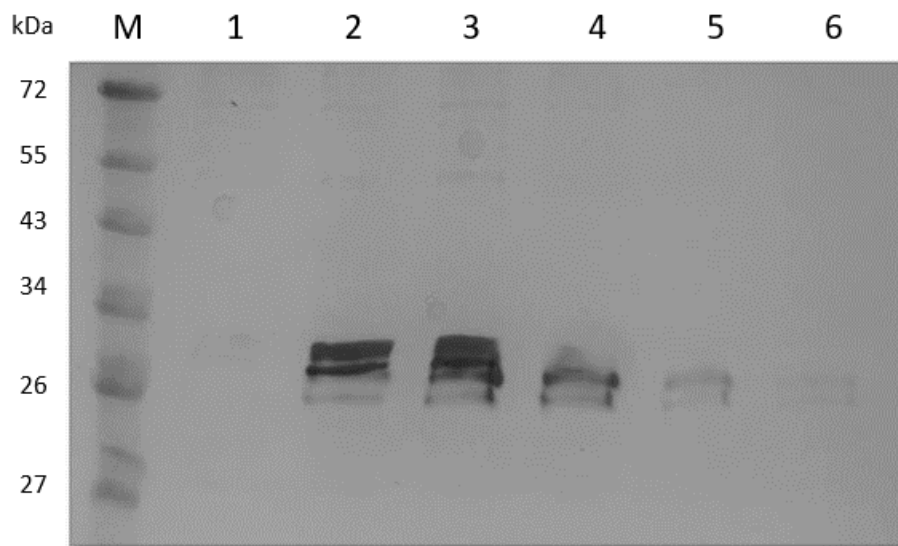


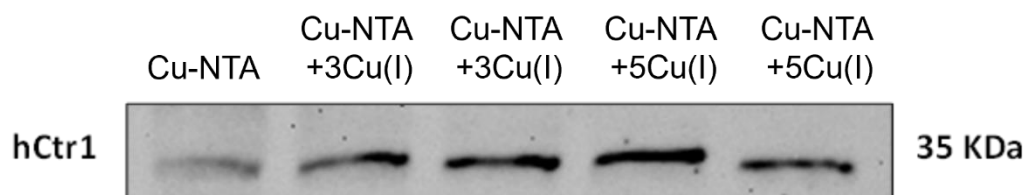
## **SUPPLEMENTARY MATERIALS**



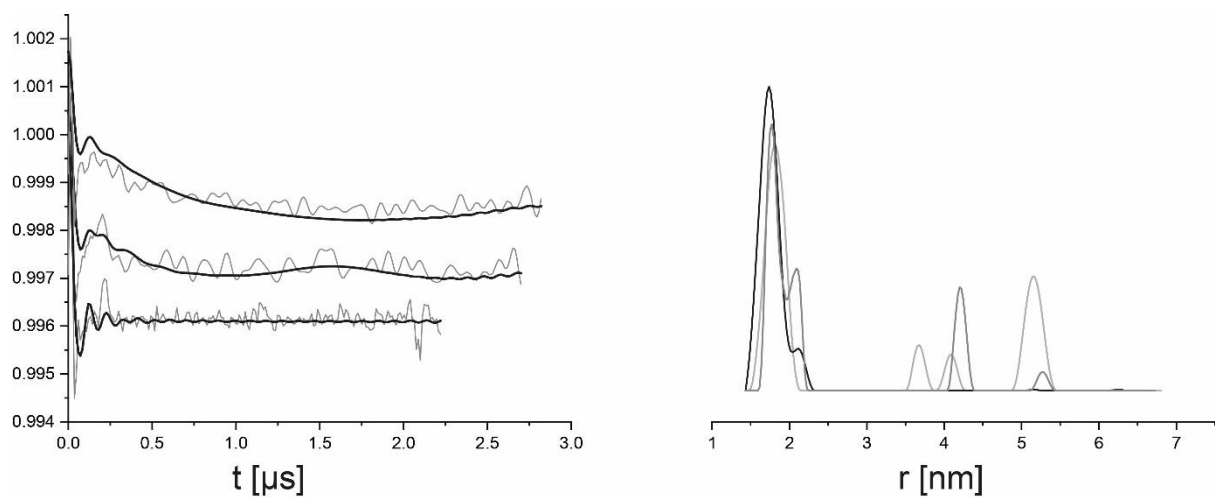
**Figure S1.** SDS-PAGE analysis of purified WT-hCtr1 following silver staining. Lanes 2–6 display the elution fractions obtained from an anti-FLAG M1 agarose affinity gel column using a solution containing 5 mM EDTA and 100  $\mu$ g/mL FLAG peptide.

