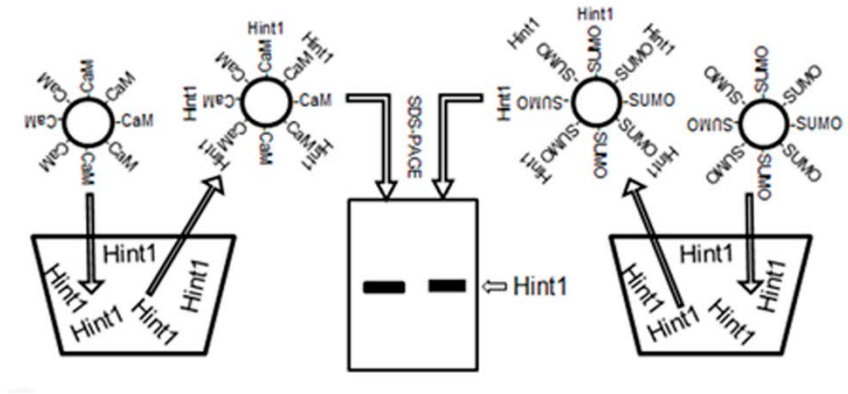
Human HINT1 mutant proteins that cause axonal motor neuropathy exhibit anomalous interactions with partner proteins

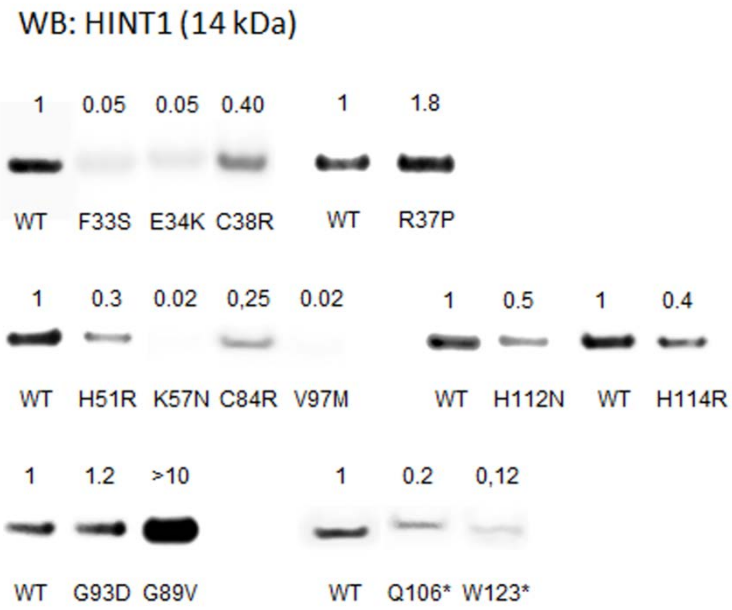
Elsa Cortés-Montero, María Rodríguez-Muñoz, Pilar Sánchez-Blázquez, Javier Garzón-Niño

Supplemental Figures

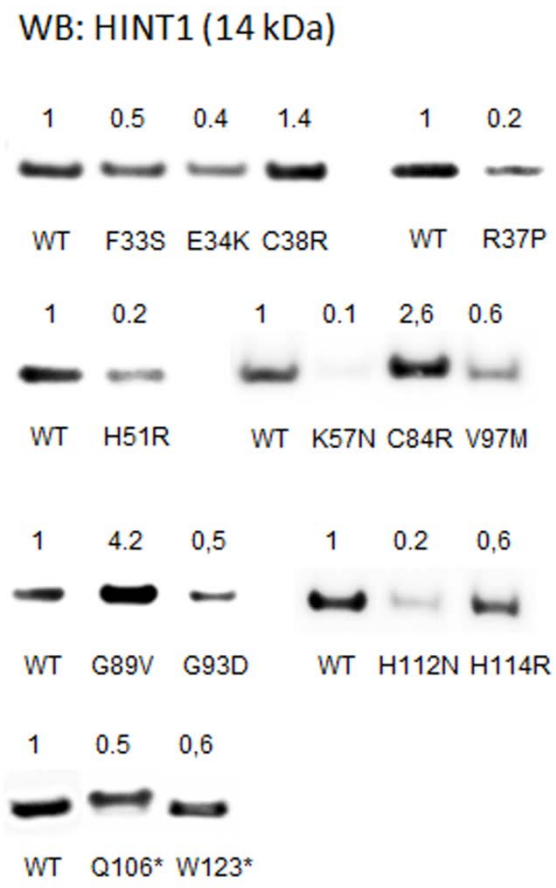


○ Agarose beads; HINT1 interacting proteins are covalently attached. CaM and SUMO are depicted as examples. Target proteins: HINT1 wild type and its human mutants.

