Supplementary Online Content

Rinaldi C, Schmidt T, Situ AJ, et al. Mutation in *CPT1C* associated with pure autosomal dominant spastic paraplegia. *JAMA Neurol*. Published online March 9, 2015. doi:10.1001/jamaneurol.2014.4769.

eFigure 1. Diagram of the filtering strategy for the exome sequencing data.
eFigure 2. Comparison of spectral and structural parameter of Nβ and Nβ (Arg37Cys) of human CPT1C.
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eFigure 4. Western blot analysis of CPT1C.
eFigure 5. COS7 cells were transfected with 1.5 µg HA-tagged CPT1C and signal was detected using anti-HA (Covance, clone 16B12, 1:5,000), anti-Calreticulin antibody (Calbiochem, 208910, 1:1,000), and anti-AIF antibody (Millipore, AB16501, 1:1,000).
eFigure 6. COS7 cells were co-transfected with 1.5 µg GFP-tagged sec61, HA-tagged CPT1C wild-type or mutant, HA-empty vector and GFP-empty vector as specified in the picture.

This supplementary material has been provided by the authors to give readers additional information about their work.

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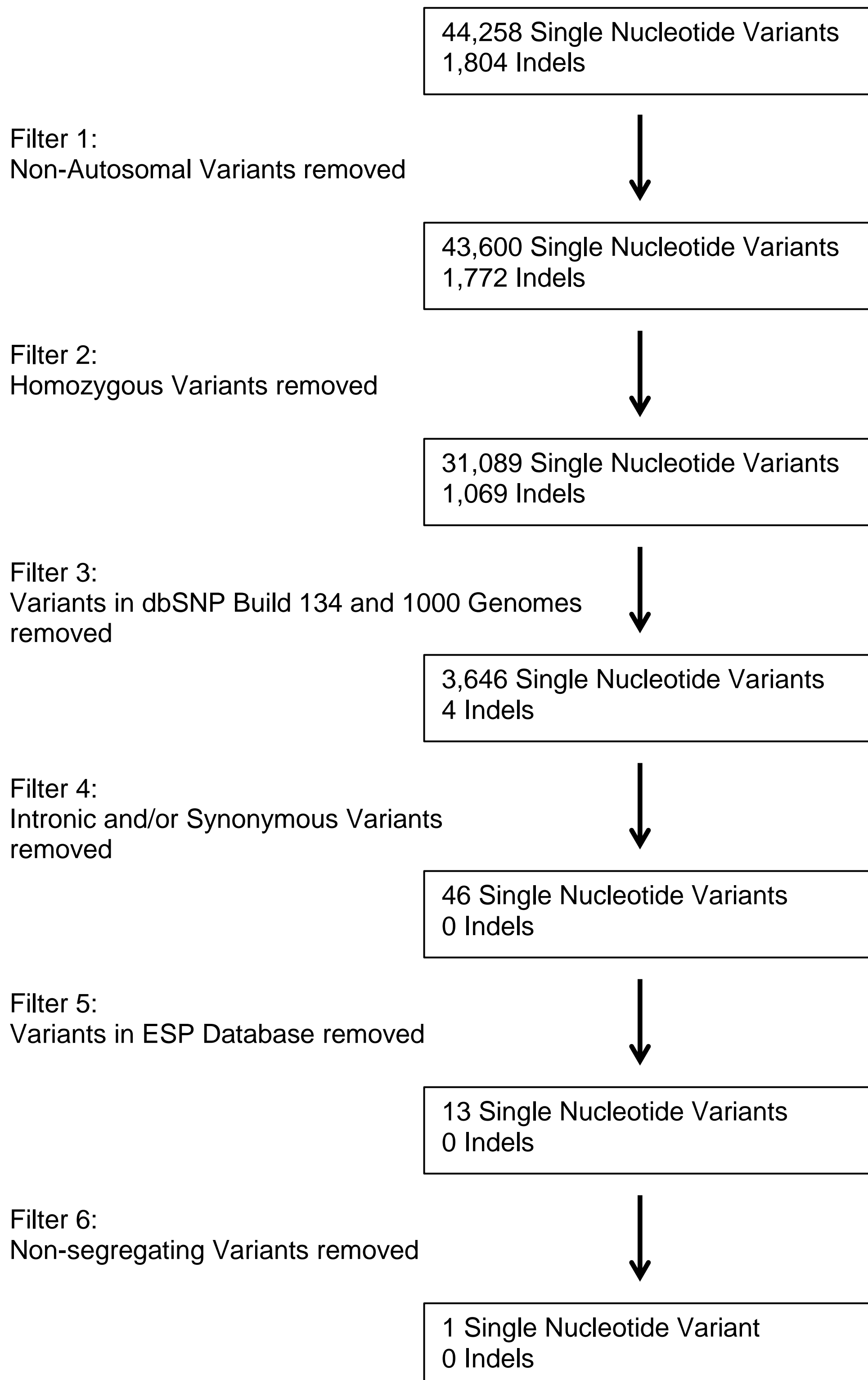
eFigure 1. Diagram of the filtering strategy for the exome sequencing data.



