Supplementary information

Knocking out alpha-synuclein in melanoma cells dysregulates cellular iron metabolism and suppresses tumor growth

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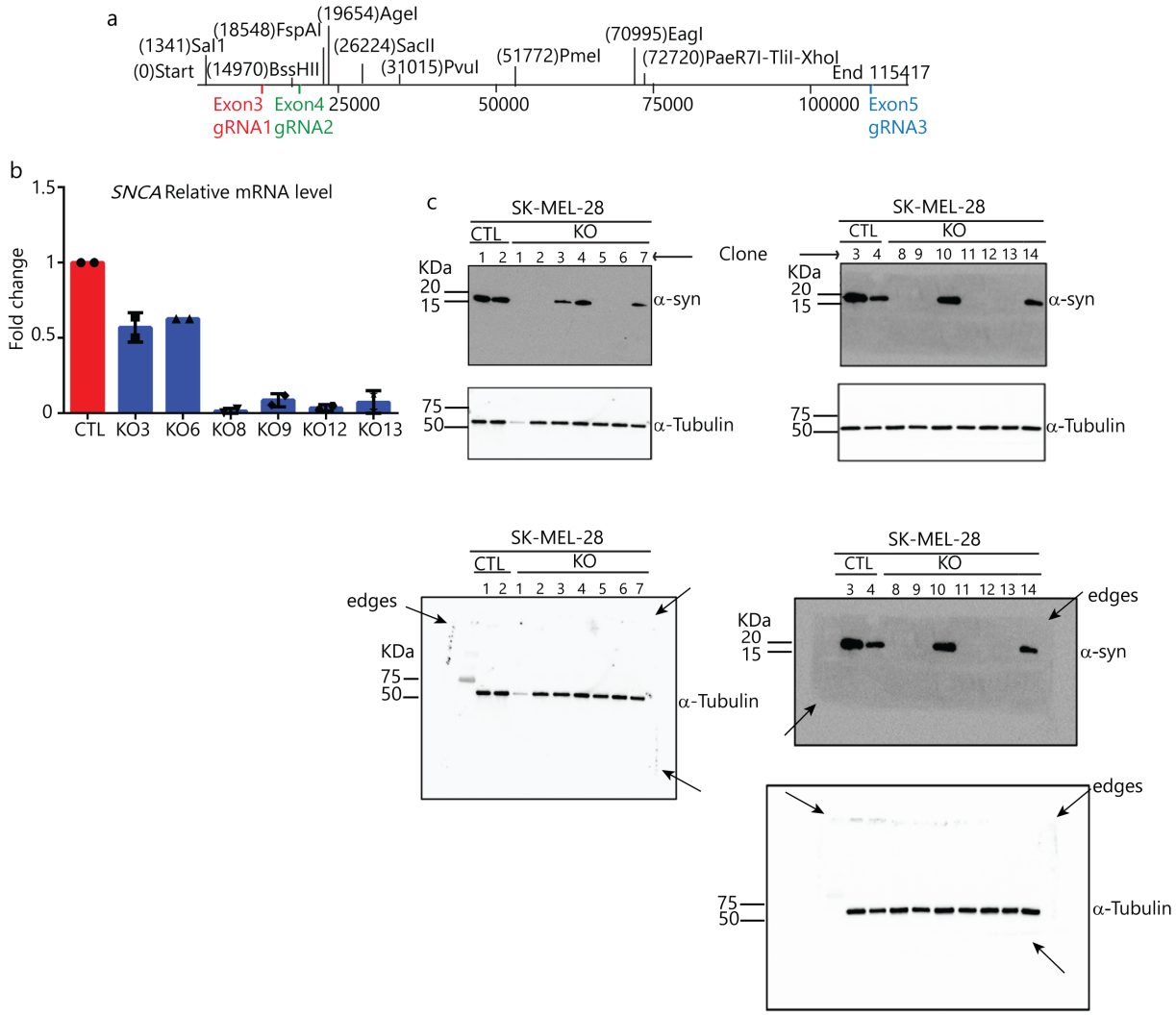
Figure S1

