Supplementary text and figures to:

# ZSCAN21 mediates the transcriptional induction of α-synuclein in models of Parkinson's disease

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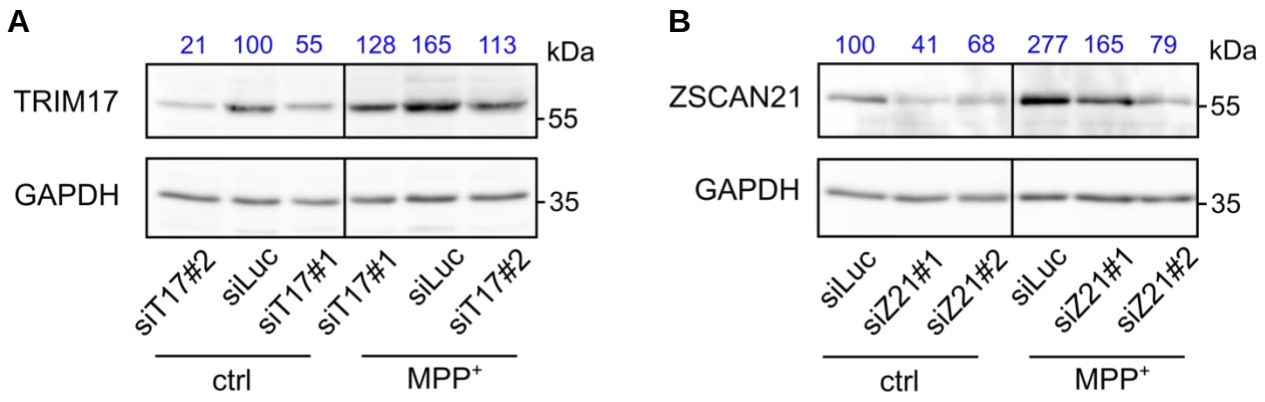


**Figure S1: Knock-down of TRIM17 and ZSCAN21 in LUHMES cells.** LUHMES cells were transfected with a control siRNA against Luciferase (siLuc), two different siRNAs against TRIM17 (siT17#1 and siT17#2) or two different siRNAs against ZSCAN21 (siZ21#1 and siZ21#2) at the time of plating for 3D-differentiation. After 6 days of differentiation, LUMHES spheroids were treated or not with 2 µM $MPP^+$ for 24 h and total proteins were analysed by western blot using antibodies against GAPDH and TRIM17 (**A**) or ZSCAN21 (**B**). The intensity of the TRIM17 or ZSCAN21 bands was quantified, normalized by the intensity of the GAPDH bands and expressed relative to the values obtained with non-treated cells transfected with the control siRNA (siLuc): values in blue above the corresponding bands. For each protein, the two parts separated by a vertical line are from the same gel with the same exposure. These data are representative of two independent experiments.

