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Supplemental information

MFN2 coordinates mitochondria motility with α-tubulin acetylation and this regulation is disrupted in CMT2A

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Figure S1

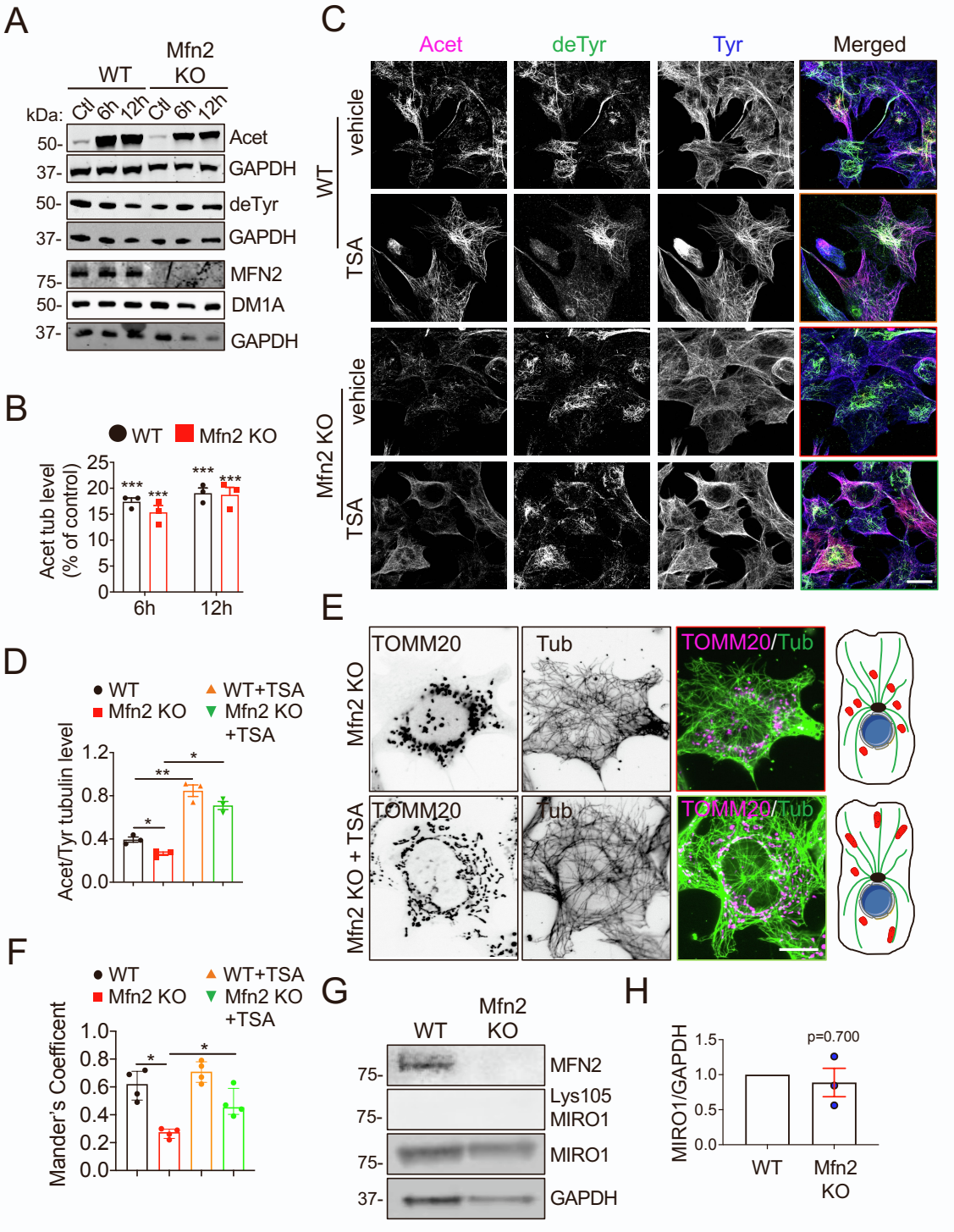

Figure S1. HDAC inhibition restores acetylated tubulin levels and mitochondria association to MTs in Mfn2 KO MEFs, Related to Figure 1. (A) Representative immunoblot of acetylated (Acet), detyrosinated (deTyr), total α-tubulin (DM1A), mitofusin-2 (MFN2) and GAPDH levels

in whole cell lysates from WT and Mfn2 KO MEFs incubated with control vehicle or 10 nM of trichostatin A (TSA) for 6 h and 12 h prior to lysis. (B) Quantification of acetylated tubulin relative to control levels (%) in WT and Mfn2 KO MEFs treated as in A. (C) Representative immunofluorescence staining of acetylated (Acet), detyrosinated (deTyr) and tyrosinated (Tyr) tubulin in WT and Mfn2 KO MEFs incubated with control vehicle or TSA for 6 h. Scale bar, 10 μm. (D) Quantification of acetylated to tyrosinated tubulin (bulk tubulin marker) immunofluorescence signal ratio measured in individual cells treated as in C. (E) Representative immunofluorescence staining of mitochondria and tyrosinated tubulin (Tub) in Mfn2 KO MEFs incubated with control vehicle or 10 nM of TSA for 6 h. Scale bar, 5 μm. (F) Quantification of mitochondria associated with MTs (identified by the bulk tubulin marker tyrosinated tubulin) using Mander's coefficient. (G) Representative immunoblot analysis of MFN2, Acetylated-miro1 (Lys 105 MIRO1), total miro1 (MIRO1) and GAPDH levels in whole cell lysates from WT and Mfn2 KO MEFs. (H) Quantification of total MIRO1 levels (relative to WT) in WT and Mfn2 KO MEFs. Data are median with interquartile range from 3 independent experiments * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<<0.001$ by Kruskal-Wallis test (B,D and F) or Mann-Whitney U test (H). NS non-significant.

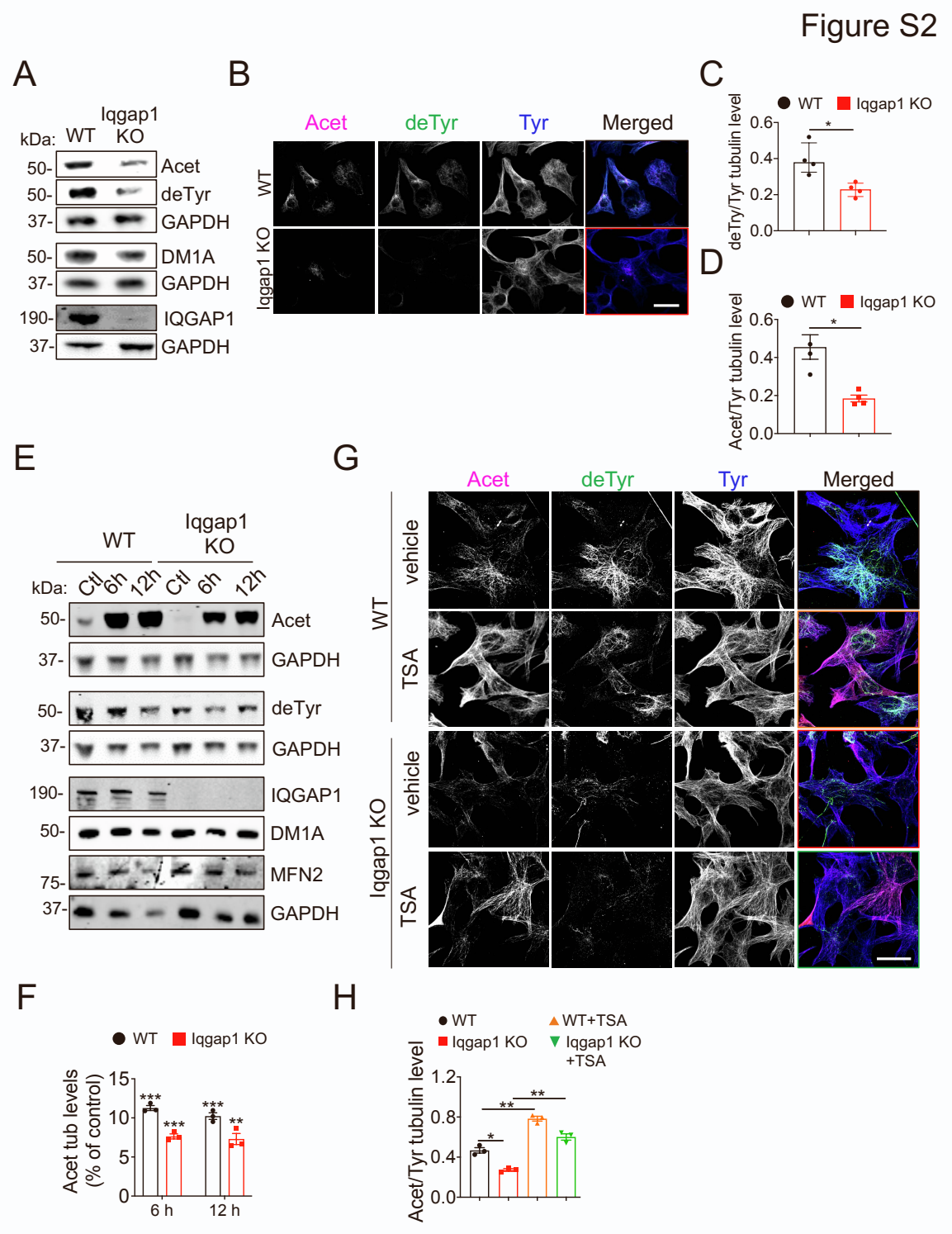

Figure S2. HDAC inhibition restores acetylated tubulin levels in Iqgap1 KO MEFs, Related to Figure 2. (A) Representative immunoblot of WT and Iqgap1 KO whole MEF lysates. DM1A, total α-tubulin, Acet, acetylated tubulin, deTyr, detyrosinated tubulin. GAPDH, loading control.