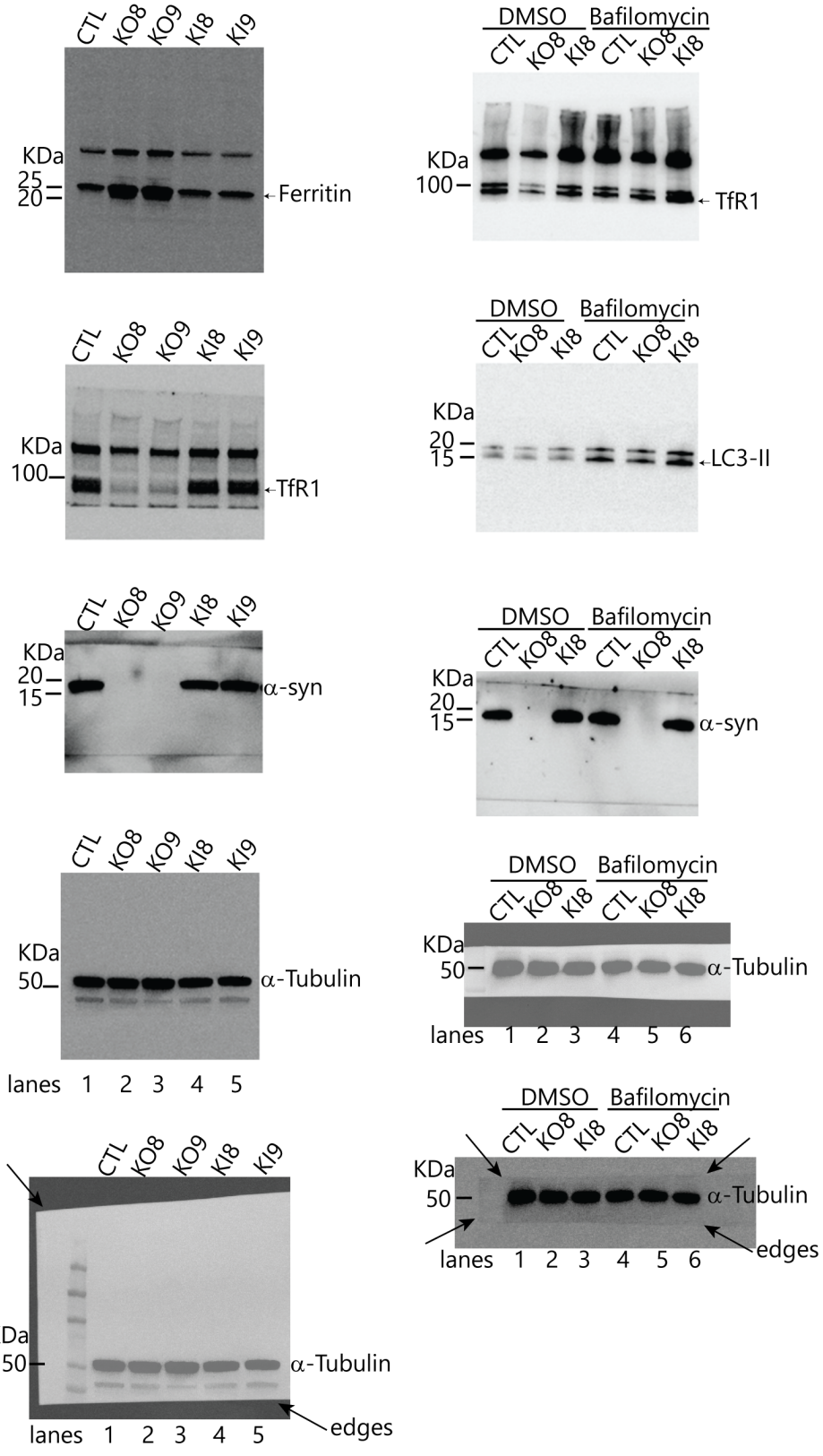


Supplementary Figure S1.

CRISP-Cas9-mediated KO of *SNCA* in SK-Mel-28 melanoma cells. (**a**) Human *SNCA* DNA sequence. gRNA 1, 2 and 3 are complementary to exon 3, 4 and 5, respectively. (**b**) Quantitative data showing RT-qPCR analysis of α-syn transcript level (n=2) in KO cells normalized to control cells and β-actin by ΔΔCt method. (**c**) Western blot analysis of *SNCA* in KO clones (n=3). Cell

lysates were subjected to SDS PAGE followed by western blotting for the indicated proteins. α-Tubulin was used as loading control.

