).

Cell fractionation, protein extraction and Western blotting. Cultured cells (5x 10^7) collected and spun at 1000 xg. Cell pellet was washed twice in PBS^{++} and re-suspended in 250 μl homogenization (H) buffer [20 mM HEPES, pH 7.4; 1 mM $MgCl_2$; 0.32 M sucrose; 43 mM 2-mercaptoethanol; and 1 x protease inhibitor mix (Sigma-Aldrich, Rehovot, Israel)]. Cells were incubated on ice for 20 minutes and homogenized by eight passages through a 25-gauge needle.

Fractionation: All procedures were performed at 4°C. Cell homogenate was centrifuged at 1,700 xg for 10 min. The supernatant was transferred to a clean tube and spun at 25,000 xg for one hour. The resultant cytosolic fraction was removed to a clean tube. The pellet was washed in H-buffer and re-spun at 1,700 xg for 15 minutes. The washed pellet was re-suspended in H-buffer; brought to 2.1 M sucrose and spun at 25,000 xg for 1 hour. The pellet (consisting of nuclei) was collected, washed and re-suspended in H-buffer containing 1% Nonidet P-40.

Western blotting: Protein samples of cytosolic or nuclear fractions (20 μg) were loaded on a 10% SDS-PAGE and following electrophoresis, proteins were transferred to nitrocellulose membrane (Whatman, Sigma-Aldrich, Rehovot, Israel). The membrane was blotted with an anti α-Syn antibody C-20 (1:2000; Santa Cruz Biotechnology; Petach Tiqva, Israel) or MJF-1 (1:2000, Abcam, Zotal, Tel-Aviv, Israel), anti CRABP2 antibody (1:2000; Millipore, CA, USA), anti Lamin B2 (1:2000; Abcam, ab8983, Zotal, Tel-Aviv, Israel) anti RanBP1 (Abcam, ab8983, Zotal, Tel-Aviv, Israel) or anti α-Tubulin (1:2000; Serotec, Oxford, UK). The immuno-blots were reacted with

a secondary, HRP-conjugated, antibody and visualized with Clarity Western ECL Substrate (Bio-Rad, Rishon Le Zion, Israel), scanned with ChemiDoc XRS$^+$ imaging system (Bio-Rad, Rishon Le Zion, Israel) and the density of signal was quantified using UN-SCAN-IT GEL 3.1 software (Silk Scientific, Orem, UT, USA). The quality of fractionation was determined according to the signal obtained for the marker proteins: Lamin B2 for nuclear fraction and α-tubulin for cytosolic fraction.

Immunocytochemistry (ICC). Cultured cells were seeded on cover slides pre-coated with Poly-D Lysine (0.1 mg/ml for 1 hour at 37°C). On the next day doxycycline was added to the conditioning medium to induce α-Syn expression (SH-SY5Y); or cells were transfected as indicated (MCF7 cells). Cells were washed in warm PBS and fixed in 4% paraformaldehyde for 10 minutes on ice. The slides were then blocked in a solution containing 1.5% BSA in PBS. For α-Syn detection, either C-20 ab (1:500; Santa Cruz Biotechnology; Almog Diagnostic, Petach Tiqva, Israel) or MJF-1 (Abcam; Zotal, Tel-Aviv, Israel) were used (as indicated). The secondary antibodies were Texas Red (1:200, Jackson Immuno Research, PA, USA) or Dy light 649 (1:150 Jackson ImmunoResearch, Baltimore, USA). Mounting with Vectashield (Vector Laboratories, Burlingame, CA, USA).

Mice. The human PrP-A53T α-Syn tg mouse line(Giasson et al., 2002) was purchased from Jackson Laboratory (Bar Harbor, ME, USA) as hemizygous; cross-bred with C57BL/6JOlaHsd α-Syn$^{-/-}$ mice (Harlan Laboratories, Jerusalem, Israel (Specht and Schoepfer, 2001)) to silence endogenous mouse α-Syn; and then bred to achieve homozygosity of the human A53T α-Syn transgene. C57BL/6 wt mouse brains were used as control mice. All animal welfare and experimental protocols were approved by the Committee for the Ethics of Animal Experiments of the Hebrew University of Jerusalem NIH approval # OPRR-A01-5011 (Permit number: MD-16-14826-3).