(B) Representative immunofluorescence staining (images are maximum projections from z-stacks) of acetylated (Acet), detyrosinated (deTyr) and tyrosinated (Tyr) tubulins in WT and Iqgap1 KO MEFs. Scale bar, 10 μm. (C) Quantification of detyrosinated tubulin signal in Iqgap1 KO MEFs relative to WT levels. (D) Quantification of acetylated tubulin signal in Iqgap1 KO MEFs relative to WT levels. (E) Representative immunoblot of acetylated (Acet), detyrosinated (deTyr), total (DM1A) α-tubulin, IQGAP1, mitofusin-2 (MFN2) and GAPDH levels in WT and Iqgap1 KO MEFs incubated with 10 nM of the HDAC inhibitor trichostatin A (TSA) or vehicle control for 6 h or 12 h prior to lysis. (F) Quantification of acetylated tubulin (Acet) levels relative to vehicle control (%) in WT and Iqgap1 KO MEFs incubated with 10 nM TSA for 6 or 12 h. (G) Representative immunofluorescence staining (images are maximum projections from z-stacks) of WT and Iqgap1 KO MEFs incubated with 10 nM of TSA or vehicle control for 6 h prior to fixation. Scale bar, 10 μm. (H) Quantification of acetylated (Acet) to tyrosinated (Tyr) tubulin immunofluorescence signal ratio measured in WT and Iqgap1 KO MEFs as in G. Data are median with interquartile range. *, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; ****, $p \leq 0.0001$; ns non-significant by Mann–Whitney U test (C and D) or Kruskal-Wallis test (F and H). (n=3-4 independent experiments).

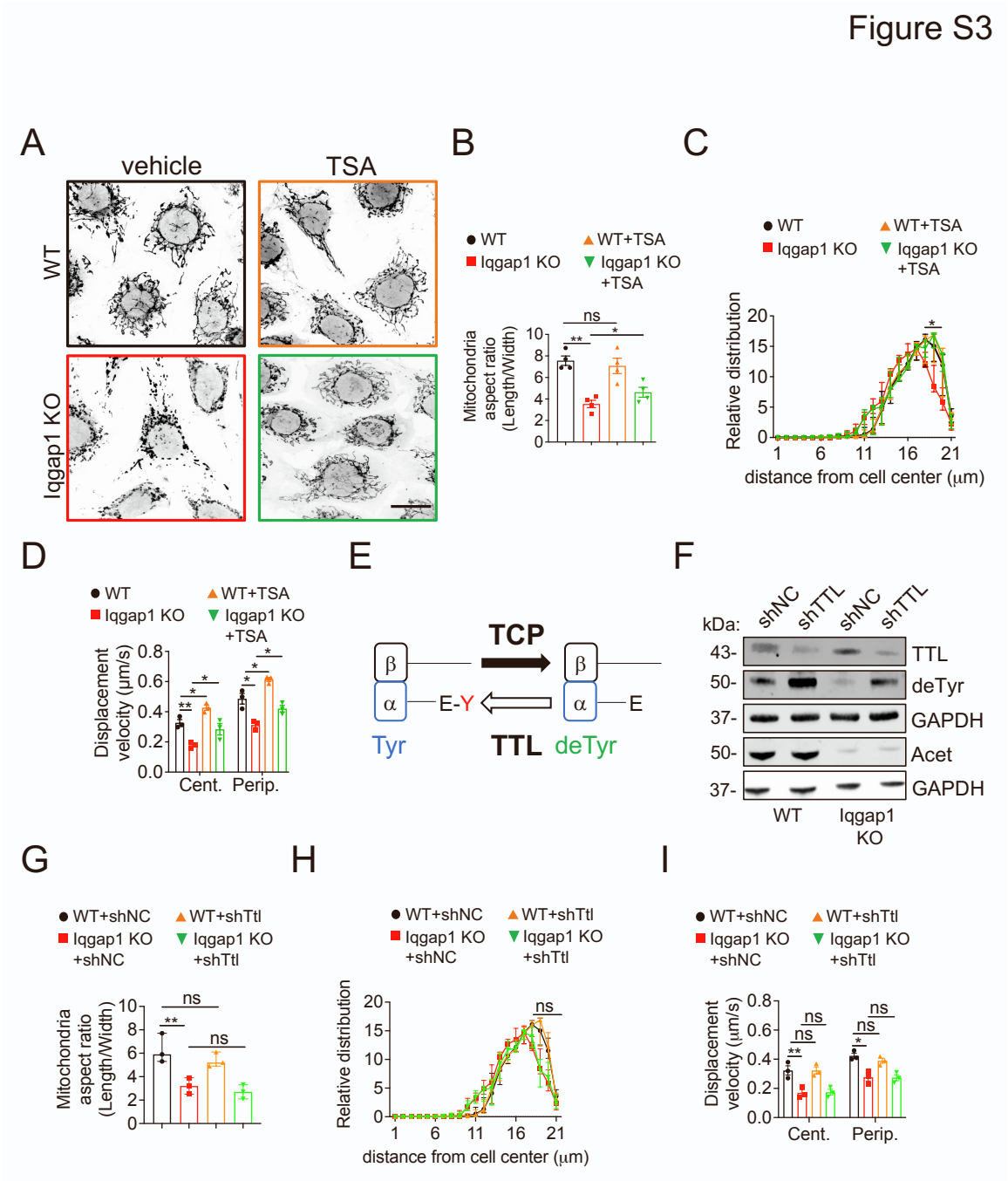

Figure S3. **Restoring tubulin acetylation levels rescues mitochondria dynamics in Iqgap1 KO MEFs, Related to Figure 2.** (A) WT and Iqgap1 KO control and trichostatin A (TSA) treated (10 nM, 6 h) MEFs were stained with mitoTracker Red. Scale bar, 10 μm. (B) Mitochondria morphology is calculated as ratio of length /width in cells treated as in A. (C) Relative distribution of mitochondria from the cell center in MEFs treated as in A. (D) Mitochondrial displacement velocity near the cell center and periphery in MEFs as in A. Live-imaging was performed for 3

min (1f/2s). (E) Schematic of the enzymes involved in the detyrosination/retyrosination α-tubulin cycle. Note that detyrosination of α-tubulin occurs on MTs while α-tubulin retyrosination occurs on the soluble tubulin heterodimer. TCPs, tubulin carboxypeptidases; deTyr, detyrosinated α-tubulin; Tyr, tyrosinated α-tubulin. TTL, tubulin tyrosine ligase. (F) Representative immunoblot of whole cell lysates from WT and Iqgap1 KO MEFs depleted of tubulin tyrosine ligase (TTL) expression by shRNA transfection for 3 days. TTL, tubulin tyrosine ligase; DeTyr, detyrosinated tubulin; Acet, acetylated tubulin; GAPDH, loading control. (G) Mitochondria morphology is calculated as ratio of length/width in cells depleted of TTL expression. (H) Relative distribution of mitochondria from the cell center in MEFs depleted of TTL expression. (I) Mitochondrial displacement velocity near the cell center and periphery in MEFs depleted of TTL expression. Live-imaging was performed for 3 min (1f/2s). Data are median with interquartile range n = 150-250 mitochondria from 15-30 cells in 3-4 independent exp. *, $p \leq 0.05$; **, $p \leq 0.01$; ns non-significant by Kruskal-Wallis test.