Figure S2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KO9
KI8
KI9
KDa
25
20
Ferritin
DMSO
Bafilomycin
CTL
KO8
KI8
CTL
KO8
KI8
KDa
100
TfR1
CTL
KO8
KO9
KI8
KI9
KDa
100
TfR1
DMSO
Bafilomycin
CTL
KO8
KI8
CTL
KO8
KI8
KDa
20
15
LC3-II
CTL
KO8
KO9
KI8
KI9
KDa
20
15
α-syn
DMSO
Bafilomycin
CTL
KO8
KI8
CTL
KO8
KI8
KDa
20
15
α-syn
CTL
KO8
KO9
KI8
KI9
KDa
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α-Tubulin
lanes 1 2 3 4 5
DMSO
Bafilomycin
CTL
KO8
KI8
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KI8
KDa
50
α-Tubulin
lanes 1 2 3 4 5 6
CTL
KO8
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α-Tubulin
lanes 1 2 3 4 5
edges
DMSO
Bafilomycin
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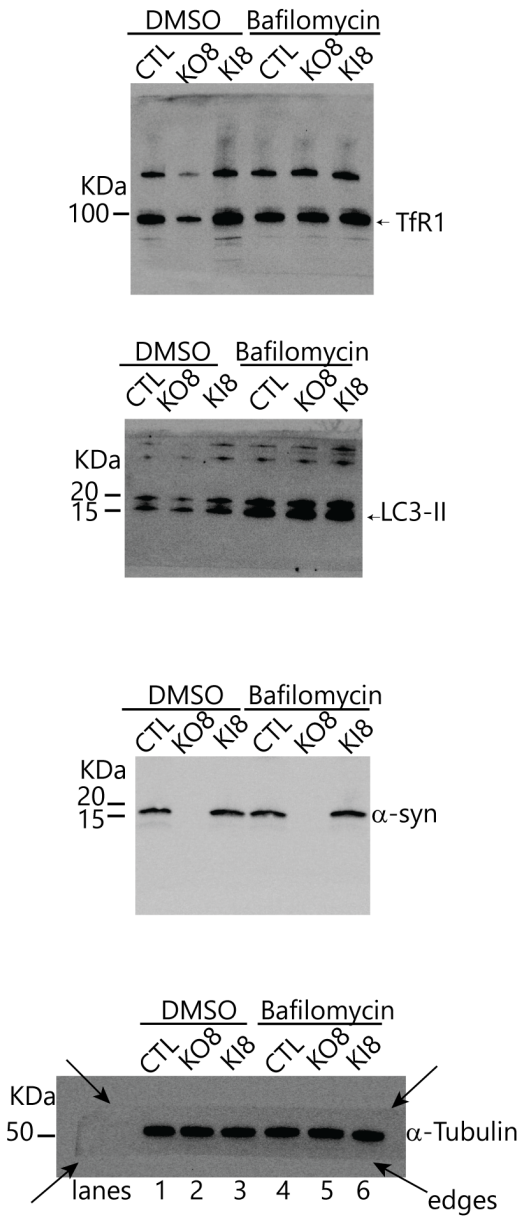
50
α-Tubulin
lanes 1 2 3 4 5 6
edges