#### Western blot:

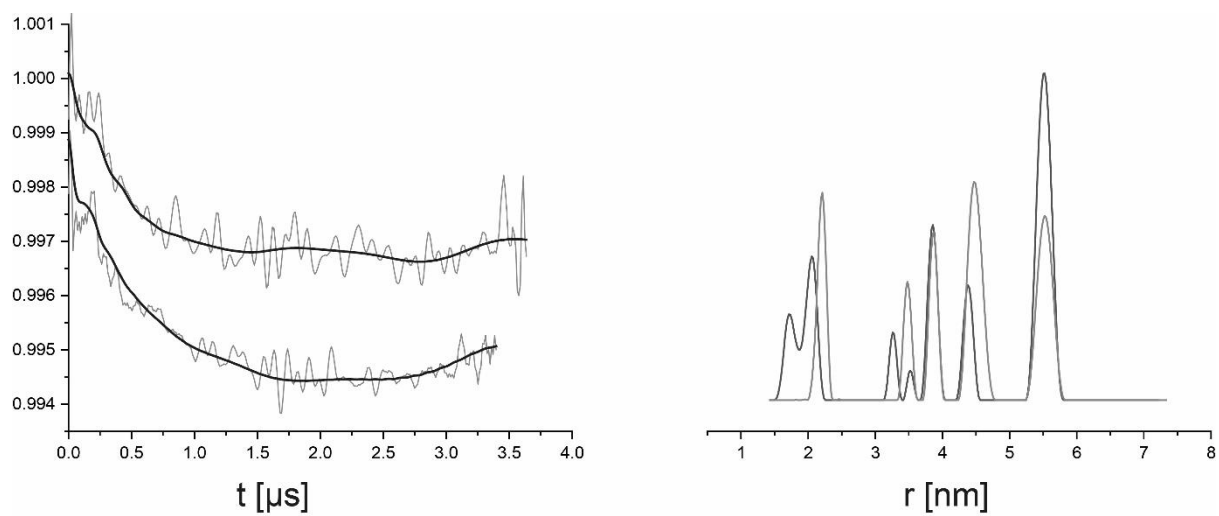
Western blot experiments were carried out according to protocol published earlier [1] for overexpressed hCtr1 protein in insect cells in the presence of Cu(II)-NTA complex and Cu(I)-tetrakis (Sigma-Aldrich) to ensure that the presence of Cu(II)-NTA and Cu(I) still express hCtr1 protein.



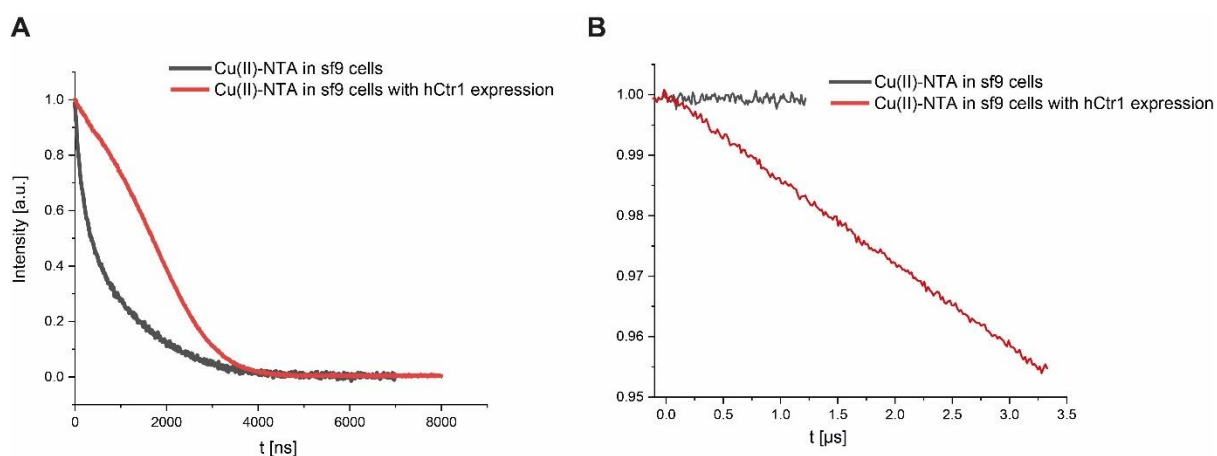
**Figure S2.** Western blot of WT-hCtr1 with Cu(II)-NTA complex and Cu(I)-tetrakis at different ratios.



