Expanded View Figures

Figure EV1. Tau affects mitochondrial morphology and ER-mitochondria association.

A Representative microscopy images of the mitochondrial network morphology in neurons of the amygdala (top) and the Ca1 region of the hippocampus (bottom) in mitoCFP$^+$/P301L$^-$ mice and mitoCFP$^+$/P301L$^+$ mice. MitoCFP signal is displayed in gray on the images. Squares represent the cropped area presented in Fig 1B and D.

B–E Complementary metrics of mitochondrial network morphology: form factor (mitochondrial elongation) (B, D), and area-weighted form factor, a variant of form factor with a bias toward larger mitochondria (C, E) in neurons of the amygdala (B, C) and in Ca1 (D, E) of mitoCFP$^+$/P301L$^-$ and mitoCFP$^+$/P301L$^+$ mice. Values represent the mean ± SEM of $n = 11$ MitoCFP$^+$/P301L$^-$ mice and $n = 13$ MitoCFP$^+$/P301L$^+$ mice. Each gray open circle represents the mean of 6 technical replicates (data from 6 images) per animal. *$P < 0.05$, Student unpaired t-test.

F Representative microscopy images of the endoplasmic reticulum stained with calnexin (in red) and mitochondria (mitoCFP) in neurons of the amygdala (top) and Ca1 (bottom) in mitoCFP$^+$/P301L$^-$ and mitoCFP$^+$/P301L$^+$ mice. Squares represent the cropped area presented in Fig 1F and H.

G–L Complementary metrics of mitochondrial network morphology: form factor (mitochondrial elongation) (G, I, K) and area-weighted form factor (H, J, L) in WT-GFP, wtTau-GFP and P301L-GFP expressing SH-SY5Y cells (G, H), WT and Tau KO SH-SY5Y cells (I, J), and iPSC-WT and iPSC-P301L (K, L). On average 1,000–2,500 mitochondrial organelles were analyzed per group ($n = 20$–45 images per group, 3 experiments).

Data information: (G, H) *$P < 0.05$; One-way ANOVA + Tukey's *post hoc* test. (I–L) *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; Student unpaired t-test.