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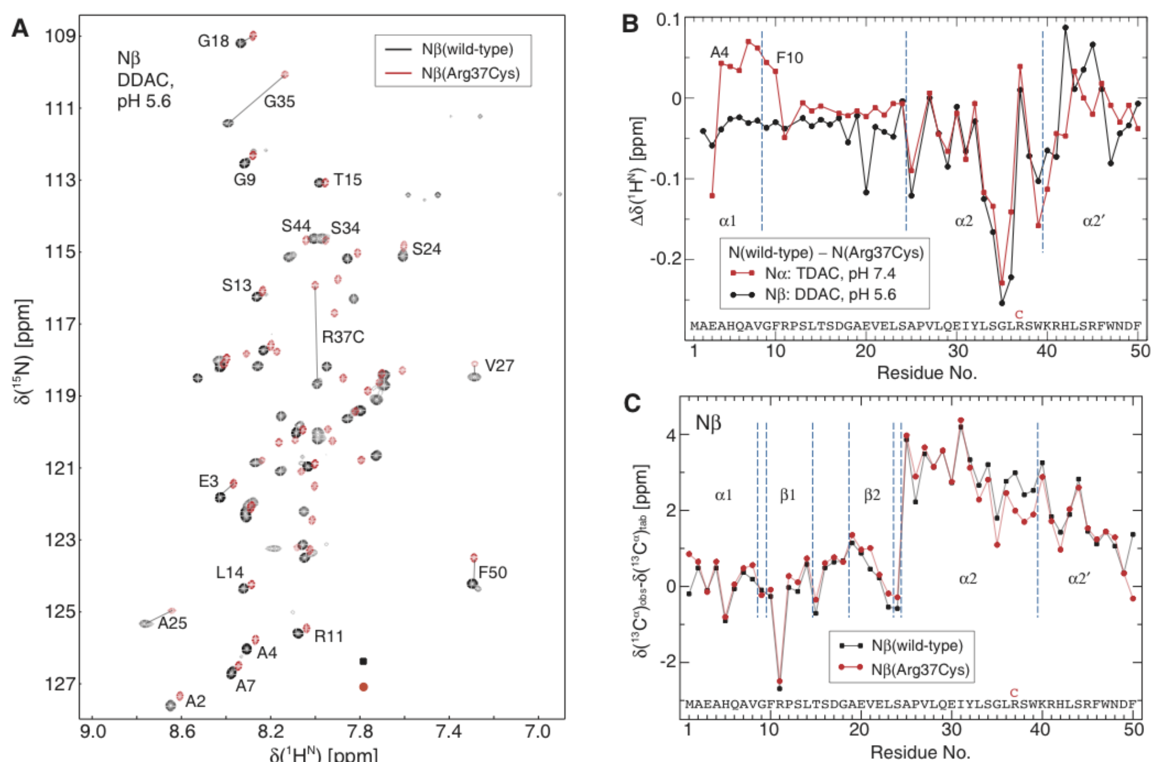
eFigure 2. Comparison of spectral and structural parameter of Nβ and Nβ (Arg37Cys) of human CPT1C.