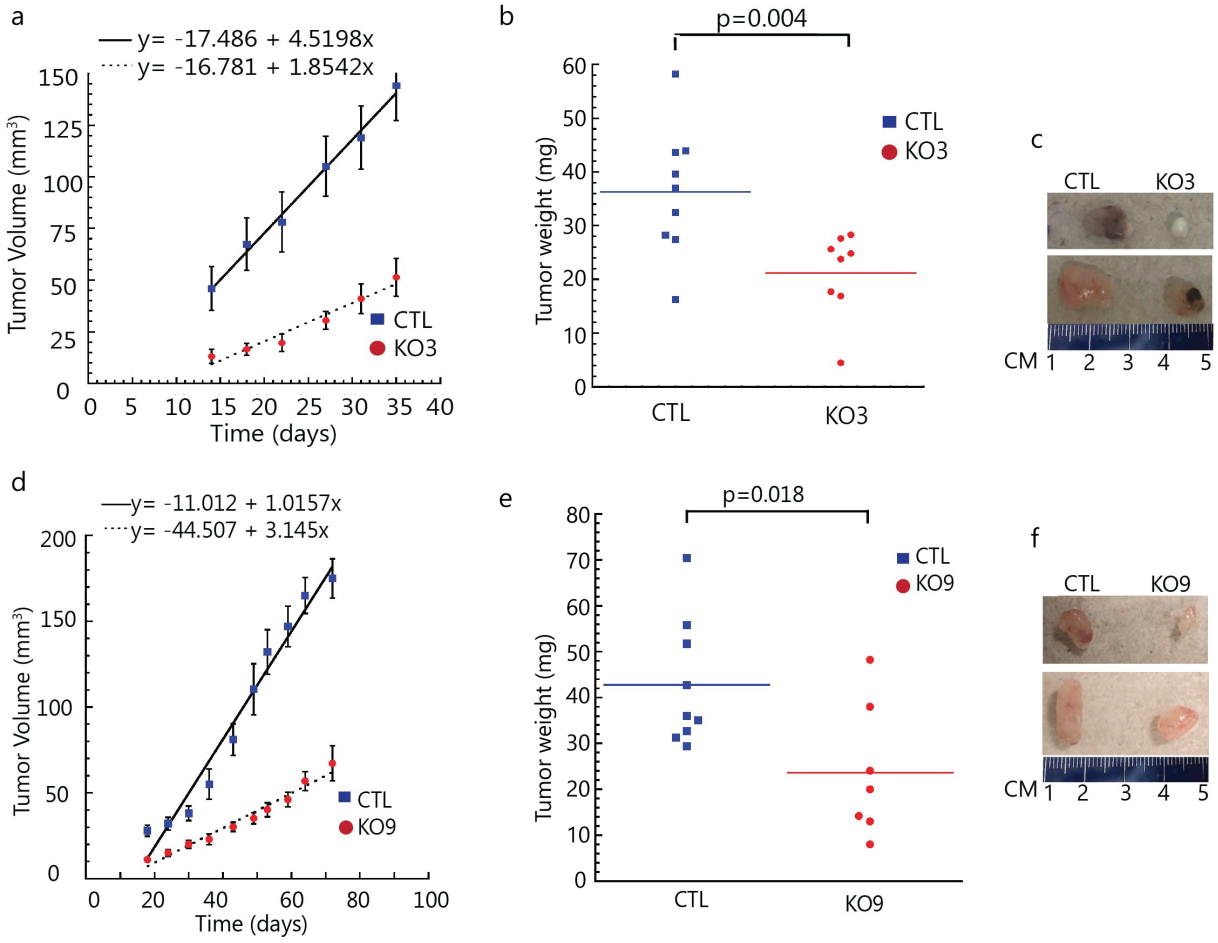
Figure S2 continued



Supplementary Figure S2.

Full length original uncontrasted Western blots which are shown cropped in Figure 2 a, d.