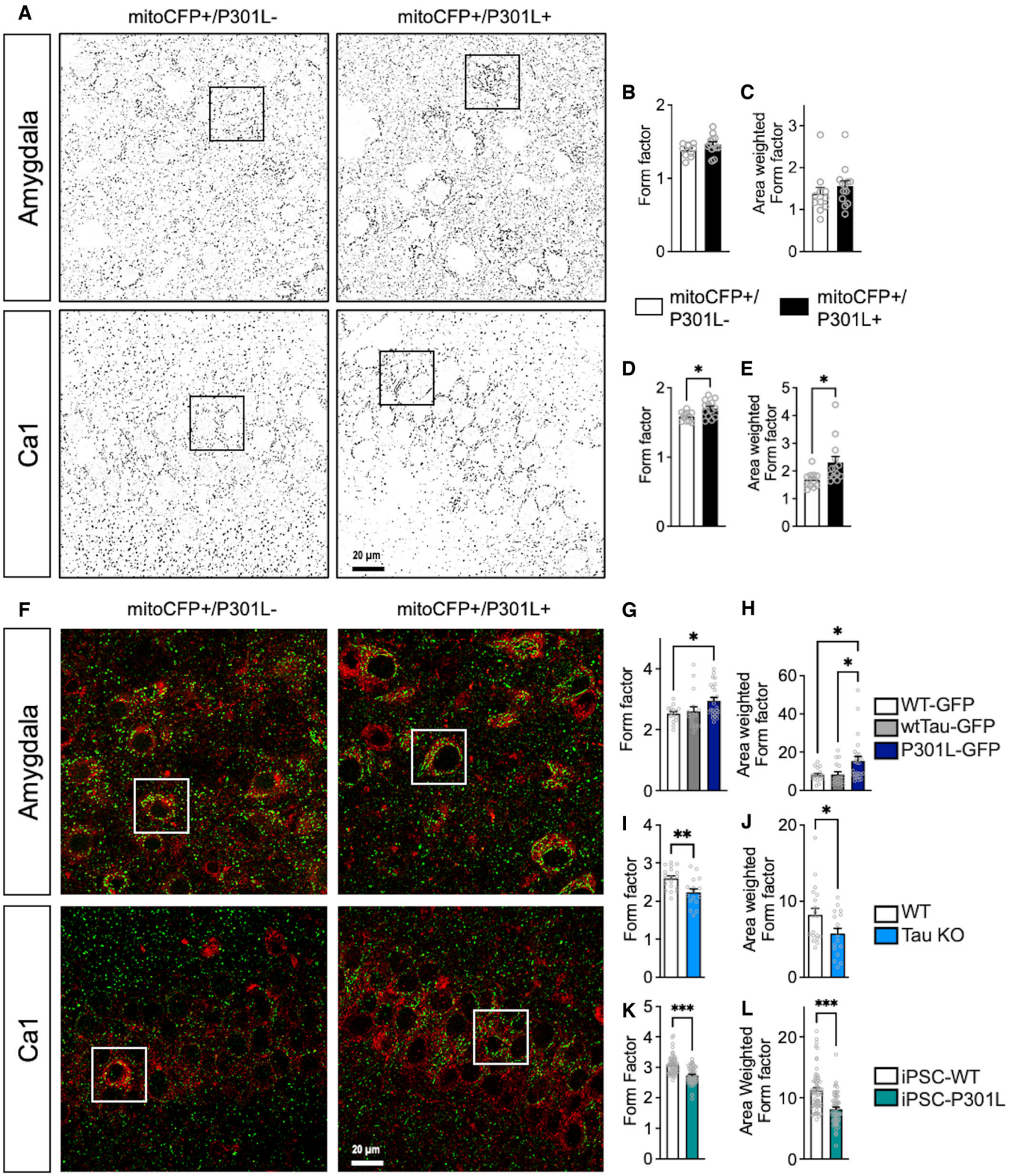
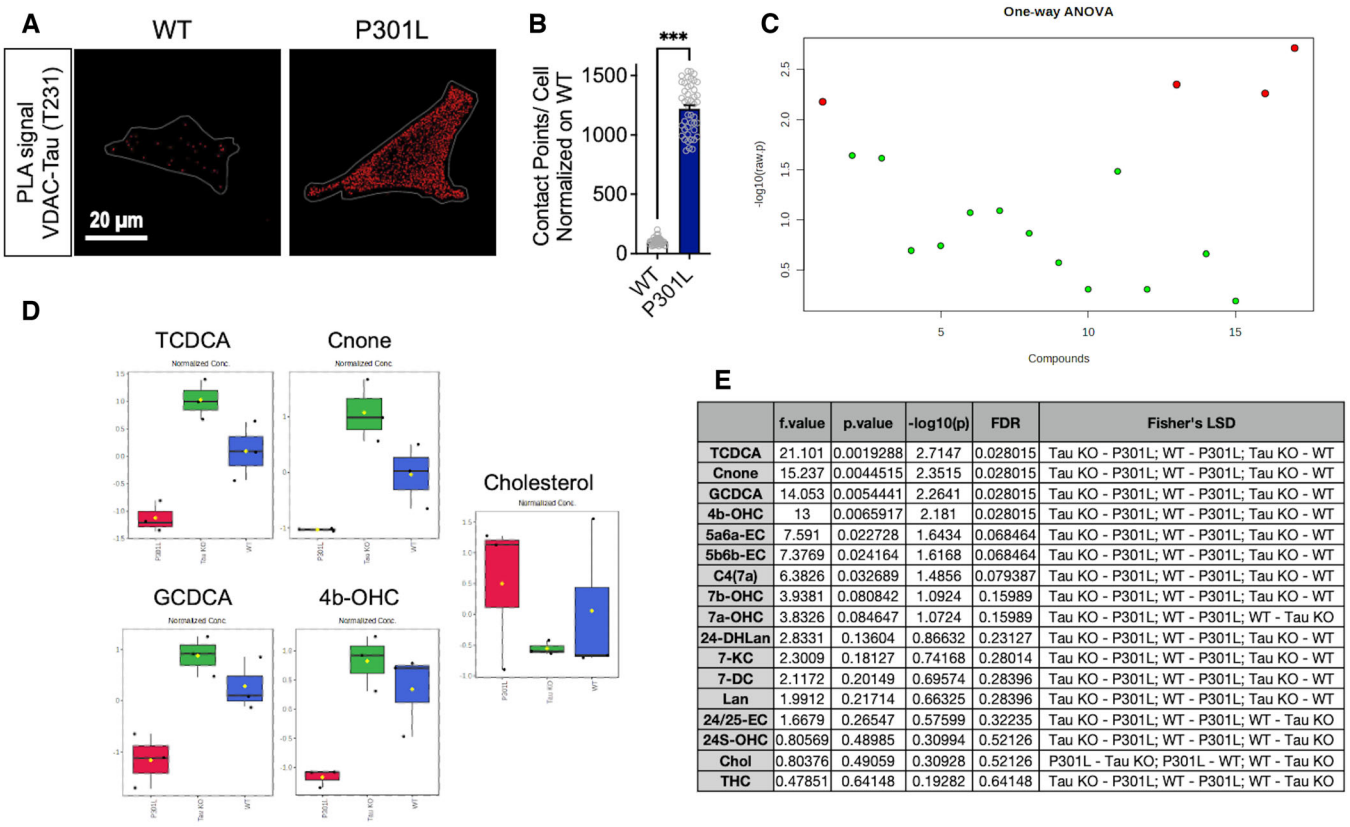