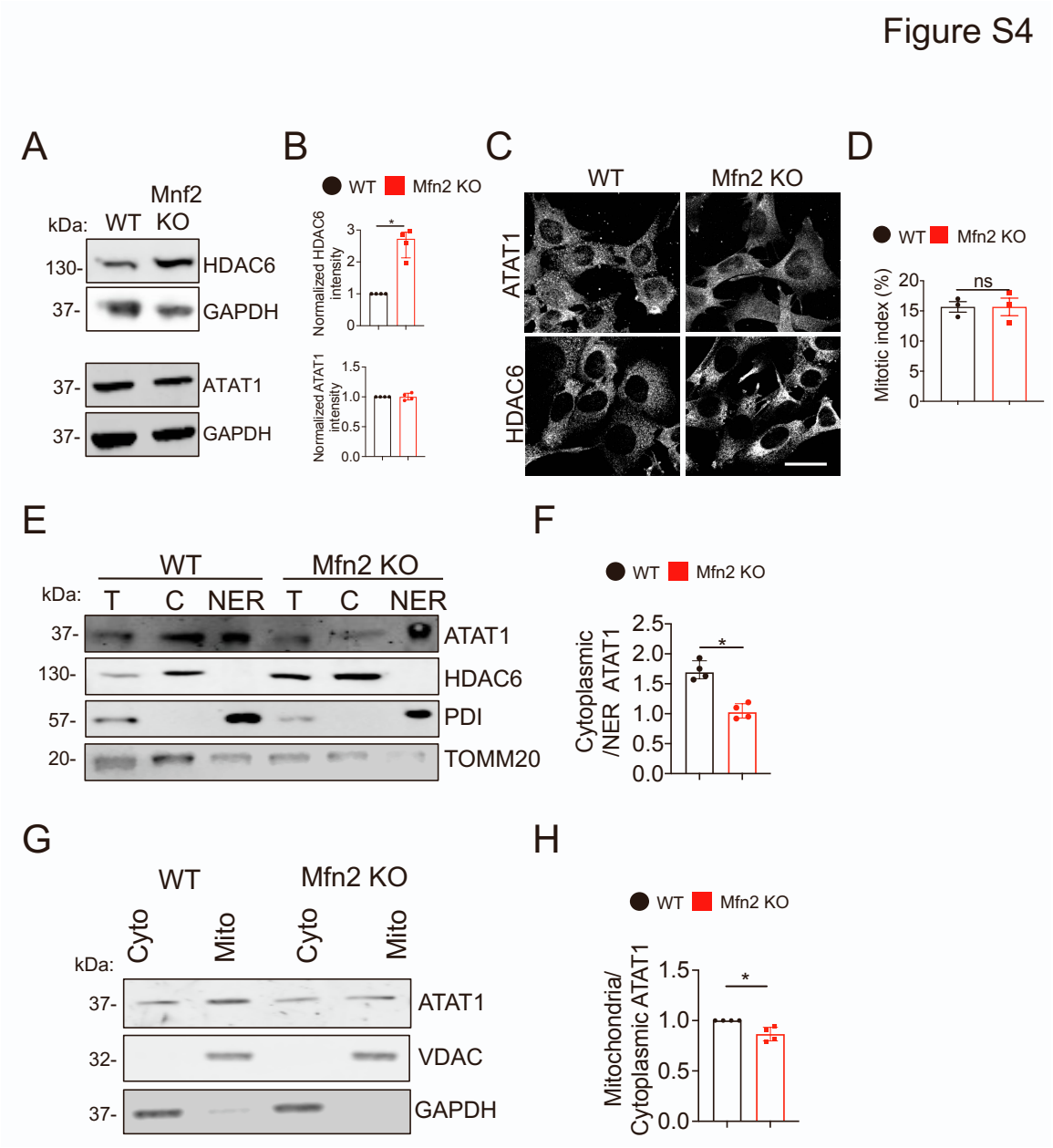

Figure S4. MFN2 regulates HDAC6 levels and localization of ATAT1, Related to Figure 3. (A) Representative immunoblot of HDAC6 and ATAT1 levels in WT and Mfn2 KO whole MEF lysates. GAPDH, loading control. (B) Quantification of expression level of HDAC6 and ATAT1 in Mfn2 KO MEFs relative to WT MEFs. (C) Representative immunofluorescence staining of ATAT1 and HDAC6 in WT and Mfn2 KO MEFs. (n=200-225 cells). Scale bar, 10 µm. (D) Mitotic index assessed by nuclear DAPI staining in WT and Mfn2 KO MEFs. (n=150 cells). (E) Representative immunoblot of cytosolic and nuclear envelope/endoplasmic reticulum (ER)

fractions (T=total lysate; C=cytoplasmic fraction; NER= nuclear ER fraction) from WT and Mfn2 KO MEF lysates. (F) Quantification of ATAT1 levels in cytoplasmic versus NER fraction expressed as ratio of intensity values from analysis as in E. (G) Representative immunoblot analysis of mitochondrial and cytoplasmic fraction from WT and Mfn2 KO MEF lysates. (H) Quantification of ATAT1 level in mitochondria versus cytoplasmic fraction expressed as ratio of intensity values. Data are median with interquartile range from 3-4 independent experiments, * $p \leq 0.05$, ** $p < 0.01$; ns: non-significant by Mann-Whitney U test.

Figure S5

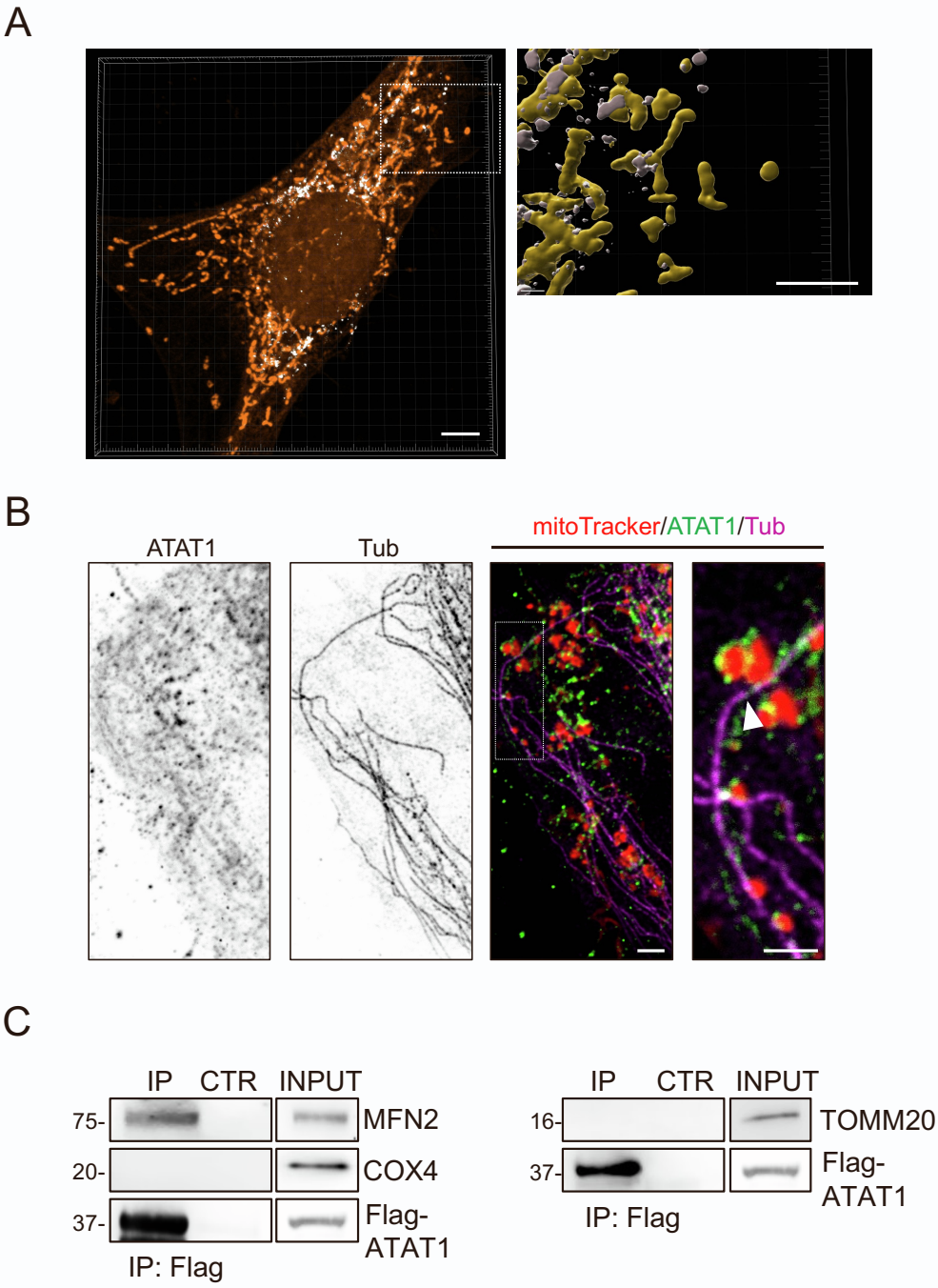

Figure S5. ATAT1 associates with mitochondria outer membranes at sites of contact with nicked MTs, Related to Figure 3. (A) Mitochondrial networks (mitoTracker Red) and MFN2/ATAT1 co-localizing puncta in a WT MEF cell imaged by Airyscan confocal microscopy (maximum projection is shown from a z-stack) and displayed by volume rendering and pseudocolors using Imaris software (Bitplane, Concord, MA). In the 3D rendering mitochondria

are represented in yellow and MFN2/ATAT1 co-localizing puncta are represented in white. Scale bars, 5 µm. (B) Airyscan confocal image (maximum projection is shown from a z-stack) showing ATAT1 punctuate localization at mitochondria (mitoTracker Red) and tyrosinated tubulin (tub) in a WT MEF cell. The white arrowhead shows a nick in the MT lattice. Scale bars, 10 µm and 5 µm in the zoomed image. (C) Interaction between transfected Flag-ATAT1 and MFN2, but not the outer mitochondrial protein TOMM20 or the inner mitochondrial protein COX4, was detected in HEK293T cells by immunoprecipitation (IP) of FLAG-ATAT1 followed by immunoblot with the indicated antibodies.