Primary neuronal cultures.
Cortical neurons were prepared from C57BL/6 wt mouse brains as in (Ben Gedalya et al., 2009) with minor modifications. Briefly, hippocampal CA1-CA3 regions were dissected from 1 day-old C57BL/6 mouse brains, dissociated by trypsin treatment, followed by trituration with a siliconized Pasteur pipette and then plated onto coverslips coated with poly-D-lysine (Sigma-Aldrich, Rehovot, Israel) inside 24-well dish. Culture medium consisted of MEM (Invitrogen, Rhenium,

Israel), 0.6% glucose, 0.1 gm/L bovine Tf (Sigma-Aldrich), 0.25 gm/L insulin (Sigma-Aldrich), 0.3 gm/L glutamine, 5–10% fetal calf serum (Sigma- Aldrich) and 2% B-27 supplement (Invitrogen, Rhenium, Israel). To eliminate the glial cells, 8 mM cytosine b-D-arabinofuranoside (Sigma-Aldrich) was added to the culture 3 days after preparation and removed after additional 3–4 days. Cultures were maintained at 378C in a 95% air/5% CO2 humidified incubator, and culture medium was replaced every 4–7 days.

Mesencephalic neurons were prepared from brains of mice at E13.5 embryos as described (Zaltieri et al., 2015) with minor modifications. Briefly, ventral mesencephalic tissues were dissected from C57BL/6J and C57BL/6JOlaHsd mice at embryonic day 13.5. After mechanical dissociation, the single cell suspension was resuspended in Neurobasal medium (Biological Industries, Bet Haemek, Israel) containing 100 µg/ml penicillin, 100 µg/ml streptomycin (Sigma-Aldrich, Rehovot, Israel), 2 mM glutamine (Biological Industries) and 1% B27 supplement (Biological Industries); cells were then centrifuged. Cell count and viability assays were performed using the Trypan Blue exclusion test. Cells were seeded onto poly-D-Lysine-coated glass coverslides in 24-well plates for immunocytochemistry, or onto poly-D-Lysine coated Petri dishes for western blot or transmission electron microscopy analyses. Cells were maintained at 37°C under a humidified atmosphere of 5% CO_2 and 95% O_2 in Neurobasal medium for 10 days *in vitro*.

Electroporation of primary neurons was performed on day of preparation using Amaxa Nucleofecto (Lonza, Tuas, Singapore) according to manufacture's protocol (Vieira et al., 2016). 0.2- 1.0 × 10^6 cells were suspended in 100µl of Ingenio electroporation solution (Mirus Bio LLC, Madison, WI, USA) containing 2.5 µg of DNA, in a nucleofection cuvette using program O-05. Cells were then plated as above.
Images were acquired using a Zeiss LSM 710 Axio Observer confocal Z1 laser scanning microscope, equipped with an argon laser 488, Diode 405-430 laser and HeNe 633 laser. Per each experiment, exciting laser, intensity, background levels, photo multiplier tube (PMT) gain, contrast and electronic zoom were maintained constant. For each antibody, the background was subtracted (determined by a negative control consisting of the secondary antibody alone). The zoom of each picture was obtained by choosing the plane with greatest fluorescent signal.

Quantification of nuclear localization were performed blinded to treatments. To reduce experimental error, comparisons were made within slides that were processed and analysed in parallel. Image series were analyzed with Fiji (Image J, National Institutes of Health). For nuclear localization of α-Syn, the area of the nucleus was defined according to the signal obtained for DAPI. α-Syn signal that colocalized with the area stained with DAPI was defined as nuclear α-Syn signal and subtracted from whole cell α-Syn signal to obtain the cytosolic α-Syn. In each experiment a mean ± SD of minimum n=20 cells is presented.

Determination of luciferase activity. MCF7 cells were transfected to express the indicated DNA plasmids. Twenty four hours post transfection, the conditioning medium was replaced to contain the activating ligands, or their corresponding controls in 0.1% FBS-supplemented DMEM. For RA or other retinoids, cells were treated with 50 nM, or 0.05% DMSO. For fatty acid treatment, either one of the following fatty acids: OA (18:1), LA (18:2), ALA (18:3) or DHA (22:6), were pre-incubated with BSA in binding buffer containing 150 mM NaCl and 10 mM Tris-Cl pH 7.5 at 37°C, for 30 minutes and filtered through 48 micron filters. Cells were conditioned with a final concentration of 0.25 mM fatty acid/0.05 mM BSA. Control cells were treated with BSA only at 0.05 mM. At 48 hours post-transfection cells were harvested, washed twice in clean DMEM and lysed in a luciferase assay-buffer, containing 100 mM Tris-acetate pH 7.5, 10 mM magnesium acetate, 1 mM EDTA, 2 mM DTT, protease inhibitor cocktail (1X; Sigma Aldrich, Rehovot, Israel) and 1% Triton X-100, by incubation for 20 minutes on ice. The homogenate was centrifuged at 10,000 xg for 5 minutes. The supernatant was transferred to a clean tube. Each sample was tested in triplicate. Samples of 15 µl lysed cells were loaded on a white Greiner 96-wells plate (Sigma-Aldrich, Rehovot, Israel) and 100 µl of luciferase assay buffer, containing 10 mM luciferine (Sigma-Aldrich) and 100 mM ATP (Sigma-Aldrich) were added to each well, in triplicates. Luminescence was determined using Infinite M200 Pro plate-reader (Thermo Fisher, Qiryat Shemona, Israel).