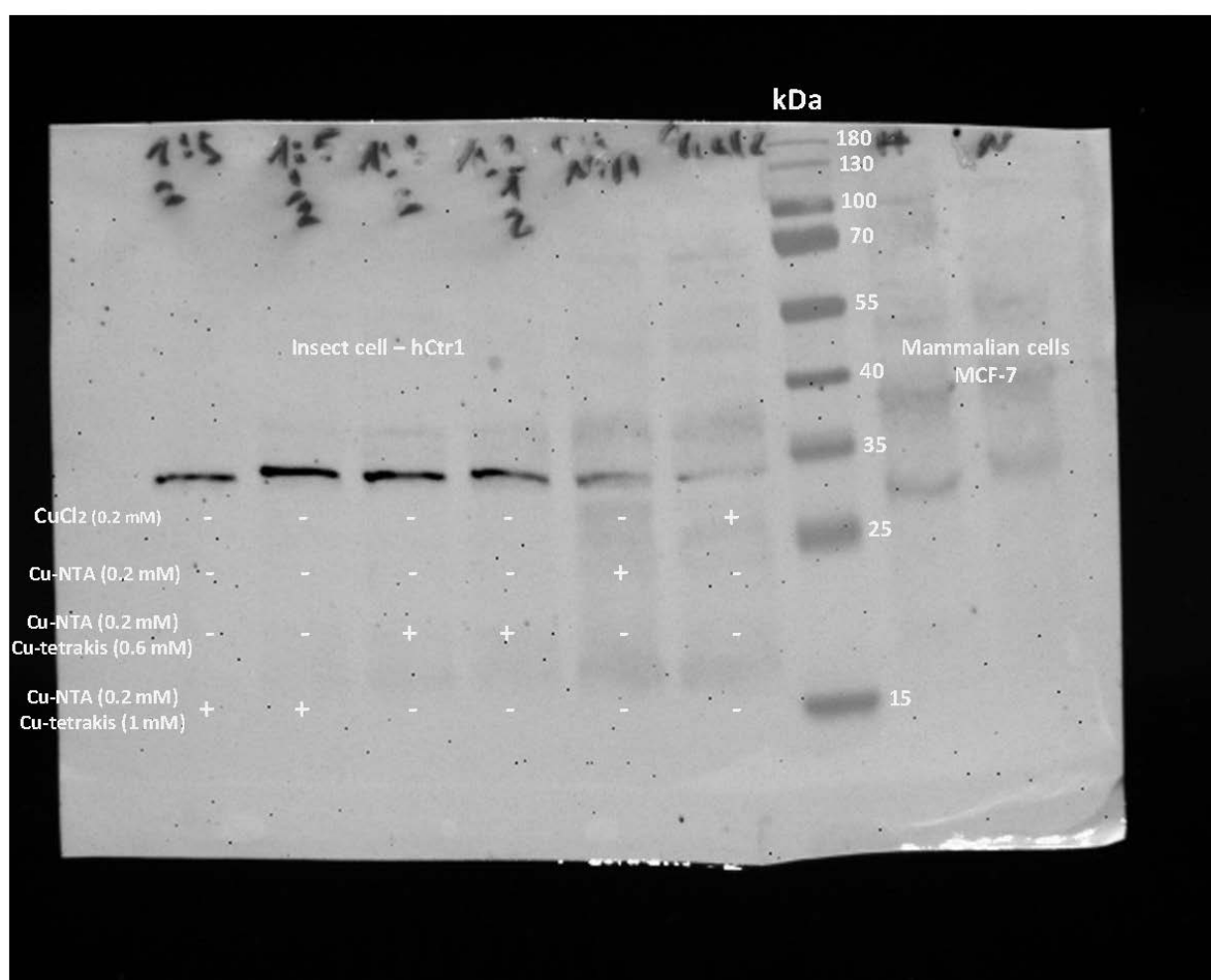
**Figure S3.** Various DEER time domain signals and corresponding distance distributions functions were acquired on different samples of purified hCtr1 in the presence of Cu(II)-NTA and 3Cu(I).



**Figure S4.** Various DEER time domain signals and corresponding distance distributions functions were acquired on different samples of overexpressed hCtr1 in the cells, in the presence of Cu(II)-NTA and 3Cu(I).



**Figure S5. A.** Two-pulse echo decay for Cu(II)-NTA in sf9 cells with (red) and without hCtr1 expression (black). **B.** DEER time domain signal for Cu(II)-NTA in sf9 cells with (red) and without hCtr1 expression (black).



**Figure S6.** Original Western Blot image ctr1 in insect cells.

## Reference

1. Meron, S.; Peleg, S.; Shenberger, Y.; Hofmann, L.; Gevorkyan-Airapetov, L.; Ruthstein, S. Tracking Disordered Extracellular Domains of Membrane Proteins in the Cell with Cu(II)-Based Spin Labels. *J Phys Chem B* 2024, 128, 8908-8914, doi:10.1021/acs.jpcc.4c03676.