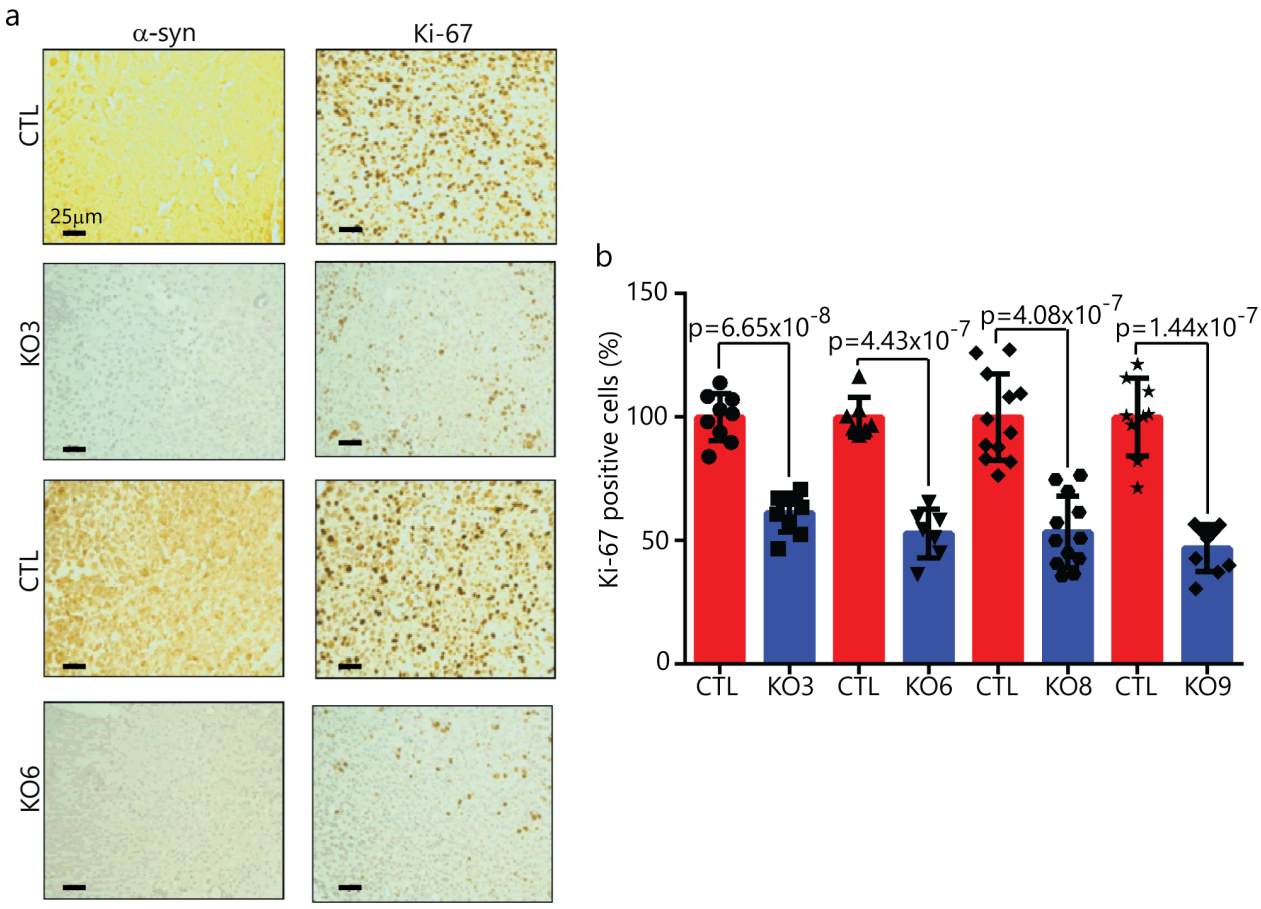
Figure S3



Supplementary Figure S3.

Loss of α-syn expression suppresses cell growth in a mouse xenograft model. (**a**) and (**d**) Tumor volume over time for the control, KO3 and KO9 xenografts. Tumor volume was assessed every 2 days, and average tumor weight was determined after the mice were sacrificed at the end of 72-day experiment. (**b**) and (**e**) Weight of the excised tumors are shown. (**c**) and (**f**) Representative photographs of xenograft mice tumors. Tumor volume and weight were analyzed using two-tailed Student's t test (n = 7-9 mice).

