Supporting Information

Model Peptides Provide New Insights into the Role of Histidine Residues as Potential Ligands in Human Cellular Copper Acquisition via Ctr1

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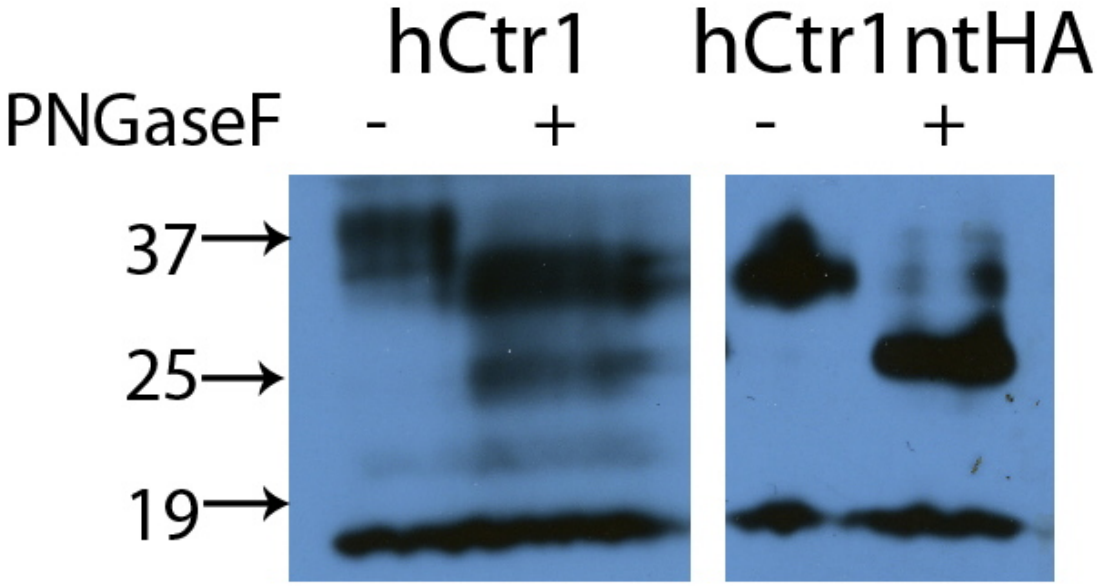

Figure S1. Ctr1ntHA protein contains N-linked sugars. Cell lysates from cells expressing transgenic Ctr1 and Ctr1ntHA were treated with buffer (-) or PNGase F (+) for 72 hours at 37°C . PNGaseF is a glycosidase that specifically deamidates glycosylated asparagine residues to aspartic acid.[1] Upon treatment with PNGase F, a shift in the bands around 35 KDa to lower molecular weight in both cell lines indicates a loss of glycosylation due to asparagine deamidation from proteins originally containing the expected N-linked glycosylation at Asn-15.

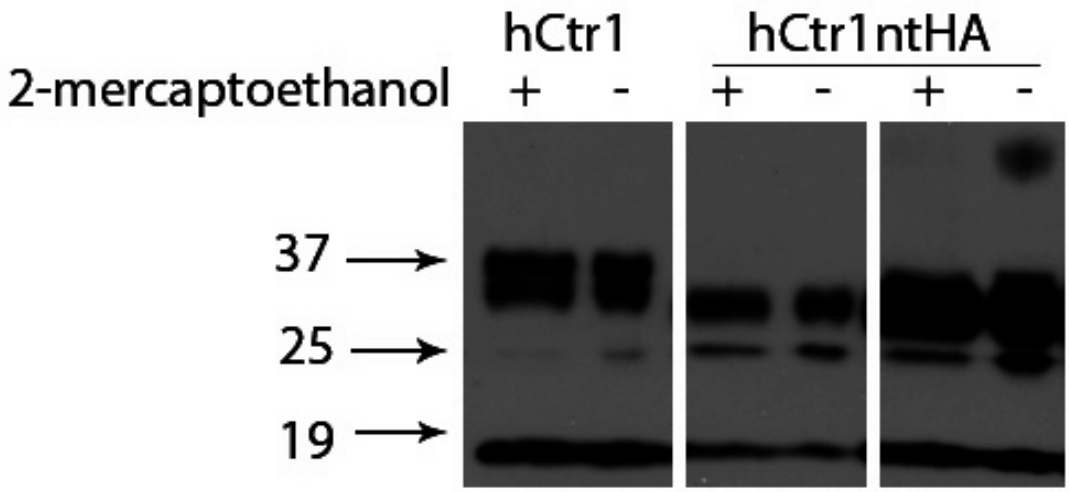

Figure S2. Ctr1 mobility between 17 and 37 kDa does not change with reducing agent. Cell lysates from cells expressing transgenic Ctr1 or Ctr1ntHA were treated with (+) or without (-) the reducing agent, 2-mercaptoethanol, before loading on an SDS-PAGE gel. In cells highly overexpressing Ctr1ntHA (far right), a higher band around 60 kDa shows a dramatic decrease in intensity upon addition of reducing agent, indicating that the 60 kDa species corresponds to a multimer that dissociates in the presence of reducing agent. Bands between 17 and 37 kDa do not change significantly upon addition of reducing agent.

(1) Kuster, B.; Mann, M. *Anal. Chem.* **1999**, *71*, 1431-1440.