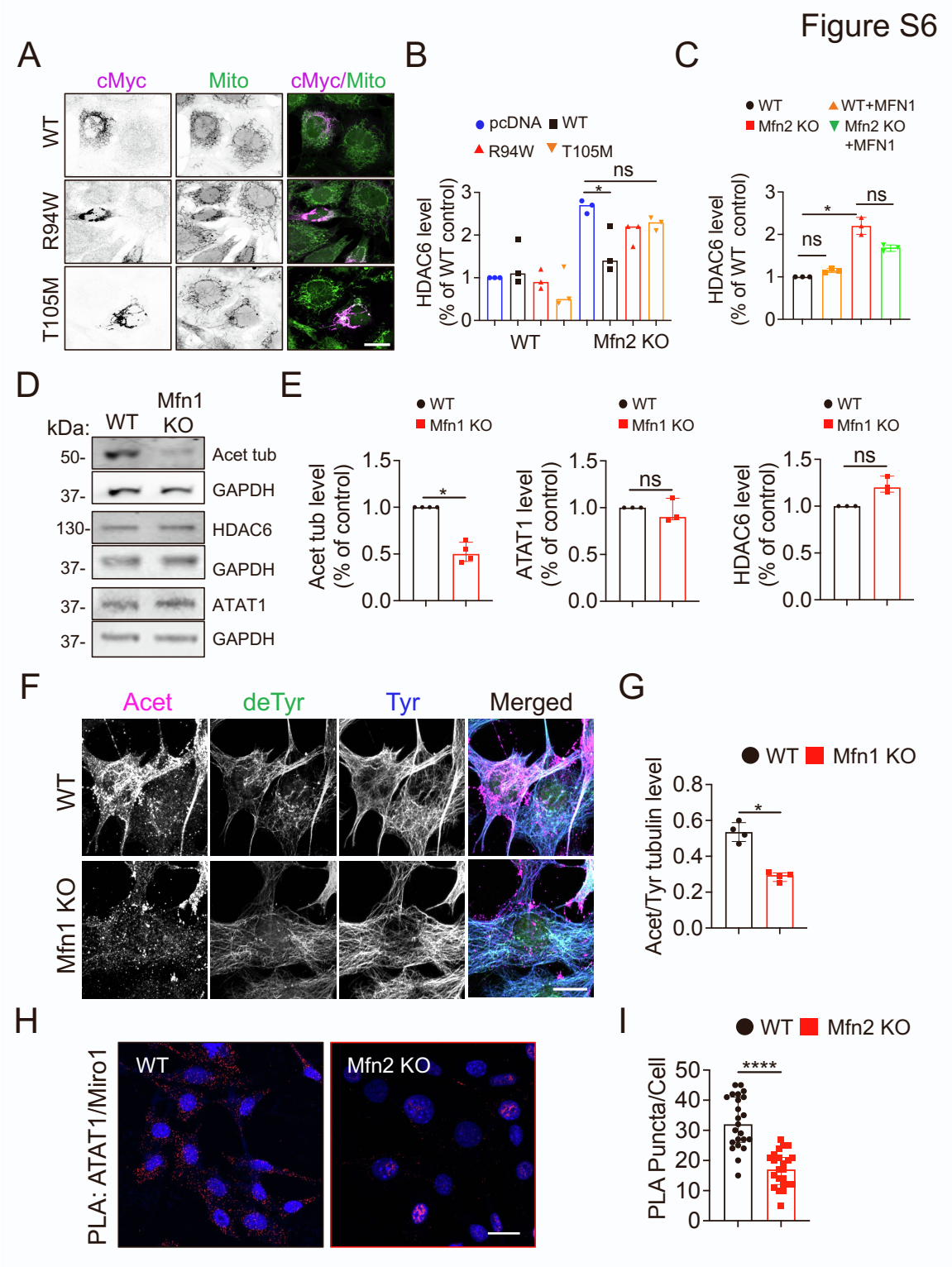

Figure S6. MFN1-dependent regulation of acetylated tubulin levels has no impact on HDAC6 protein levels and loss of Mfn2 expression suppresses endogenous ATAT1/miro binding in

MEFs, Related to Figure 4. (A) Representative confocal immunofluorescence images (maximum projections from z-stacks) showing mitochondria (mitodsRed) in WT MEF cells overexpressing Myc-MFN2 WT, Myc-MFN2 R94W, Myc-MFN2 T105M. Scale bar, 10 μm. (B) Quantification of HDAC6 levels in WT and Mfn2 KO MEFs upon overexpression of WT and mutant MFN2. (C) Quantification of HDAC6 levels in WT and Mfn2 KO MEFs upon overexpression of MNF1. (D) Representative immunoblot of acetylated tubulin (Acet tub), HDAC6 and ATAT1 levels in WT and Mfn1 KO cells. (E) Quantification of Acet tub, HDAC6 and ATAT1 levels in WT and Mfn1 KO cells as in D. (F) Representative immunofluorescence staining (maximum projections from z-stacks, 0.2 mm step size) of acetylated (Acet), detyrosinated (deTyr) and tyrosinated (Tyr) MTs in WT and Mfn1 KO MEFs. Scale bar, 10 μm. (G) Quantification of acetylated tubulin signal in Mfn11 KO MEFs relative to WT levels from images as in F. (H) Representative confocal immunofluorescence images (maximum projection from z-stacks) of Miro1 and ATAT1 PLA puncta in WT and Mfn2 KO MEFs. Scale bar, 10 μm. (I) Quantification of PLA puncta per cell in WT and Mfn2 KO MEFs as in H. Data are median with interquartile range from 3 independent experiments * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by Kruskal Wallis test (B,C) and Mann-Whitney U test (E,G,I).

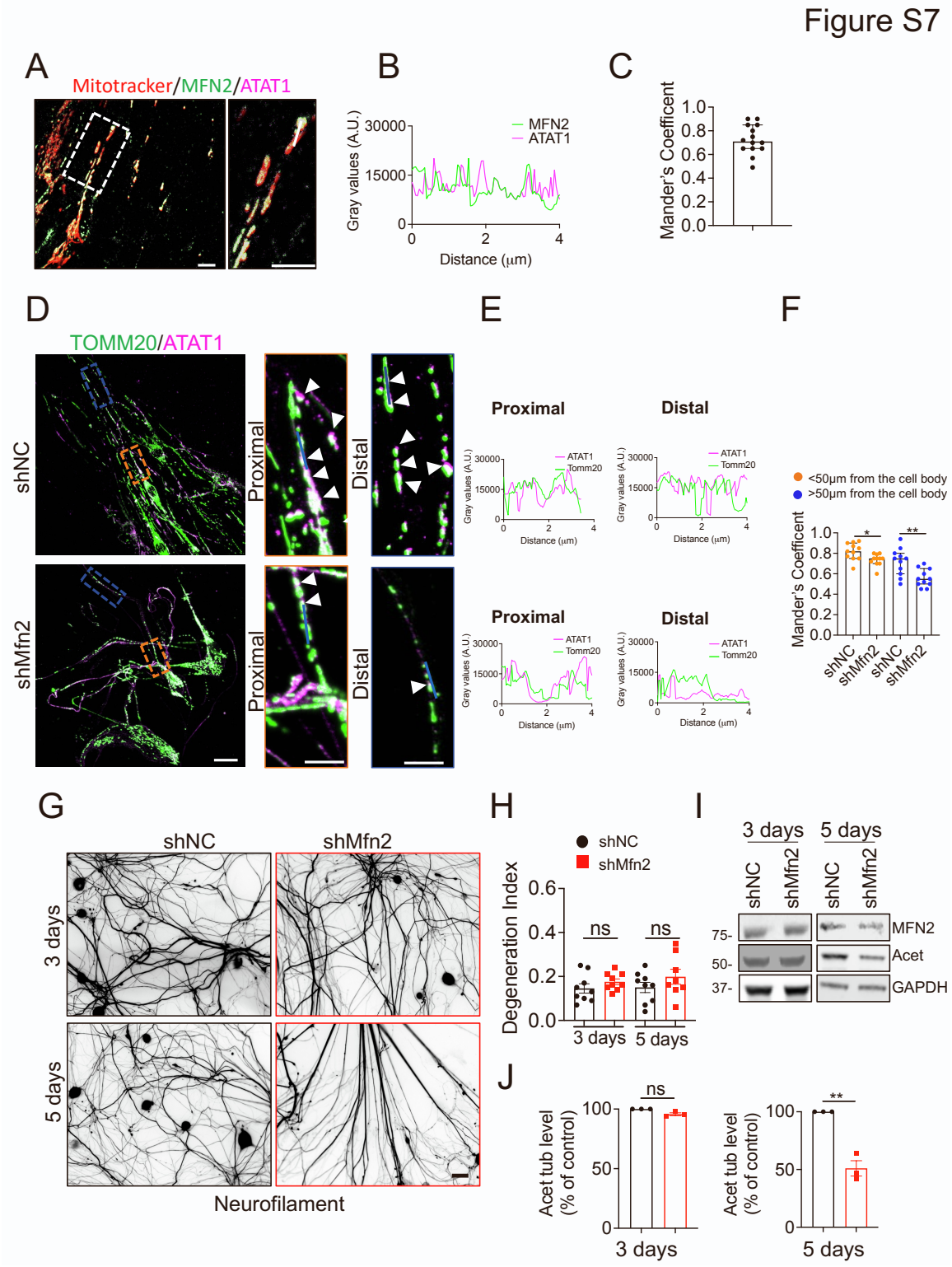

Figure S7. ATAT1 localization to mitochondria is dependent on MFN2 expression in primary adult DRG neurons and loss of acetylated tubulin precedes axonal degeneration in Mfn2 KD