(**A**) Superposition of $^1H^N$-^{15}N correlation NMR spectra of wild-type Nβ and Nβ(Arg37Cys). The spectra were recorded in the presence of dodecyltrimethylammonium chloride (DDAC) at pH 5.6, 35 °C and a ^1H frequency of 700 MHz. (**B**) $^1H^N$ chemical shift differences, Dd($^1H^N$), between Nα and Nα (Arg37Cys), and Nβ and Nβ(Arg37Cys). The borders of the secondary structure elements of Nα, helices α1, α2, and α2′, are indicated. (**C**) Comparison of Nβ and Nβ(Arg37Cys) secondary $^{13}C^{\alpha}$ chemical shifts, d($^{13}C^a$), defined as the difference between observed and tabulated, random coil $^{13}C^a$ shifts. Positive and negative shifts indicate helical and extended backbone conformations, respectively (Wishart & Case, 2001). The borders of all possible Nα and Nβ secondary structure elements, helices α1, α2, α2′, and sheets β1, β2 are indicated (Rao et al, 2011).

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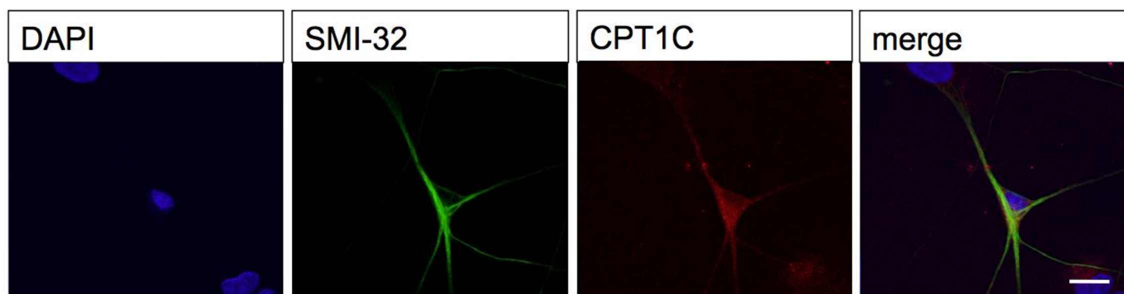
eFigure 3. iPSC-derived motor neurons from an unaffected subject were stained for SMI-32 (Covance, SMI-32R, 1:1,000) and CPT1C (Proteintech, 12969-1-AP; 1:1,000).

The nuclear DAPI stain is blue. Scale bar=20 μm.



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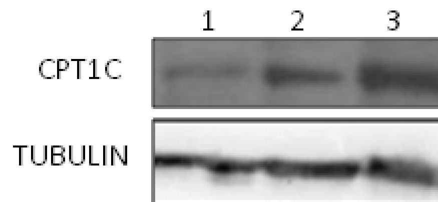
eFigure 4. Western blot analysis of CPT1C.

(antibody developed following the indications in the previous study by Carrasco et al., 2012, by Sigma-Genosys; cropped image) expression in different samples. 1) Ganglion (the majority are sensory neurons); 2) ventral horn of the spinal cord (the majority are motor neurons); and 3) cortex from WT mice.