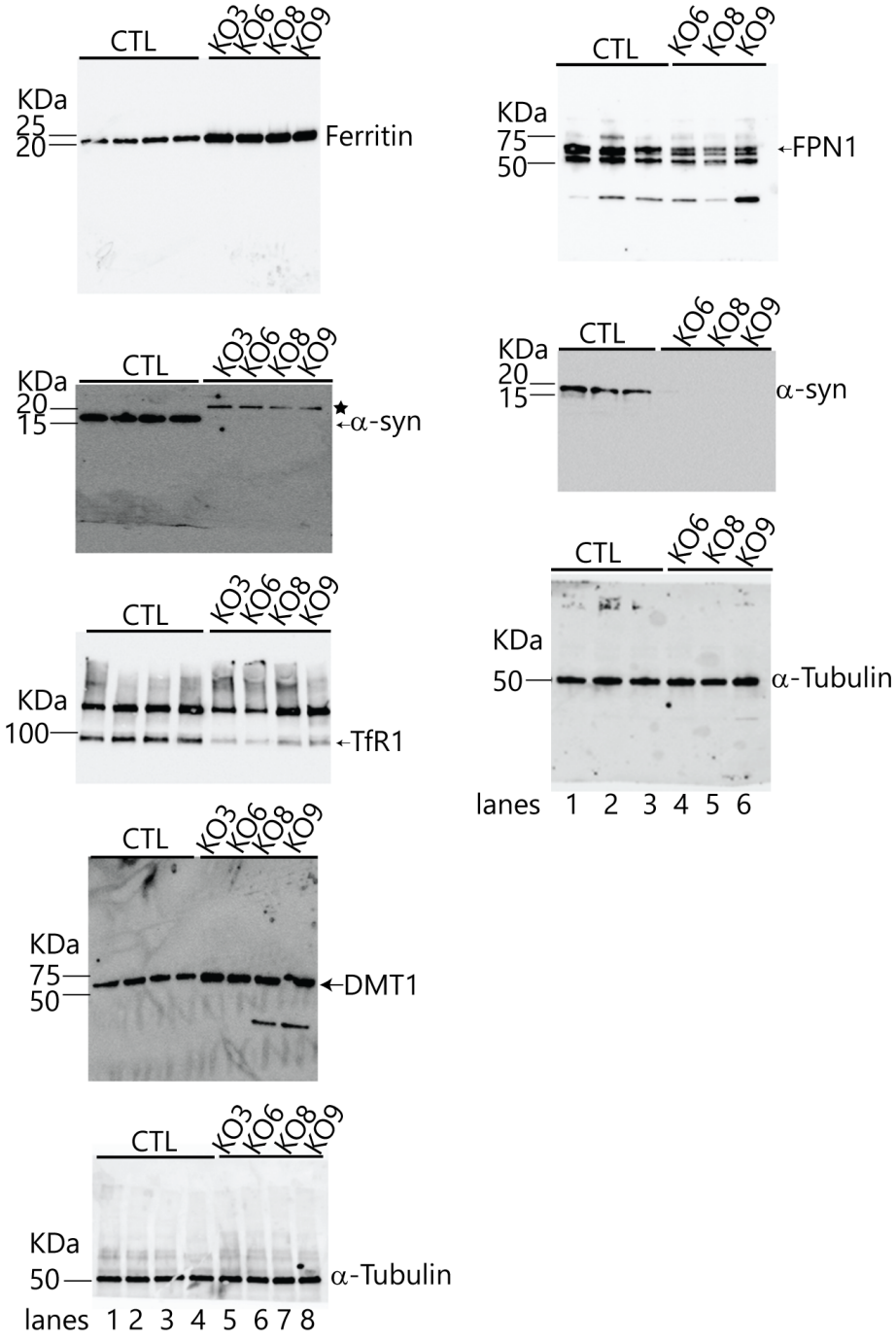
Figure S4



Supplementary Figure S4.

α-syn and Ki67 staining of tumor tissue from SK-Mel-28 xenografts. (**a**) Representative images of immunostained sections of xenografts generated from subcutaneous injection of the *SNCA* KO clone 3, 6 and control cells into mice. Tumor sections were probed with antibodies specific for α-syn and Ki-67. Magnification 20×, scale bar = 25 μm. (**b**) Plot of the number of Ki-67 immunopositive cells per 20x field. For each condition, n = 3 (KO3, 6), with 1-3 fields counted for each slide. The two tailed student's t test was performed to calculate p values. Error bars are ± s.d. This is the same plot as in Figure 6.

Figure S5



Supplementary Figure S5.

Full length original uncontrasted Western blots which are shown cropped in Figure 7 a, e. *, denotes residual ferritin.

Table S1. SK-Mel-28 *SNCA* KO clones

Crispr KO clones #	**WB of α-syn protein**	**RT-qPCR of α-syn mRNA**	**DNA sequencing of *SNCA***	**validation**
Control	yes	yes	All exons	Full expression
3	partial	partial	No exon 4	Monoallelic deletion clone (heterozygous deletion)
6	no	partial	No exon 3; no exon 4	Biallelic deletion clone (homozygous deletion)
8	no	no	No exon 3; no exon 4	Biallelic deletion clone (homozygous deletion)
9	no	no	No exon 3; no exon 4	Biallelic deletion clone (homozygous deletion)

Table S2 Antibodies

Name	Antibody type	Application	Dilution	Catalog Number and Company
α-synuclein	Monoclonal (mouse)	Western Blot / IHC	1:1000/ 1:500	610786, BD Biosciences
Transferrin Receptor	Monoclonal (mouse)	Western Blot	1:1000	13-6800, Thermo Fisher Scientific
Ferroportin	Polyclonal (rabbit)	Western Blot	1:1000	PA522993,Thermo Fisher Scientific
LC3-II	Polyclonal (rabbit)	Western Blot	1:1000	2775S, Cell Signaling Technology
α-Tubulin	Monoclonal (mouse)	Western Blot	1:1000	T9026, Santa Cruz
HRP- conjugated anti-rabbit	rabbit	Western Blot	1:1000	sc-516102, Santa Cruz
HRP- conjugated anti-mouse	mouse	Western Blot	1:1000	sc-2357, Santa Cruz
DMT1	Monoclonal (mouse)	Western Blot	1:1000	sc-166884, Santa Cruz
Ferritin	Monoclonal (mouse)	Western Blot	1:1000	sc-74513, Santa Cruz
Ki-67	Monoclonal (rabbit)	Western Blot / IHC	1:1000/ 1:500	12202S, Cell Signaling Technology