DRG neurons, Related to Figure 5. (A) Representative Airyscan confocal image (single plane) of ATAT1 and MFN2 at mitochondria (mitoTracker Red) in adult DRG neurons (14 days). Zoomed image is shown from the boxed area. Scale bars, 10 μm. (B) Linescan analysis of ATAT1 and MFN2 signals from the white line shown in the zoomed image. (C) Quantification of MFN2 and ATAT1 co-localization as in (A) by Mander's correlation coefficient. N= 20 cells (D) Airyscan confocal images (single plane) of TOMM20 (red) and ATAT1 (green) signals in shNC and shMfn2 KD adult DRG neurons (14 days) infected at 7 DIV**.** Scale bars, 20 μm and 5 μm in the zoomed image. (E) Line scan analysis (from selected blues bars shown in D) at proximal and distal axonal segments in DRG neurons treated as in (D). (F) Mander's coefficient analysis of TOMM20 and ATAT1 signals from images of neurons treated as in (D) N=25 cells**.** (G) Neurofilament staining of shNC and shMfn2 KD DRG neurons infected for either 3 or 5 days starting at 7 DIV. Scale bar, 50 μm. (H) Degeneration index of axons in shNC and shMfn2 silenced DRG neurons treated as in (G). (I) Representative immunoblots of MFN2 and acetylated tubulin (Acet) levels in DRG neurons treated as in (G). (J) Quantification of acetylated tubulin (Acet tub) levels from immunoblot analysis as shown in (I). GAPDH, loading control. Data are median with interquartile range from 3 independent experiments. * $p \leq 0.05$, ** $p < 0.01$; *** $p < 0.001$ ns: non-significant by Mann–Whitney U test.

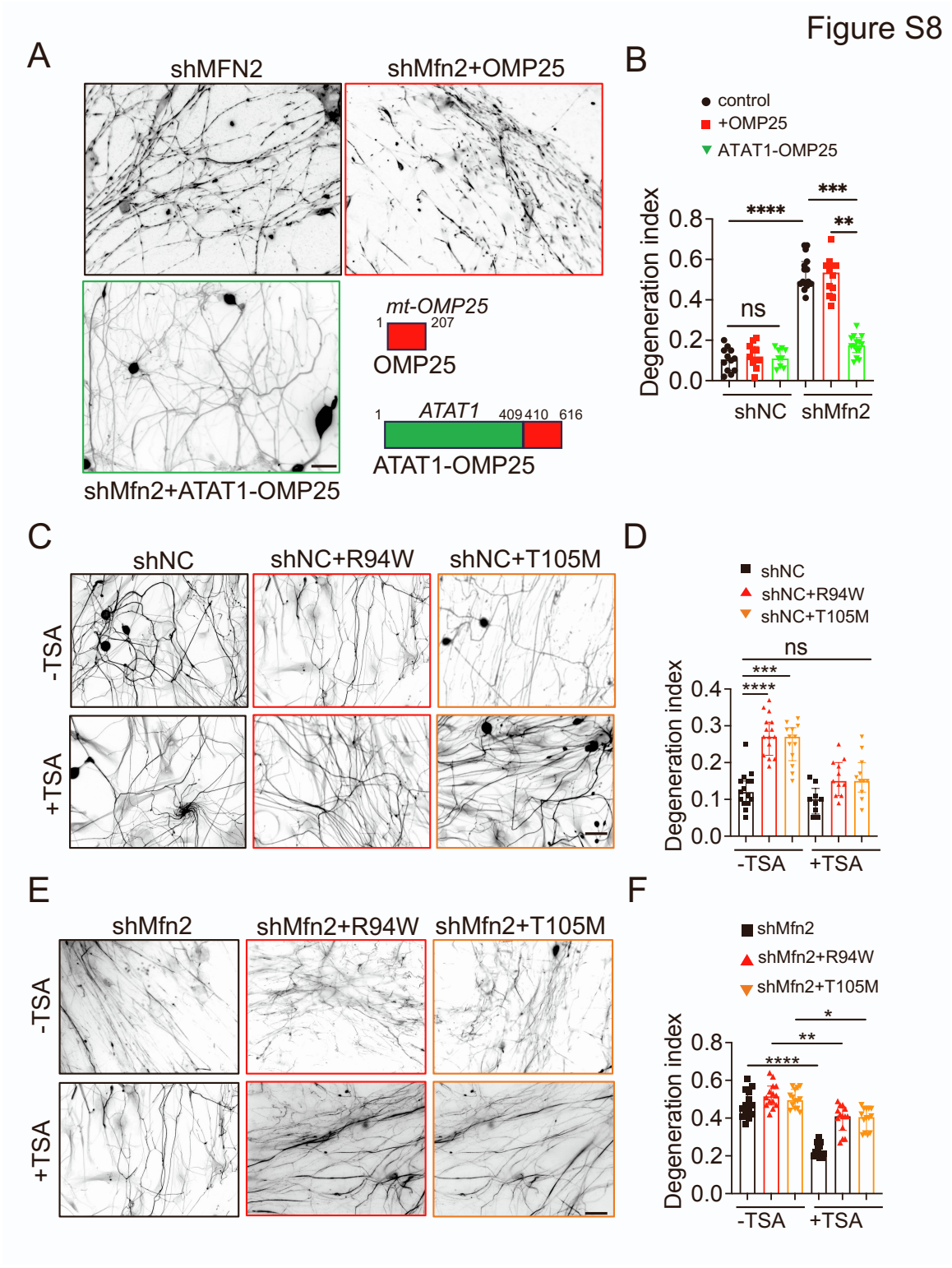

Figure S8. ATAT1, but not mutant MFN2, rescues axonal degeneration in MFN2 depleted DRG neurons, Related to Figure 5. (A) Neurofilament staining of shMfn2 KD DRG neurons at

14DIV overexpressing the mitochondrial targeting domain of OMP25 alone or the fusion protein ATAT1-OMP25 by lentiviral infection for 5 days. Scale bar, 50 µm. (B) Quantification of degree of axonal degeneration in shNC and shMfn2 KD DRG neurons as in A (9-19 random fields from 3 independent experiments). (C) Neurofilament staining of shNC DRG neurons at 7DIV overexpressing R94W or T105M Mfn2 mutants by retroviral infection for 5 days prior to treatment with TSA (10 nM, 6 h), fixation and immunostaining. Scale bar, 50 µm. (D) Quantification of degree of axonal degeneration in adult DRG neurons (14DIV) as in C (15-20 random field from 3 independent experiments). (E) Neurofilament staining of shMfn2 KD DRG neurons overexpressing R94W or T105M Mfn2 mutants by retroviral infection for 5 days prior to treatment with TSA (10 nM, 6 h), fixation and immunostaining. Scale bar, 50 µm (F) Quantification of degree of axonal degeneration in adult DRG neurons (14DIV) as in E (15 random field from 3 independent experiments). Data are median with interquartile range from 3 independent experiments. * $p \leq 0.05$, ** $p < 0.01$; *** $p < 0.001$ ns: non-significant by Kruskal Wallis test.