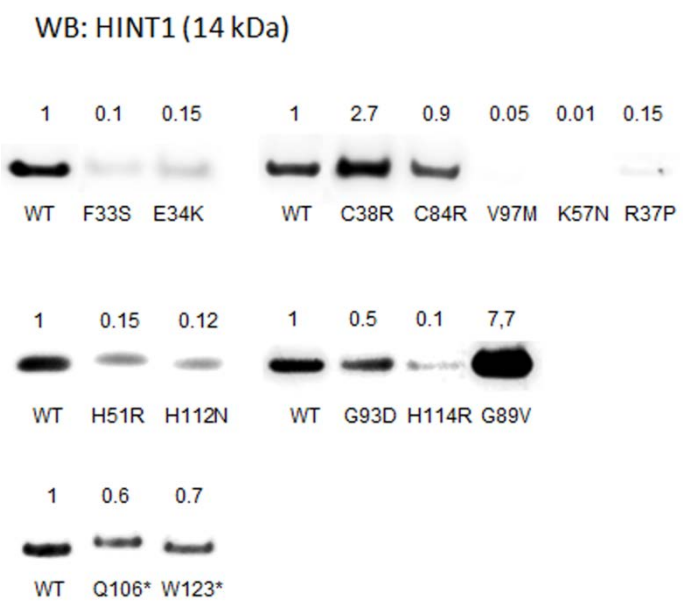
Supplemental Figure 1. Typical pull-down assays.



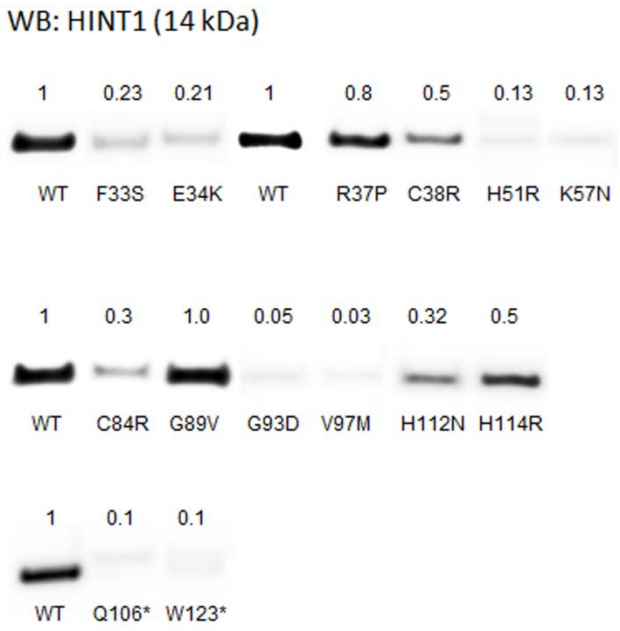
Supplemental Figure 2. Binding of HINT1 wild type and its human mutants to SUMO1-agarose**.** The Western blot images, antibody binding, were visualized by chemiluminescence and recorded using an ImageQuant™ LAS 500 (GE). Because the assays performed with recombinant proteins produce the expected protein sizes, the capture of the data was optimized. Thus, for each blot, the area containing the recombinant protein was typically selected. The device automatically captures the selected area and calculates the optimal exposure time to provide the highest possible signal to enable accurate comparison between the samples. For quantification, protein immunosignals were measured using the area of the strongest signal of each studied group of samples (AlphaEase FC software). HINT1 wild type was assigned a value of 1, and the human mutants were referred to this control. Details in Supplemental Figures 1, & 8. Further details in Cortés-Montero et al., The Axonal Motor Neuropathy-Related HINT1 Protein Is a Zinc- and Calmodulin-Regulated Cysteine SUMO Protease. *Antioxid Redox Signal* 31: 503-520, 2019.