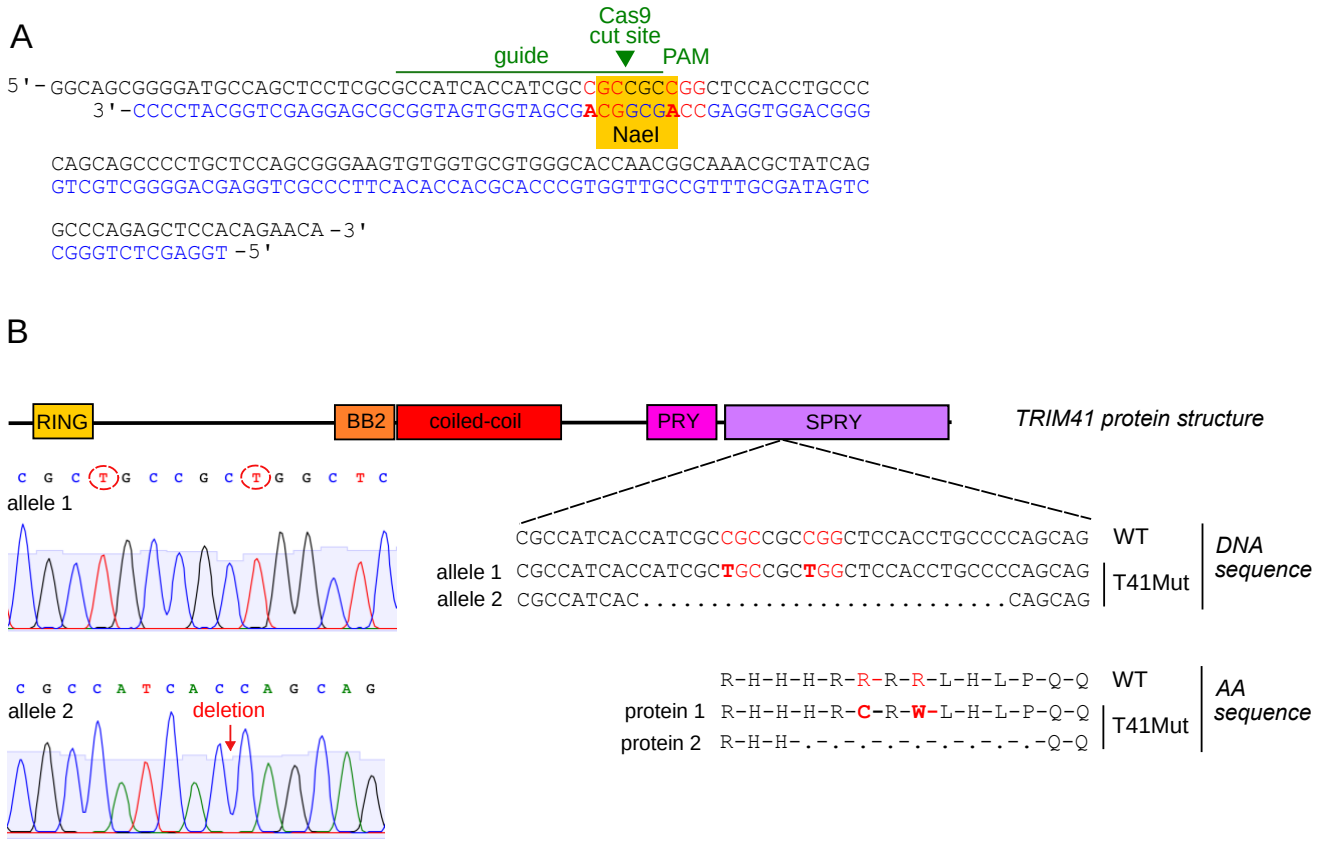


**Figure S2: CRISPR-Cas9 genome editing of *TRIM41* in LUHMES cells. A.** Genomic DNA sequence of the targeted locus in human *TRIM41* with the binding site of the guide RNA, Cas9 cleavage site and the PAM in green. The codons corresponding to R534 and R536 are in red. The sequence of the donor oligonucleotide is represented in blue, with the nucleotides modified in order to introduce the desired point mutations in genomic DNA in bold. The yellow box underlines the NaeI restriction site that is disrupted if the point mutations are introduced or if small insertions/deletions occur after Cas9 cleavage. Note that introducing the desired mutations disrupts the PAM, preventing further cleavage by Cas9 following DNA repair. **B.** Schematic representations of the different domains of the TRIM41 protein (BB2: B-Box2) indicating the location of the amino acid substitutions. Chromatograms of DNA sequencing for the two alleles of the clone T41Mut are shown, with the two-point mutations in red circles and the location of the deletion indicated with an arrow. The DNA sequences of WT TRIM41 and the two alleles of clone T41Mut are compared, as well as the amino acid sequences of the protein products.

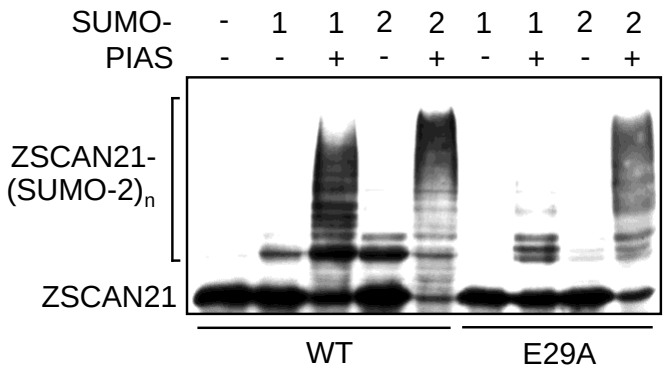


**Figure S3: ZSCAN21 is SUMOylated on K27 consensus site.** *In vitro* translated 3×Flag-ZSCAN21, either the WT form or the E29A mutant, was incubated with *in vitro* SUMOylation reaction mix (containing E1 and E2 enzymes), in the presence or the absence of SUMO-1 or SUMO-2, in the presence or the absence of the E3 enzyme PIASxα, as indicated, for 1h at 37 °C. Multi-SUMOylated forms of ZSCAN21 were detected by immunoblotting using anti-Flag antibody.

**Supplementary Table 1: list of sequences targeted by siRNAs and shRNAs**

| **siRNAs** | **cDNA targeted sequences (5' – 3')** |
|---|---|
| siRNA-Luc | CGTACGCGGAATACTTCGA |
| siRNA-TRIM17#1 | CCAAGTACTTATCCACCTT |
| siRNA-TRIM17#2 | GGTATACTGACAGATGCTT |
| siRNA-TRIM41 | AAGGAGACTTTCAATAGGTGT |
| siRNA-ZSCAN21#1 | CTTAGAGAGGCAGTGCGTAAA |
| siRNA-ZSCAN21#2 | GATCCAAGAAAGGTCCGAGAT |
| **shRNAs** | **cDNA targeted sequences (5' – 3')** |
| shRNA-Luc | CGCTGAGTACTTCGAAATGTC |
| shRNA-eGFP | TACAACAGCCACAACGTCTAT |
| shRNA-Trim17 | CTGTTACCCAATTCCACTCTA |
| shRNA-Zscan21 | GCCAGCCCTAAATATGAGTTT |