Table S1

	WT	Mfn2 KO	WT+ TSA	Mfn2 KO + TSA
Growth rate (µm/s)	0.06 ± 0.01	0.11 ± 0.008 *	0.04 ± 0.007	0.08 ± 0.005 *
Shrinkage rate (µm/s)	0.08 ± 0.004	0.11 ± 0.009 *	0.07 ± 0.008	0.08 ± 0.005 *
Catastrophe freq. (s-1)	0.06 ± 0.006	0.06 ± 0.006	0.05 ± 0.004	0.05 ± 0.006
Rescue freq. (s-1)	0.08 ± 0.006	0.08 ± 0.006	0.07 ± 0.006	0.09 ± 0.004
% Growth	39.5 ± 1.32	49.95 ± 0.95 ***	20.04 ± 1.28 ****	22.4 ± 0.90 ****
% Shrinkage	32.05 ± 0.87	37.68 ± 1.77 *	18.25 ± 1.37 ****	16.05 ± 1.42 ****
% Pause	20.68 ± 0.71	17.1 ± 1.37	63.5 ± 0.64 ****	62.13 ± 1.68 ****
MT lifetime (s)	60.25 ± 2.05	61.5 ± 1.93	56.5 ± 3.66	60.25 ± 1.43
MT dynamicity (µm/min)	6.6 ± 0.64	11.63 ± 0.43 ***	4.17 ± 0.44 *	4.858 ± 0.46 ****
Number of MTs	22	22	24	24

Table S1. HDAC inhibition normalizes MT dynamics in Mfn2 KO MEFs, Related to Figure 2. MT dynamics were measured from time-lapse analysis of GFP-tubulin-labeled MTs using epifluorescence microscopy in WT and Mfn2 KO MEFs treated with 10 nM trichostatin A (TSA) for 6 h. Live imaging of MT dynamics was performed for 5 min (1f/5s). * in black represents statistical comparison with WT control while * in red represents statistical comparison with Mfn2 KO control. Data are mean ± SEM from 3 independent experiments. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; **** $p<<0.001$ by 2-way ANOVA with Dunnett's multiple comparison.

Table S2

	WT	Iqgap1 KO	WT+ TSA	Iqgap1 KO + TSA
Growth rate (µm/s)	0.06 ± 0.008	0.13 ± 0.004 ***	0.04 ± 0.004	0.095 ± 0.006 **
Shrinkage rate (µm/s)	0.04 ± 0.010	0.12 ± 0.006 ***	0.03 ± 0.004	0.09 ± 0.004
Catastrophe freq. (s-1)	0.07 ± 0.004	0.06 ± 0.007	0.06 ± 0.003	0.06 ± 0.004
Rescue freq. (s-1)	0.07 ± 0.006	0.08 ± 0.010	0.07 ± 0.007	0.08 ± 0.006
% Growth	45.5 ± 2.021	45 ± 1.354	23.75 ± 0.85 ***	25.25 ± 1.54 ***
% Shrinkage	31.5 ± 2.63	30.18 ± 2.254	27.25 ± 3.19	22.5 ± 0.86 ***
% Pause	22.5 ± 1.041	24.75 ± 1.652	50 ± 2.48 ***	51.75 ± 1.10 ***
MT lifetime (s)	47.5 ± 2.533	51 ± 1.472	49.5 ± 2.32	51.25 ± 1.79
MT dynamicity (µm/min)	5.22 ± 0.317	8.17 ± 0.606 **	3.32 ± 0.24 **	3.77 ± 0.33 **
Number of MTs	20	21	25	25

Table S2. HDAC inhibition rescues MT dynamics in Iqgap1 KO MEFs, Related to Figure 2. MT dynamics were measured from time-lapse analysis of WT and Iqgap1 KO MEFs transfected with EGFP-tubulin prior to treatment with vehicle or 10 nM trichostatin A (TSA) for 6 h. Live imaging was performed for 5 min (1f/5s). * black represents statistical comparison with WT control and * red represents statistical comparison with Iqgap1 KO control. Data are mean ± SEM from 3 independent experiments. * $p<0.05$; ** $p<0.01$; *** $p<0.001$; by 2-way ANOVA with Dunnett's multiple comparison.

INTERNAL STANDARD	Corresponding Lipid Class	Concentration (ug/ul)
IS AcylPG 14:0-28:0	Acyl PG, NAPE, NAPS	**0.046799614**
IS BMP 28:0	BMP	**0.015298133**
IS CE C17	CE	**78.59098931**
IS Cer C17:0	Cer, dhCer	**0.758320608**
IS Chol d7 b	Free Cholesterol	**63.78791732**
IS DG 4ME	diacylglycerols	**0.640874053**
IS dhSM d18:0/12:0	dihydrosphingomyelins	**2.579623778**
IS DMPC	AC	**12.34642208**
IS GalCer d18:1/12:0	MhCer	**1.039897431**
IS LacCer d18:1/12:0	LacCer	**0.259594347**
IS LPC 13:0	LPC	**12.34642208**
IS LPE 14:0	LPE	**0.098349468**
IS LPI 13:0	LPI	**0.07642123**
IS MG C17	MG	**0.242952978**
IS PA 28:0	PA	**0.068072997**
IS PC 28:0	PC	**12.34642208**
IS PE 25:0	PE	**8.839285714**

IS PG 12:0/13:0	PG	**0.446428571**
IS PI 12:0/13:0	PI	**2.232142857**
IS PS 28:0	PS	**11.92531331**
IS SM d18:1/12:0	SM	**13.39285714**
IS Sulf d18:1/12:0	Sulf	**0.225924621**
IS TG 50:0 d5	TG	**0.498018035**

Table S3. List of internal standards used to calculate the concentrations of corresponding lipid classes, Related to Figure 2.

Data S1. Map of the newly generated lentiviral plasmid expressing the mitochondrially targeting OMP25 domain alone, Related to Figure S8

GenBank: AF107295.1

GenBank Graphics

>AF107295.1 Rattus norvegicus outer membrane protein (OMP25) mRNA, complete cds; nuclear gene for mitochondrial product; cds 456…1076; c-terminal domain

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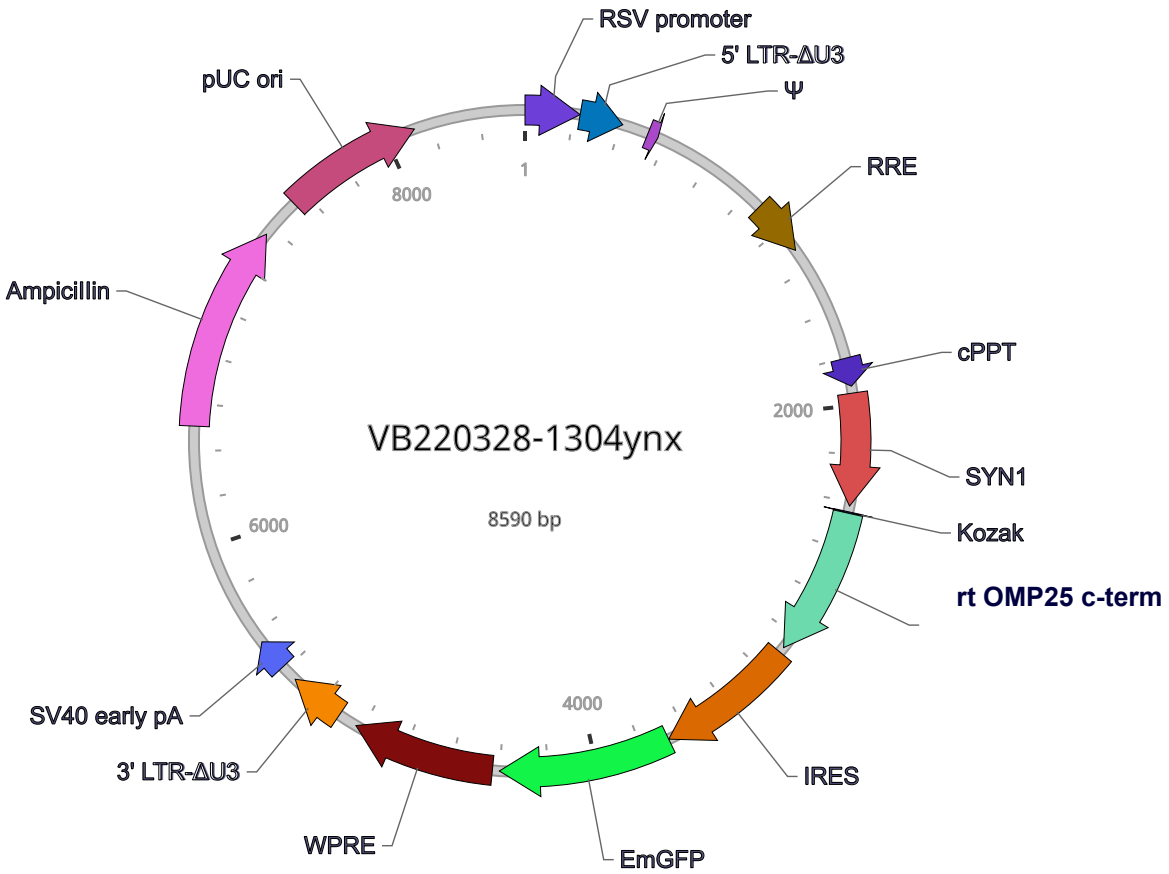
TTAGTCCCAGCGTTAGGATGCAGAGATCCATTTCT