Figure EV1.



E

	f.value	p.value	-log10(p)	FDR	Fisher's LSD
TCDCA	21.101	0.0019288	2.7147	0.028015	Tau KO - P301L; WT - P301L; Tau KO - WT
Cnone	15.237	0.0044515	2.3515	0.028015	Tau KO - P301L; WT - P301L; Tau KO - WT
GCDCA	14.053	0.0054441	2.2641	0.028015	Tau KO - P301L; WT - P301L; Tau KO - WT
4b-OHC	13	0.0065917	2.181	0.028015	Tau KO - P301L; WT - P301L; Tau KO - WT
5a6a-EC	7.591	0.022728	1.6434	0.068464	Tau KO - P301L; WT - P301L; Tau KO - WT
5b6b-EC	7.3769	0.024164	1.6168	0.068464	Tau KO - P301L; WT - P301L; Tau KO - WT
C4(7a)	6.3826	0.032689	1.4856	0.079387	Tau KO - P301L; WT - P301L; Tau KO - WT
7b-OHC	3.9381	0.080842	1.0924	0.15989	Tau KO - P301L; WT - P301L; Tau KO - WT
7a-OHC	3.8326	0.084647	1.0724	0.15989	Tau KO - P301L; WT - P301L; WT - Tau KO
24-DHLan	2.8331	0.13604	0.86632	0.23127	Tau KO - P301L; WT - P301L; Tau KO - WT
7-KC	2.3009	0.18127	0.74168	0.28014	Tau KO - P301L; WT - P301L; Tau KO - WT
7-DC	2.1172	0.20149	0.69574	0.28396	Tau KO - P301L; WT - P301L; Tau KO - WT
Lan	1.9912	0.21714	0.66325	0.28396	Tau KO - P301L; WT - P301L; Tau KO - WT
24/25-EC	1.6679	0.26547	0.57599	0.32235	Tau KO - P301L; WT - P301L; WT - Tau KO
24S-OHC	0.80569	0.48985	0.30994	0.52126	Tau KO - P301L; WT - P301L; WT - Tau KO
Chol	0.80376	0.49059	0.30928	0.52126	P301L - Tau KO; P301L - WT; WT - Tau KO
THC	0.47851	0.64148	0.19282	0.64148	Tau KO - P301L; WT - P301L; WT - Tau KO

Figure EV2. Tau binds to VDAC and affects intracellular cholesterol metabolism.

A Visualization of the VDAC–phospho-Tau (T231) interactions by *in situ* proximity ligation assay (PLA) in wild-type (WT) and P301L cells. Interactions between the two targeted proteins are depicted in red (63× magnification) and cell surfaces are delimited by a white line.