**Supplementary Table 2: list of the primers used for quantitative RT-PCR**

| **cDNA** | **Forward (5' - 3')** | **Reverse (5' - 3')** |
|---|---|---|
| human SNCA pre-mRNA | GCAGAAGCAGCAGGAAAGAC | CCTACCTACACATACCTCTGAC |
| human SNCA | GCAGAAGCAGCAGGAAAGAC | ACCACTGCTCCTCCAACATT |
| human TRIM17 pre-mRNA | AGGACATGGAGTACCTTCGG | ACAGACGTGGAAATGCAGGA |
| human TRIM17 | GACATGGAGTACCTTCGGGA | GCAGTCTCCTCTTCTTCCGT |
| human TRIM41 | AGCTCTTCTGCGAGGTAGAC | CTCTTCAGCCAGAAACCGTG |
| human ZSCAN21 | CAAATACGAGTCTTGGGGGC | TCTAAGCTGGCCTCAGGTTT |
| human B2M | GCGCTACTCTCTCTTTCTGG | AGAAAGACCAGTCCTTGCTGA |
| human GAPDH | CCATCTTCCAGGAGCGAGAT | GGTTCACACCCATGACGAAC |
| human GUSB | TGGGTCTGGATCAAAAACGC | TTGTCTCTGCCGAGTGAAGA |
| human HMBS | ACACACAGCCTACTTTCCAAG | AGAATCTTGTCCCCTGTGGT |
| human HPRT1 | TGACACTGGCAAAACAATGCA | GGTCCTTTTCACCAGCAAGCT |
| human RPL31 | ACGAAGTGGTAACCCGAGAA | TCCCATCTCCTTCATGGCAA |

| | | |
|---|---|---|
| human RSP16 | GGTCTCATCAAGGTGAACGG | CACGGATGTCTACACCAGCA |
| human SDHA | TGGGAACAAGAGGGCATCTG | CCACCACTGCATCAAATTCATG |
| human TBP | GTTCTGGGATTGTACCGCAG | TTATATTCGGCGTTTCGGGC |
| human YWHAZ | GTCATCTTGGAGGGTCGTCT | GCTTGTGAAGCATTGGGGAT |
| mouse B2M | TATGCTATCCAGAAAACCCCTCAA | GTATGTTCGGCTTCCCATTCTC |
| mouse Hmbs | ACTCTGCTTCGCTGCATTG | AGTTGCCCATCTTTCATCACTG |
| mouse Trim17 | ACTGAGTGGCAGGAGAGAGTGAA | CCAGAAACAAGACCACCTTCTGA |
| mouse Zscan21 | GACTGGTGAGGAGGAGGTTT | ATCATGGGGATGACGGTTCT |

**Supplementary Table 3: list of the primers used for ChIP analysis by quantitative PCR**

| **DNA** | **Forward (5' - 3')** | **Reverse (5' - 3')** |
|---|---|---|
| *SNCA* promoter | GCTTGTTCATGAGTGATGGGT | CCTCTGACTCAGTCCACCTTT |
| *SNCA* "enhancer 1" | GCCCCGGTGTTATCTCATTC | CCTCTCCAATTACGATTTTAGCAC |
| *SNCA* "enhancer 2" | GTGCAGCGGTAGTTTAGCTT | TTTCCTGGATGCTCAGTGGT |
| *SNCA* "enhancer 4" | CTGCTTGGTTTTCCCTTTTG | CTGCAAACGCTTCTGTTTTG |
| *SNCA* "enhancer 7" | TATATTCTCCAAATTTCCTGTCC TTCC | TAAGGTTCCTCTTGAATCACAATTTA TC |

**Supplementary Table 4: targeted sequence, DNA template and primers for CRISPR Cas9 genome editing of TRIM41**

| **DNA** | **Forward (5' - 3')** | **Reverse (5' - 3')** |
|---|---|---|
| Primers to amplify the genomic DNA around Cas9 cut site | GAGGTTGCTGACCATCCCAA | GACCAGGCAGCTGTTCTCAT |
| Sequencing primer | ACTACTGGGAGGTAGAGGTG | |
| sequence targeted by the crRNA (PAM in red) | GCCATCACCATCGCCGCCGCCGG | |
| DNA template (targeted codons in red, desired point mutations in bold, *: phosphorothioates) | T*G*GAGCTCTGGGCCTGATAGCGTTTGCCGTTGGTGCCCACG CACCACACTTCCCGCTGGAGCAGGGGCTGCTGGGGCAGGTGG AGCCAGCGGCAGCGATGGTGATGGCGCGAGGAGCTGGCATCC *C*C | |

**Supplementary Table 5: PCR primer pairs for directed mutagenesis of ZSCAN21**
(modified codons in red, mutations in bold)

| DNA | Forward (5' - 3') | Reverse (5' - 3') |
|---|---|---|
| ZSCAN21 K27R | GCCTCTGATGGTAAGAGTCGAG GAG | CTCCTCGACTCTTACCATCAGAGGC |
| ZSCAN21 E29A | GATGGTAAAAGTCGCGGAGAAA GAAGAG | CTCTTCTTTCTCCGCGACTTTTACCATC |