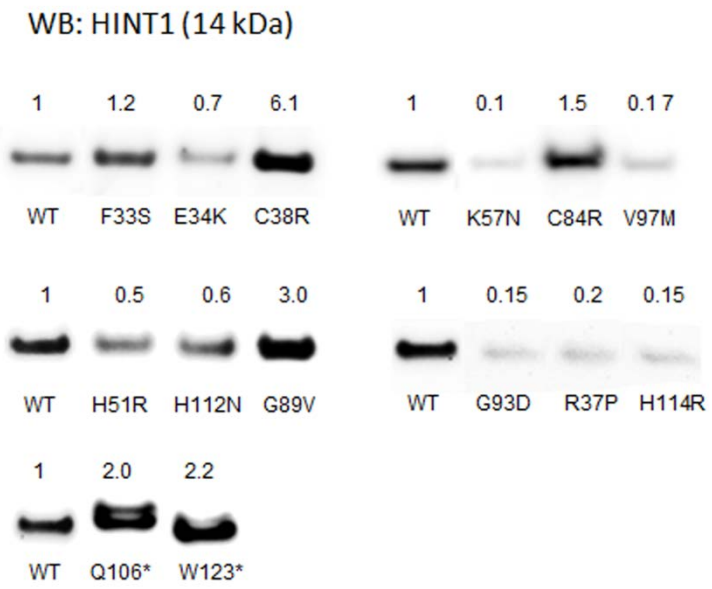
Supplemental Figure 3. Binding of HINT1 wild type and its human mutants to CaM-agarose**.** Details in Supplemental Figures 1, 2 & 8.



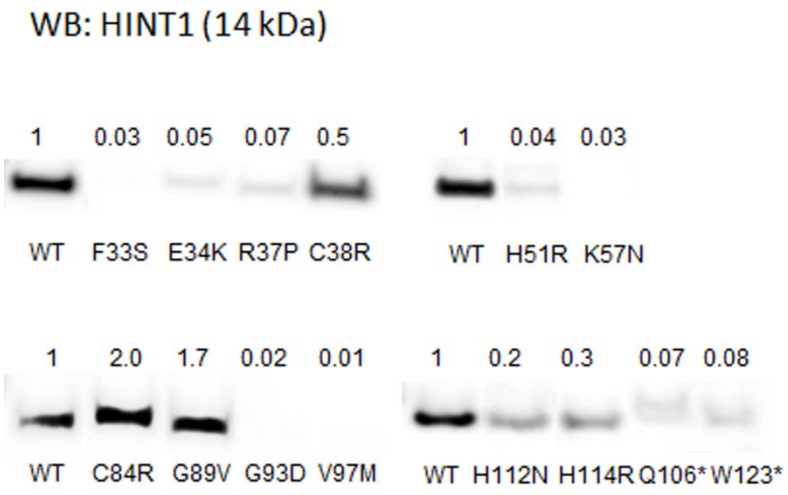
Supplemental Figure 4. Binding of HINT1 wild type and its human mutants to NR1-agarose**.** Details in Supplemental Figures 1, 2 & 8.



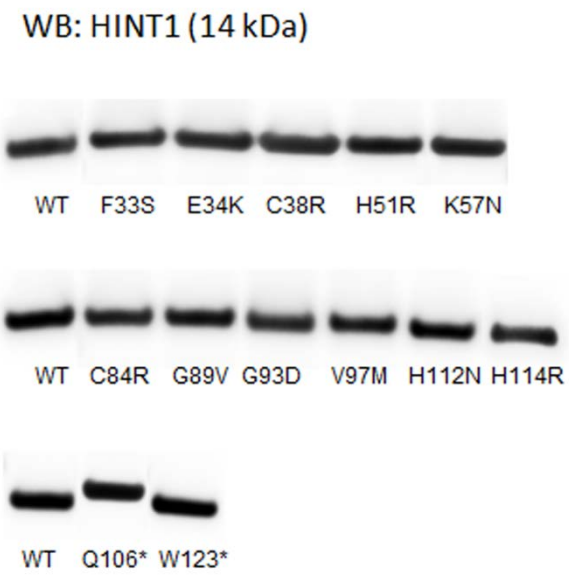
Supplemental Figure 5. Binding of HINT1 wild type and its human mutants to MOR C terminus-agarose**.** Details in Supplemental Figures 1, 2 & 8.



Supplemental Figure 6. Binding of HINT1 wild type and its human mutants to RGSZ2-agarose**.** Details in Supplemental Figures 1, 2 & 8.



Supplemental Figure 7. Binding of HINT1 wild type and its human mutants to ICD teneurin 1-agarose**.** Details in Supplemental Figures 1, 2 & 8.



Supplemental Figure 8. The assay was conducted with 1/3 the amount of HINT1 wild type and its human mutants used to study their interaction with the proteins described in Figs 2-7**.**