B Quantitative analysis of the PLA signal represented as number of contact points between VDAC–phospho-Tau per cell. In total, 45–50 cells were analyzed per group (3 independent experiments). Data are presented as mean ± SEM. ***$P < 0.001$; Student unpaired *t*-test.

C Important features of cholesterol-related metabolites selected by ANOVA plot with *P*-value threshold 0.05. Red dots represent feature for which a statistically significant difference was detected between WT, Tau KO, and P301L cells. One-way ANOVA + Fisher's least significant difference (Fisher's LSD).

D Corresponding graphs of features for which a statistically significant difference was detected between WT, Tau KO, and P301L cells. Of note, no statistical difference was detected regarding cholesterol (probably due to high variation within samples), but the corresponding graph is shown for information. $n = 3$ independent cell culture/group. Data are presented as boxplot, the central band represents the median, the box represents the 10–90 percentile, and the whiskers represent the minimum to maximum. One-way ANOVA + Fisher's least significant difference (Fisher's LSD).

E Table showing the details of the features depicted in (C).

Data information: 24/25-EC, 24,25-Epoxycholesterol; 24-DHLan, 24,25-Dihydro-lanosterol; 24S-OHC, 24S-Hydroxy-cholesterol; 4b-OHC, 4-beta-Hydroxy-cholesterol; 5a6a-EC, 5-alpha,6-alpha-Epoxycholesterol; 5b6b-EC, 5-beta,6-beta-Epoxycholesterol; 7DC, 7-Dehydrocholesterol; 7a-OHC, 7-alpha-Hydroxy-cholesterol; 7b-OHC, 7-beta-Hydroxy-cholesterol; 7KC, 7-Ketocholesterol; C4(7a), 7-alpha-Hydroxy-cholestenone; Chol., Cholesterol; Cnone, Cholestenone; GCDCA, Glycochenodeoxycholic acid; Lan, Lanosterol; TCDCA, Taurochenodeoxycholic acid; THC, 5-alpha,6-beta-Dihydroxycholestanol; VDAC, voltage-dependent anion channel.

Figure EV3. Supplementary data obtained in Tau KO cells.

A Representative microscopy images (z-projections) of WT and Tau KO cells co-stained with Filipin III (cholesterol) in green and TOMM20 (mitochondria) in red with xy- and xz-axis orthogonal views framing the corresponding image. Scale bar: 10 μm.

B, C Manders' coefficients M1 (B) representing the proportion of Filipin III overlapping with TOMM20, and M2 (C) representing the proportion of TOMM20 overlapping with Filipin III in WT versus Tau KO cells. Data are presented as mean ± SEM ($n = 20$–23 images per group, 2 independent experiments). $^{**}P < 0.01$, Student unpaired *t*-test.

D Visualization of the VAPB–PTPIP51 interactions by *in situ* proximity ligation assays (PLAs) in wild-type (WT) cells and Tau KO cells transfected with the control (Ctrl) siRNA, as well as in MFN2 siRNA-transfected cells. Interactions between the two targeted proteins are depicted in red (63× magnification) and cell surfaces are delimited by a white line. Scale bars: 20 μm.

E Quantitative analysis of the PLA signal represented as number of contact points between VAPBPTPIP51 per cell in percentage of the WT cells. In total, 60 cells were analyzed per group (4 independent experiments). Data are presented as mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$; One-way ANOVA + Tukey's *post hoc* test.

F, G Complementary metrics of mitochondrial network morphology: form factor (mitochondrial elongation) (F) and area-weighted form factor (G) in WT and P301L transfected with MFN2 siRNA or control (Ctrl) siRNA. On average 500–1,500 mitochondrial organelles were analyzed per group ($n = 15$–20 images per group, 2 independent experiments). Data are presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$; One-way ANOVA + Tukey's *post hoc* test.

H Visualization of the VAPB–PTPIP51 interactions by *in situ* proximity ligation assay (PLA) in untreated wild-type (WT) cells and Tau KO cells as well as in CHIR99021 (CHIR)-treated cells. Interactions between the two targeted proteins are depicted in red (63× magnification) and cell surfaces are delimited by a white line. Scale bar: 20 μm.