β-galactosidase (β-gal) assay, sample (20 µl) were loaded on a Thermo-Fisher flat transparent 96-well plate in triplicates. Eighty µl β-gal buffer [50 mM KH_2PO_4, 1 mM $MgCl_2$, and 1 mM chlorophenol red-β-D-galactopyranoside (CPRG), pH 7.5] were added to each well. The plate was incubated for 10 minutes at 37°C and absorbance was measured using Infinite M200 Pro plate

reader (ThermoFisher, Petah Tiqva, Israel). Protein concentration was determined using Pierce BCA Protein Assay Kit (Thermo Fisher). Luciferase counts were normalized to protein concentration and β-gal activity for each test sample.

Isothermal titration calorimetry (ITC) data collection. All experiments were performed at 25 °C on high-feedback mode with a stirring speed of 750 r.p.m. (Malvern PEAQ-ITC). Purified α-Syn protein (10 μM in 150 mM NaCl, 10 mM Tris-cl pH 7.5) was added to the reaction cell and the ligand to the injection syringe. The concentration of RA in the injection syringe was 100 μM and the concentration of OA (18:1) or ALA (18:3) was 200 μM. The solutions were allowed to reach 25 °C for ~5 minutes prior to starting the experiment. Injections were monitored at a volume of 2 μl and in 180 seconds intervals. In reactions containing the organic DMSO solvent, both α-Syn protein and the ligand were brought to the same final DMSO concentration of 1%. A baseline correction procedure was implemented.

ImageStream Analysis. MCF7 cells were washed with clean DMEM and fixed in 2% paraformaldehyde for 10 minutes on ice. Cells were washed three times with DMEM and re-suspended in permeabilization buffer containing 0.1% Triton X-100 and 1% BSA in PBS for 5 minutes at room temperature. Cells were then incubated in blocking solution containing 1% BSA in PBS for 10 minutes and then incubated with a primary antibody, MJF-1 (1:250, Abcam. Zotal, Tel Aviv, Israel) for one hour at room temperature, washed 3 timed with DMEM and incubated with secondary antibody, Dy light 649 (1:150 Jackson ImmunoResearch, Baltimore, USA) for one hour at room temperature. Diamidino-2-phenylindole (DAPI; 1 mg/ml) was added to cells for 15 minutes before the end of the incubation with the secondary antibody. Cells were washed twice in clean DMEM and then analyzed.

Images were acquired on the ImageStream X MKII imaging cytometer (Amnis Corp, WA, USA). Data analysis was performed using Nuclear Localization Wizard, IDEAS® image analysis software package (Amnis Corp). 4000-6000 events were collected for each sample on an ImageStream IS100 using 649 nm laser excitation. Cell populations were sequentially gated for single cells that were singular, round, in focus and positive for both α-Syn and DAPI. Following application of gating, the acquired images were analyzed for intensity and saturation of signals. The spatial localization of α-Syn and DAPI was measured using the “Similarity” feature in the IDEAS®

software package. The “Similarity Score”, a log transformed Pearson's correlation coefficient between the pixel values of two image pairs, provided a measure of the degree of nuclear localization of α-Syn by measuring the pixel intensity correlation between the α-Syn immunoreactivity and DAPI images.

Lipid-ELISA was performed as previously described (Abd-Elhadi et al., 2016). Briefly, a 96-well PolySorp ELISA plate was coated with phospholipids and GM-1 ganglioside at a final amount of 5 µg/well. Wells were blocked with 100 µl of 1 % BSA (fatty acid-free) in PBS. Test samples were added into the wells, in triplicates and incubated overnight at 4 °C to allow capture of proteins by the immobilized lipids. The wells were washed with PBS (Ca^{2+} and Mg^{2+} free). Bound PSer129 α-Syn detection with monoclonal antibody (pSyn#64, WAKO, Osaka, Japan) diluted 1:1000 in 1% BSA in PBS. Following incubation for one hour at 37 ºC, the wells were washed 3 times and then incubated with a secondary antibody for one hour at 37 ºC (Jackson Laboratories, ENCO, Israel) diluted 1:8000 in 1% BSA in PBS and washed three times as above. Detection with Super Signal ELISA Femto (Pierce, Ornat, Israel) for the enzymatic reaction. Luminescence was determined by Luminometer (Infinite M200 Pro. NEOTEL Scientific Instrumentation Ltd.) immediately after adding the substrate. A standard curve consisting of recombinant protein, phosphorylated at Ser129 (Proteos, Kalamazoo, US), was applied in parallel to test samples and used as a reference.

