# Hypertonicity-responsive ubiquitin ligase RNF183 promotes the lysosomal degradation of Na,K-ATPase and NKCC1

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We previously reported that RNF183, a member of the RING finger (RNF) ubiquitin ligase family, is specifically expressed in the renal collecting duct and is induced under hypertonic conditions. However, its functional role under such conditions remains unclear. In this study, we used the BirA proximity-biotinylation technique to identify candidate substrates of RNF183; these included the α1 subunit of Na,K-ATPase and the basolateral Na-K-Cl cotransporter (NKCC1). We confirmed that RNF183 interacted with the α1 subunit and with NKCC1. RNF183 ubiquitinated NKCC1 as well as the β1 subunit that, together with the α1 subunit, forms the Na,K-ATPase complex. RNF183 promoted the translocation of the α1 and β1 subunits and NKCC1 from the plasma membrane to lysosomes. In addition, RNF183 overexpression significantly reduced the expression levels of the α1 and β1 subunits and NKCC1 compared to treatment with mock control or an RNF183 mutant. Chloroquine treatment of RNF183-expressing cells inhibited this reduction in the protein levels of Na,K-ATPase and NKCC1. These results suggest that Na,K-ATPase and NKCC1 are degraded in lysosomes by RNF183-mediated ubiquitination. Thus, RNF183 may play an important role in the adaptation to continuous hypertonic conditions through the downregulation of osmoregulatory transporters.