I Quantitative analysis of the PLA signal represented as number of contact points between VAPB–PTPIP51 per cell in percentage of the WT cells. In total, 45 cells were analyzed per group (3 independent experiments). Data are presented as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; One-way ANOVA + Tukey's *post hoc* test.

J, K Complementary metrics of mitochondrial network morphology: form factor (mitochondrial elongation) (J) and area-weighted form factor (K) in WT and P301L untreated or treated with CHIR99021 in percentage of the WT cells. On average 500–1,500 mitochondrial organelles were analyzed per group ($n = 15$–20 images per group, 2 independent experiments). Data are presented as mean ± SEM. $^{*}P < 0.05$; One-way ANOVA + Tukey's *post hoc* test.

L Clustering result of cholesterol metabolites shown as heat map (distance measure using Euclidean, and clustering algorithm using ward.D).

M, N Corresponding graphs of *GCDCA (glycochenodeoxycholic acid) and TCDCA: (taurochenodeoxycholic acid) levels* in WT cells, P301L cells, and P301L cells + CHIR. $N = 3$ independent cell culture/group. Data are presented as boxplot, the central band represents the median, the box represents the 10–90 percentile, and the whiskers represent the minimum to maximum. (M) $P = 0.018483$, One-way ANOVA + Fisher's LSD: P301L + CHIR vs P301L; WT vs P301L; WT vs P301L + CHIR. (N) $P = 0.022415$, One-way ANOVA + Fisher's LSD: P301L + CHIR vs P301L; WT vs P301L; WT vs P301L + CHIR. (L–N) Graphs were generated on Metaboanalyst.ca.

O Expression level of MFN2 (mRNA expression) in SH-SY5Y cells transfected with the control (Ctrl) siRNA, as well as in MFN2 siRNA-transfected cells. Data represent the mean and SEM, normalized to 100% of the Ctrl siRNA condition ($n = 4$ replicates per group).

Data information: 24/25-EC, 24,25-Epoxycholesterol; 24S-OHC, 24S-Hydroxy-cholesterol; 4b-OHC, 4-beta-Hydroxy-cholesterol; 5a6a-EC, 5-alpha,6-alpha-Epoxycholesterol; 5b6b-EC, 5-beta,6-beta-Epoxycholesterol; 7DC, 7-Dehydrocholesterol; 7a-OHC, 7-alpha-Hydroxy-cholesterol; 7b-OHC, 7-beta-Hydroxy-cholesterol; 7KC, 7-Ketocholesterol; C4(7a), 7-alpha-Hydroxy-cholestenone; Chol., Cholesterol; Cnone, Cholestenone; GCDCA, Glycochenodeoxycholic acid; Lan, Lanosterol; TCDCA, Taurochenodeoxycholic acid; THC, 5-alpha,6-beta-Dihydroxycholestanol; TOMM20, translocase of the outer mitochondrial membrane complex subunit 20; VDAC, voltage-dependent anion channel.