Statistical analyses

Comparisons between two groups were performed by two tailed T test. Additional comparisons were performed by one way ANOVA. Data presented as mean ± SD or mean ± SE, as indicated. Significant differences were considered with $P < 0.05$.

Supplemental Table 1. PCR primers

Gene	Forward (5→3)	Reverse (5→3)
m. α-Syn	CATCTTTAGCCATGGATGTG	CCCATCTGGTCCTTCTTGAC
m. CRABP2	GATCCTGACAATGACAGCAGATG	TCGTCTCAGGCAGTTCTTGGA
m. RBP1	GCGCTCGACGTCAACGT	TCAGCGTGCGGATGATCAT
m. CRABP1	CGCCGCTACAGCCAACAC	TTCACACCCAACGCCTTGA
m. RBP4	TCCAGCGAGGAAACGATGAC	GCACAGGTGCCATCCAGATT
m. 18S	5-GCCAGAACCTGGCTGTACTT	5-GAGCGAGTGATCACCATCAT
h. DDC	AAGTCGGTCCTATCTGCAACAAG	TTCAGAAGGTGCCGGAACTC
h. PINK1	TATGGAGCAGTCACTTACAGAAAA TCC	GGTGAAGGCGCGGAGAA
h. ADORA1	CATCCCTCTGGAGCTTACCG	CAAGCACCCAAGGTCACCAA
h. FTL	CCATGAGCTCCCAGATTCGT	GGTCGAAATAGAAGCCCAGAGA
h. ATP13A2	ATGATGGCTGGGATCCCTTT	AACTATCCTCTTTGTCTCTTATTTCGAT AAC
h. α-Syn	GCAGGGAGCATTGCAGCAGC	GGCTTCAGGTTCGTAGTCTTG
h.CRABP2	AAAATGGTCTGTGAGCAGAAGCT	CCGTCATGGTCAGGATCAGTT
h. RBP1	CGCCCTCGACGTCAATGT	CAGCGTGCGGATGATCATAT
h. CRABP1	TCCACTGCACGCAAACTCTT	CAAACGTCAGGATAAGTTCATCGT
h. RBP4	TGATCGTCCACAACGGTTACTG	AGCTGAAGACTGAGAGCTAATCAGAA
h. PIN1	GCAGCTCAGGCCGAGTGTA	CCCGTTTTTGCCACCACTG
h. STUB1	GGCCAAGCACGACAAGTACA	CAAAGCTGATCTTGCCACACA
h. RARa	GGCCTGTTTGCTCCCAGAGAA	GAGGGCTGGGCACTATCTCTT
h. RARb	ACAGCTGAGTTGGACGATCTCA	CACTGGAATTCGTGGTGTATTTACC
h. RET	GGCCGAGATGAAGCTCGTT	CAAGCCGAAATCCGAAATCTT
h. G6PD	5-CACCATCTGGTGGCTGTTC	5-TCACTCTGTTTGCGGATGTC
WPRE	CCGTTGTCAGGCAACGTG	AGCTGACAGGTGGTGGCAAT

m, mouse; h, human

Supplemental References

ABD-ELHADI, S., BASORA, M., VILAS, D., TOLOSA, E. & SHARON, R. 2016. Total alpha-synuclein levels in human blood cells, CSF, and saliva determined by a lipid-ELISA. *Anal Bioanal Chem,* 408**,** 7669-7677.

ASSAYAG, K., YAKUNIN, E., LOEB, V., SELKOE, D. J. & SHARON, R. 2007. Polyunsaturated Fatty Acids Induce alpha-Synuclein-Related Pathogenic Changes in Neuronal Cells. *Am J Pathol,* 171**,** 2000-11.

BEN GEDALYA, T., LOEB, V., ISRAELI, E., ALTSCHULER, Y., SELKOE, D. J. & SHARON, R. 2009. Alpha-synuclein and polyunsaturated fatty acids promote clathrin-mediated endocytosis and synaptic vesicle recycling. *Traffic,* 10**,** 218-34.