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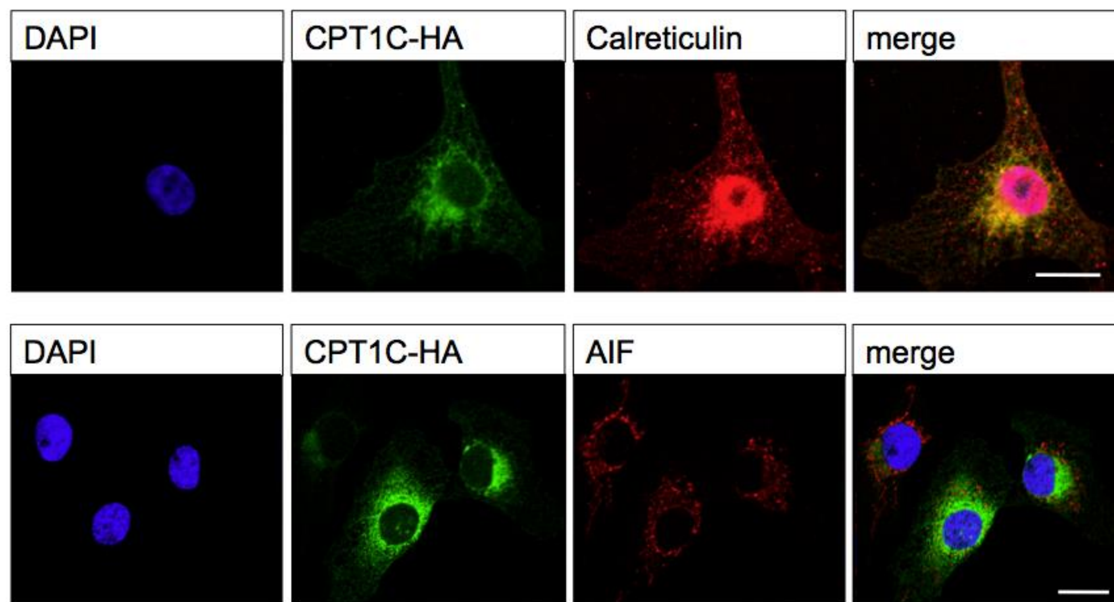
eFigure 5. COS7 cells were transfected with 1.5 µg HA-tagged CPT1C and signal was detected using anti-HA (Covance, clone 16B12, 1:5,000), anti-Calreticulin antibody (Calbiochem, 208910, 1:1,000), and anti-AIF antibody (Millipore, AB16501, 1:1,000).

The nuclear DAPI stain is blue. Scale bar=20 µm.



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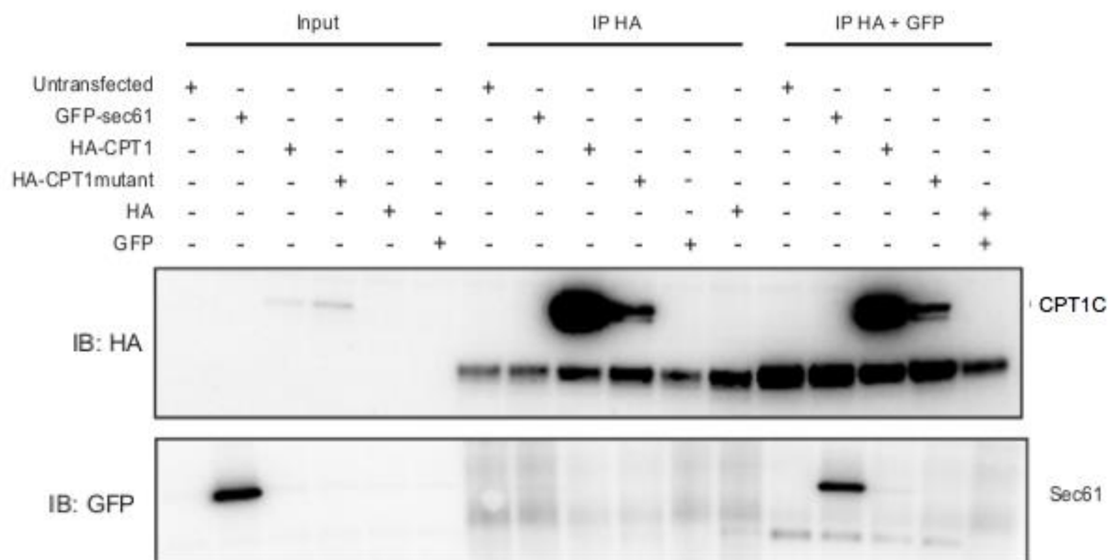
eFigure 6. COS7 cells were co-transfected with 1.5 µg GFP-tagged sec61, HA-tagged CPT1C wild-type or mutant, HA-empty vector and GFP-empty vector as specified in the picture.

Co-Immunoprecipitation was detected using an anti-HA antibody (Covance, clone 16B12, 1:5,000) and anti-GFP antibody (IP: molecular probe, A-11122; IB: abcam, ab290, 1:5,000).



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