



Cryo-EM structures of phosphorylated NKCC1. A) Co-expression of NKCC1 with the upstream WNK-SPAK kinases yielded phosphorylated transporter sample as confirmed by a phospho-specific NKCC1 antibody. Phosphorylated NKCC1 also migrates as a larger protein than the untreated NKCC1. B) Untreated human NKCC1 transporter shows no density for the cytosolic domains. C-D) Phosphorylated human NKCC1 transporter yielded two cryo-EM maps, both of which show well-resolved cytosolic domains. One NKCC1 dimer exhibits an overall 2-fold symmetry, while the other shows mismatch of 2-fold symmetry axis of the transmembrane and cytosolic domains.