FANNING, S., HAQUE, A., IMBERDIS, T., BARU, V., BARRASA, M. I., NUBER, S., TERMINE, D., RAMALINGAM, N., HO, G. P. H., NOBLE, T., SANDOE, J., LOU, Y., LANDGRAF, D., FREYZON, Y., NEWBY, G., SOLDNER, F., TERRY-KANTOR, E., KIM, T. E., HOFBAUER, H. F., BECUWE, M., JAENISCH, R., PINCUS, D., CLISH, C. B., WALTHER, T. C., FARESE, R. V., JR., SRINIVASAN, S., WELTE, M. A., KOHLWEIN, S. D., DETTMER, U., LINDQUIST, S. & SELKOE, D. 2018. Lipidomic Analysis of alpha-Synuclein Neurotoxicity Identifies Stearoyl CoA Desaturase as a Target for Parkinson Treatment. *Mol Cell.*

GIASSON, B. I., DUDA, J. E., QUINN, S. M., ZHANG, B., TROJANOWSKI, J. Q. & LEE, V. M. 2002. Neuronal alpha-synucleinopathy with severe movement disorder in mice expressing A53T human alpha-synuclein. *Neuron,* 34**,** 521-33.

GOLOVKO, M. Y., BARCELO-COBLIJN, G., CASTAGNET, P. I., AUSTIN, S., COMBS, C. K. & MURPHY, E. J. 2009. The role of alpha-synuclein in brain lipid metabolism: a downstream impact on brain inflammatory response. *Mol Cell Biochem,* 326**,** 55-66.

HOFFMAN, L. M., GARCHA, K., KARAMBOULAS, K., COWAN, M. F., DRYSDALE, L. M., HORTON, W. A. & UNDERHILL, T. M. 2006. BMP action in skeletogenesis involves attenuation of retinoid signaling. *J Cell Biol,* 174**,** 101-13.

LOVE, M. I., HUBER, W. & ANDERS, S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol,* 15**,** 550.

SHARON, R., BAR-JOSEPH, I., FROSCH, M. P., WALSH, D. M., HAMILTON, J. A. & SELKOE, D. J. 2003a. The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. *Neuron,* 37**,** 583-95.

SHARON, R., BAR-JOSEPH, I., MIRICK, G. E., SERHAN, C. N. & SELKOE, D. J. 2003b. Altered fatty acid composition of dopaminergic neurons expressing alpha-synuclein and human brains with alpha-synucleinopathies. *J Biol Chem,* 278**,** 49874-81.

SHARON, R., GOLDBERG, M. S., BAR-JOSEF, I., BETENSKY, R. A., SHEN, J. & SELKOE, D. J. 2001. alpha-Synuclein occurs in lipid-rich high molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. *Proc Natl Acad Sci U S A,* 98**,** 9110-5.

SLADEK, F. M., ZHONG, W. M., LAI, E. & DARNELL, J. E., JR. 1990. Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. *Genes Dev,* 4**,** 2353-65.

SPECHT, C. G. & SCHOEPFER, R. 2001. Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. *BMC Neurosci,* 2**,** 11.

VARGA, T., CZIMMERER, Z. & NAGY, L. 2011. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. *Biochim Biophys Acta,* 1812**,** 1007-22.

VIEIRA, M. M., SCHMIDT, J., FERREIRA, J. S., SHE, K., OKU, S., MELE, M., SANTOS, A. E., DUARTE, C. B., CRAIG, A. M. & CARVALHO, A. L. 2016. Multiple domains in the C-terminus of NMDA receptor GluN2B subunit contribute to neuronal death following in vitro ischemia. *Neurobiol Dis,* 89**,** 223-34.

WISELY, G. B., MILLER, A. B., DAVIS, R. G., THORNQUEST, A. D., JR., JOHNSON, R., SPITZER, T., SEFLER, A., SHEARER, B., MOORE, J. T., MILLER, A. B., WILLSON, T. M.

& WILLIAMS, S. P. 2002. Hepatocyte nuclear factor 4 is a transcription factor that constitutively binds fatty acids. *Structure,* 10, 1225-34.

ZALTIERI, M., GRIGOLETTO, J., LONGHENA, F., NAVARRIA, L., FAVERO, G., CASTREZZATI, S., COLIVICCHI, M. A., DELLA CORTE, L., REZZANI, R., PIZZI, M., BENFENATI, F., SPILLANTINI, M. G., MISSALE, C., SPANO, P. & BELLUCCI, A. 2015. alpha-synuclein and synapsin III cooperatively regulate synaptic function in dopamine neurons. *J Cell Sci,* 128, 2231-43.