Vector Summary

Vector ID	VB220328-1304ynx
Vector Name	pLV[Exp]-SYN1>{rtOMP25C-ter Lenti WT}:IRES:EmGFP
Vector Size	8590 bp
Viral Genome Size	5115 bp
Vector Type	Mammalian Gene Expression Lentiviral Vector
Inserted Promoter	SYN1
Inserted ORF	{hATAT1(1)rtOMP25C-ter Lenti WT}, EmGFP
Inserted Linker	IRES
Plasmid Copy Number	High
Antibiotic Resistance	Ampicillin
Cloning Host	VB UltraStable (or alternative strain)

Vector Map



Vector Components

Name	Position	Size (bp)	Type	Description	Application notes
RSV promoter	1-229	229	Promoter	Rous sarcoma virus enhancer/promoter	Strong promoter; drives transcription of viral RNA in packaging cells.
5' LTR-ΔU3	230-410	181	LTR	Truncated HIV-1 5' long terminal repeat	Allows transcription of viral RNA and its packaging into virus.
Ψ	521-565	45	Miscellaneous	HIV-1 packaging signal	Allows packaging of viral RNA into virus.
RRE	1075-1308	234	Miscellaneous	HIV-1 Rev response element	Rev protein binding site that allows Rev-dependent nuclear export of viral RNA during viral packaging.
cPPT	1803-1920	118	Miscellaneous	Central polypurine tract	Facilitates the nuclear import of HIV-1 cDNA through a central DNA flap.
SYN1	1950-2418	469	Promoter	Human synapsin I promoter	Tissue specificity: Brain. Cell type specificity: Mature neurons.
Kozak	2443-2448	6	Miscellaneous	Kozak translation initiation sequence	Facilitates translation initiation of ATG start codon downstream of the Kozak sequence.
rtOMP25C-ter Lenti W...	2449-3069	621	CDS	*None*	*None*
IRES	3094-3681	588	Linker	Encephalomyocarditis virus internal ribosome entry site	Recruits ribosome to initiate translation internally on a transcript independent of its 5' end. Multiple proteins can be made from a polycistronic transcript containing multiple ORFs separated by IRES.

Name	Position	Size (bp)	Type	Description	Application notes
EmGFP	3682-4401	720	CDS	Emerald green fluorescent protein; variant of EGFP generated by mutagenesis	Enhanced photostability and brightness compared to its predecessor EGFP.
WPRE	4431-5028	598	Miscellaneous	Woodchuck hepatitis virus posttranscriptional regulatory element	Enhances virus stability in packaging cells, leading to higher titer of packaged virus; enhances higher expression of transgenes.
3' LTR-ΔU3	5110-5344	235	LTR	Truncated HIV-1 3' long terminal repeat	Allows packaging of viral RNA into virus; self-inactivates the 5' LTR by a copying mechanism during viral genome integration; contains polyadenylation signal for transcription termination.
SV40 early pA	5417-5551	135	PolyA_signal	Simian virus 40 early polyadenylation signal	Allows transcription termination and polyadenylation of mRNA transcribed by Pol II RNA polymerase.
AmpiciIIin	6505-7365	861	CDS	AmpiciIIin resistance gene	Allows E. coli to be resistant to ampiciIIin.
pUC ori	7536-8124	589	Rep_origin	pUC origin of replication	Facilitates plasmid replication in E. coli; regulates high-copy plasmid number (500-700).

Note: Components added by user are listed in **bold red** text.

Vector Sequence

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   1 AATGTAGTCT TATGCAATAC TCTTGTAGTC TTGCAACATG GTAACGATGA GTTAGCAACA TGCCTTACAA GGAGAGAAAA
  81 AGCACCGTGC ATGCCGATTG GTGGAAGTAA GGTGGTACGA TCGTGCCTTA TTAGGAAGGC AACAGACGGG TCTGACATGG
 161 ATTGGACGAA CCACTGAATT GCCGCATTGC AGAGATATTG TATTTAAGTG CCTAGCTCGA TACATAAACG GGTCTCTCTG
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641  GAAAGAAAAA ATATAAATTA AAACATATAG TATGGGCAAG CAGGGAGCTA GAACGATTCG CAGTTAATCC TGGCCTGTTA
721  GAAACATCAG AAGGCTGTAG ACAAATACTG GGACAGCTAC AACCATCCCT TCAGACAGGA TCAGAAGAAC TTAGATCATT
801  ATATAATACA GTAGCAACCC TCTATTGTGT GCATCAAAGG ATAGAGATAA AAGACACCAA GGAAGCTTTA GACAAGATAG
881  AGGAAGAGCA AAACAAAAGT AAGACCACCG CACAGCAAGC GGCCGCTGAT CTTCAGACCT GGAGGAGGAG ATATGAGGGA
961  CAATTGGAGA AGTGAATTAT ATAAATATAA AGTAGTAAAA ATTGAACCAT TAGGAGTAGC ACCCACCAAG GCAAAGAGAA
1041 GAGTGGTGCA GAGAGAAAAA AGAGCAGTGG GAATAGGAGC TTTGTTCCTT GGGTTCTTGG GAGCAGCAGG AAGCACTATG
1121 GGCGCAGCGT CAATGACGCT GACGGTACAG GCCAGACAAT TATTGTCTGG TATAGTGCAG CAGCAGAACA ATTTGCTGAG
1201 GGCTATTGAG GCGCAACAGC ATCTGTTGCA ACTCACAGTC TGGGGCATCA AGCAGCTCCA GGCAAGAATC CTGGCTGTGG
1281 AAAGATACCT AAAGGATCAA CAGCTCCTGG GGATTTGGGG TTGCTCTGGA AAACTCATTT GCACCACTGC TGTGCCTTGG
1361 AATGCTAGTT GGAGTAATAA ATCTCTGGAA CAGATTTGGA ATCACACGAC CTGGATGGAG TGGGACAGAG AAATTAACAA
1441 TTACACAAGC TTAATACACT CCTTAATTGA AGAATCGCAA AACCAGCAAG AAAAGAATGA ACAAGAATTA TTGGAATTAG
1521 ATAAATGGGC AAGTTTGTGG AATTGGTTTA ACATAACAAA TTGGCTGTGG TATATAAAAT TATTCATAAT GATAGTAGGA
1601 GGCTTGGTAG GTTTAAGAAT AGTTTTTGCT GTACTTTCTA TAGTGAATAG AGTTAGGCAG GGATATTCAC CATTATCGTT
1681 TCAGACCCAC CTCCCAACCC CGAGGGGACC CGACAGGCCC GAAGGAATAG AAGAAGAAGG TGGAGAGAGA GACAGAGACA
1761 GATCCATTCG ATTAGTGAAC GGATCTCGAC GGTATCGCTA GCTTTTAAAA GAAAAGGGGG GATTGGGGGG TACAGTGCAG
1841 GGGAAAGAAT AGTAGACATA ATAGCAACAG ACATACAAAC TAAAGAATTA CAAAAACAAA TTACAAAAAT TCAAAATTTT
1921 ACTAGTATCA ACTTTGTATA GAAAAGTTGC TGCAGAGGGC CCTGCGTATG AGTGCAAGTG GGTTTTAGGA CCAGGATGAG
2001 GCGGGGTGGG GGTGCCTACC TGACGACCGA CCCCGACCCA CTGGACAAGC ACCCAACCCC CATTCCCCAA ATTGCGCATC
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2161 TCGCCCCCGC CTGGCGGCGC GCGCCACCGC CGCCTCAGCA CTGAAGGCGC GCTGACGTCA CTCGCCGGTC CCCCGCAAAC
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2721 GACAGATCAA CAGTATGTCT CCAATGACAG TGGCATCTAC GTCAGCCGCA TCAAAGAGGA TGGGGCTGCG GCCCGGGATG
2801 GGCGGCTCCA GGAGGGTGAT AAGATCCTCT CGGTAAATGG CCAAGACCTG AAGAACCTGT TGCACCAAGA TGCCGTAGAC
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3601 GGTTAAAAAA ACGTCTAGGC CCCCCGAACC ACGGGGACGT GGTTTTCCTT TGAAAAACAC GATGATAATA TGGCCACAAC
3681 CATGGTGAGC AAGGGCGAGG AGCTGTTCAC CGGGGTGGTG CCCATCCTGG TCGAGCTGGA CGGCGACGTA AACGGCCACA
3761 AGTTCAGCGT GTCCGGCGAG GGCGAGGGCG ATGCCACCTA CGGCAAGCTG ACCCTGAAGT TCATCTGCAC CACCGGCAAG
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8481  AAAGGGAACA AAAGCTGGAG CTGCAAGCTT
8561
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Validation by Restriction Enzyme Digestion

Restriction Enzymes	Cutting Sites	DNA Fragments (bp)
SmaI	2605, 2795	190, 8400
AvaI	1700, 2603, 2692, 2793	903, 89, 101, 7497
NheI	1798	8590
XmaI	2603, 2793	190, 8400
ApaLI	3573, 4645, 6621, 7867	1072, 1976, 1246, 4296
ApaLI+XmaI	2603, 2793, 3573, 4645, 6621, 7867	190, 780, 1072, 1976, 1246, 3326
ApaLI+SmaI	2605, 2795, 3573, 4645, 6621, 7867	190, 778, 1072, 1976, 1246, 3328
ApaLI+AvaI	1700, 2603, 2692, 2793, 3573, 4645, 6621, 7867	903, 89, 101, 780, 1072, 1976, 1246, 2423
ApaLI+NheI	1798, 3573, 4645, 6621, 7867	1775, 1072, 1976, 1246, 2521