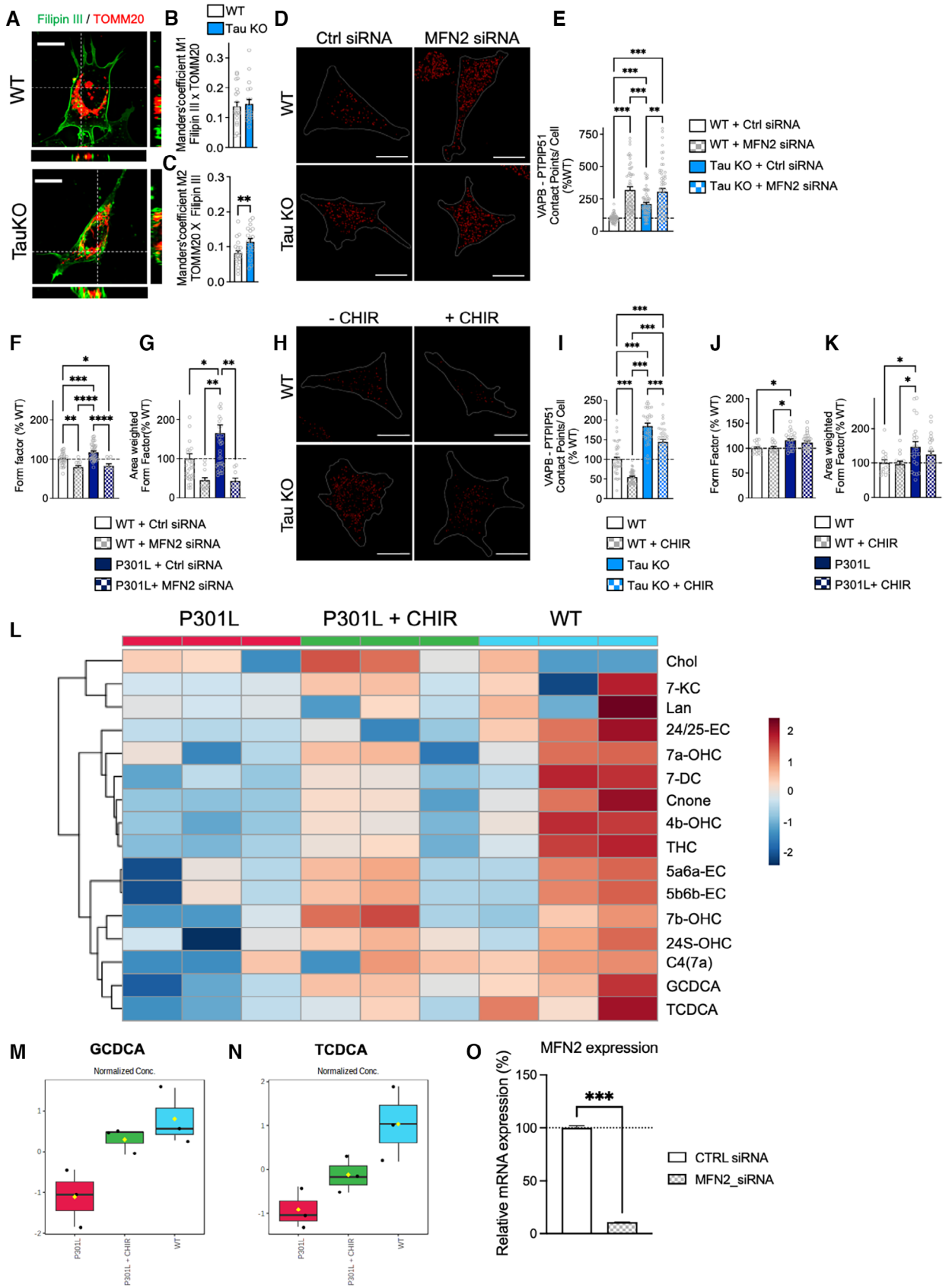

A
Filipin III / TOMM20
WT
TauKO
B
WT
Tau KO
Manders'coefficient M1 Filipin III x TOMM20
C
Manders'coefficient M2 TOMM20 X Filipin III
D
Ctrl siRNA
MFN2 siRNA
WT
Tau KO
E
VAPB - PTPIP51 Contact Points/ Cell (%WT)
WT + Ctrl siRNA
WT + MFN2 siRNA
Tau KO + Ctrl siRNA
Tau KO + MFN2 siRNA
F
Form factor (% WT)
G
Area weighted Form Factor(% WT)
WT + Ctrl siRNA
WT + MFN2 siRNA
P301L + Ctrl siRNA
P301L+ MFN2 siRNA
H
- CHIR
+ CHIR
WT
Tau KO
I
VAPB - PTPIP51 Contact Points/ Cell (% WT)
WT
WT + CHIR
Tau KO
Tau KO + CHIR
J
Form Factor (% WT)
K
Area weighted Form Factor(% WT)
WT
WT + CHIR
P301L
P301L+ CHIR
L
P301L
P301L + CHIR
WT
Chol
7-KC
Lan
24/25-EC
7a-OHC
7-DC
Cnone
4b-OHC
THC
5a6a-EC
5b6b-EC
7b-OHC
24S-OHC
C4(7a)
GCDCA
TCDCA
M
GCDCA
Normalized Conc.
N
TCDCA
Normalized Conc.
P301L
P301L + CHIR
WT
O
MFN2 expression
Relative mRNA expression (%)
CTRL siRNA
MFN2_siRNA

Figure EV3.