Data S2. Map of the newly generated lentiviral plasmid expressing ATAT1 fused to the mitochondrially targeting OMP25 domain, Related to Figure S8

>NM_024909.5 Homo sapiens alpha tubulin acetyltransferase 1 (ATAT1), transcript variant 2, mRNA
>NM_022599.2 Rattus norvegicus synaptojanin 2 binding protein (Synj2bp), mRNA

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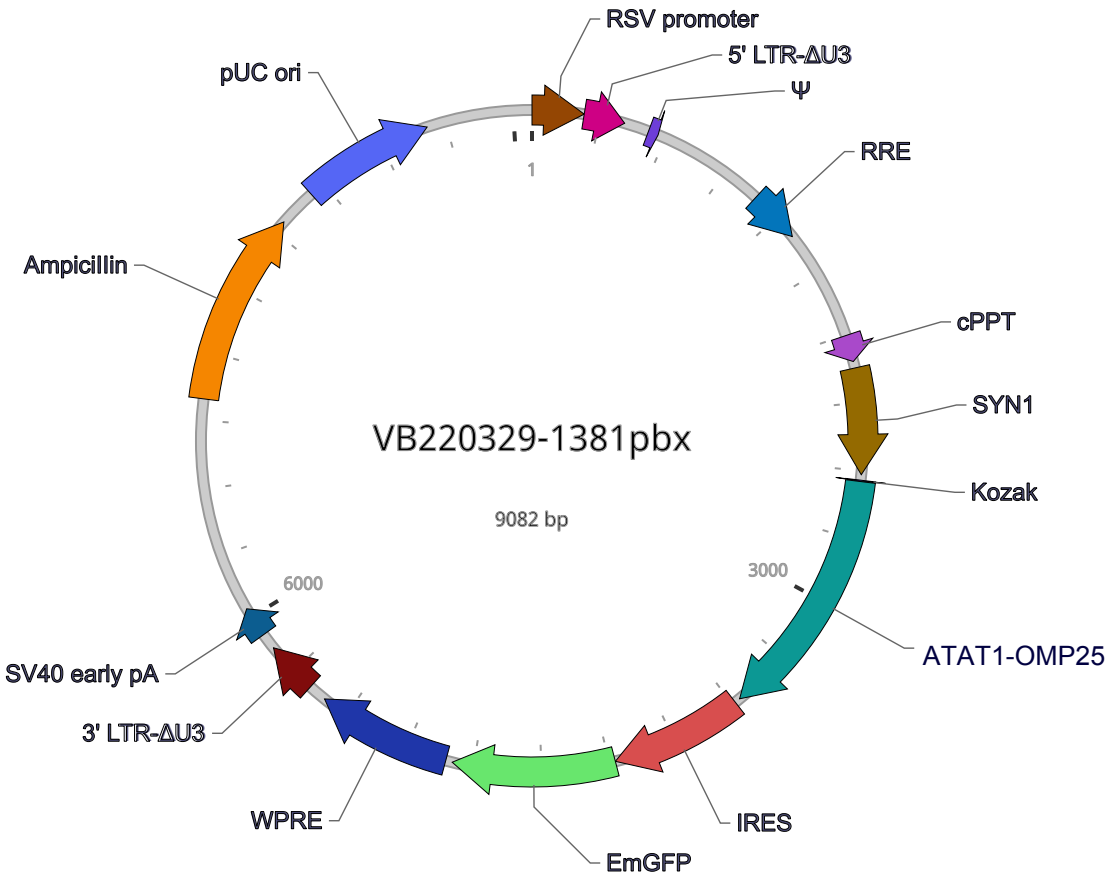
Vector Summary

Vector ID	VB220329-1381pbx
Vector Name	pLV[Exp]-SYN1>{hATAT1(2)rtOMP25C-ter Lenti WT}:IRES:EmGFP
Vector Size	9082 bp
Viral Genome Size	5607 bp
Vector Type	Mammalian Gene Expression Lentiviral Vector
Inserted Promoter	SYN1
Inserted ORF	{hATAT1(2)rtOMP25C-ter Lenti WT}, EmGFP
Inserted Linker	IRES
Plasmid Copy Number	High
Antibiotic Resistance	Ampicillin
Cloning Host	VB UltraStable (or alternative strain)

Vector Map



Vector Components

Name	Position	Size (bp)	Type	Description	Application notes
RSV promoter	1-229	229	Promoter	Rous sarcoma virus enhancer/promoter	Strong promoter; drives transcription of viral RNA in packaging cells.
5' LTR-ΔU3	230-410	181	LTR	Truncated HIV-1 5' long terminal repeat	Allows transcription of viral RNA and its packaging into virus.
Ψ	521-565	45	Miscellaneous	HIV-1 packaging signal	Allows packaging of viral RNA into virus.
RRE	1075-1308	234	Miscellaneous	HIV-1 Rev response element	Rev protein binding site that allows Rev-dependent nuclear export of viral RNA during viral packaging.
cPPT	1803-1920	118	Miscellaneous	Central polypurine tract	Facilitates the nuclear import of HIV-1 cDNA through a central DNA flap.
SYN1	1950-2418	469	Promoter	Human synapsin I promoter	Tissue specificity: Brain. Cell type specificity: Mature neurons.
Kozak	2443-2448	6	Miscellaneous	Kozak translation initiation sequence	Facilitates translation initiation of ATG start codon downstream of the Kozak sequence.
{hATAT1(2)rtOMP25C-terLenti W...	2449-3561	1113	CDS	*None*	*None*
IRES	3586-4173	588	Linker	Encephalomyocarditis virus internal ribosome entry site	Recruits ribosome to initiate translation internally on a transcript independent of its 5' end. Multiple proteins can be made from a polycistronic transcript containing multiple ORFs separated by IRES.

Name	Position	Size (bp)	Type	Description	Application notes
EmGFP	4174-4893	720	CDS	Emerald green fluorescent protein; variant of EGFP generated by mutagenesis	Enhanced photostability and brightness compared to its predecessor EGFP.
WPRE	4923-5520	598	Miscellaneous	Woodchuck hepatitis virus posttranscriptional regulatory element	Enhances virus stability in packaging cells, leading to higher titer of packaged virus; enhances higher expression of transgenes.
3' LTR-ΔU3	5602-5836	235	LTR	Truncated HIV-1 3' long terminal repeat	Allows packaging of viral RNA into virus; self-inactivates the 5' LTR by a copying mechanism during viral genome integration; contains polyadenylation signal for transcription termination.
SV40 early pA	5909-6043	135	PolyA_signal	Simian virus 40 early polyadenylation signal	Allows transcription termination and polyadenylation of mRNA transcribed by Pol II RNA polymerase.
AmpiciIIin	6997-7857	861	CDS	AmpiciIIin resistance gene	Allows E. coli to be resistant to ampiciIIin.
pUC ori	8028-8616	589	Rep_origin	pUC origin of replication	Facilitates plasmid replication in E. coli; regulates high-copy plasmid number (500-700).

Note: Components added by user are listed in **bold red** text.

Vector Sequence

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   1 AATGTAGTCT TATGCAATAC TCTTGTAGTC TTGCAACATG GTAACGATGA GTTAGCAACA TGCCTTACAA GGAGAGAAAA
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8801  GCGGGCAGTG AGCGCAACGC AATTAATGTG AGTTAGCTCA CTCATTAGGC ACCCCAGGCT TTACACTTTA TGCTTCCGGC
8881  TCGTATGTTG TGTGGAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCTATGACCA TGATTACGCC AAGCGCGCAA
8961  TTAACCCTCA CTAAAGGGAA CAAAAGCTGG AGCTGCAAGC TT
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Validation by Restriction Enzyme Digestion

Restriction Enzymes	Cutting Sites	DNA Fragments (bp)
DrdI	6689, 8571	1882, 7200
SpeI	1922, 2642	720, 8362
ApaLI	4065, 5137, 7113, 8359	1072, 1976, 1246, 4788
EcoRI	5522	9082
DraIII	3951, 6645	2694, 6388
ApaLI+SpeI	1922, 2642, 4065, 5137, 7113, 8359	720, 1423, 1072, 1976, 1246, 2645
ApaLI+DrdI	4065, 5137, 6689, 7113, 8359, 8571	1072, 1552, 424, 1246, 212, 4576
ApaLI+EcoRI	4065, 5137, 5522, 7113, 8359	1072, 385, 1591, 1246, 4788
ApaLI+DraIII	3951, 4065, 5137, 6645, 7113, 8359	114, 1072, 1508, 468, 1246, 4674