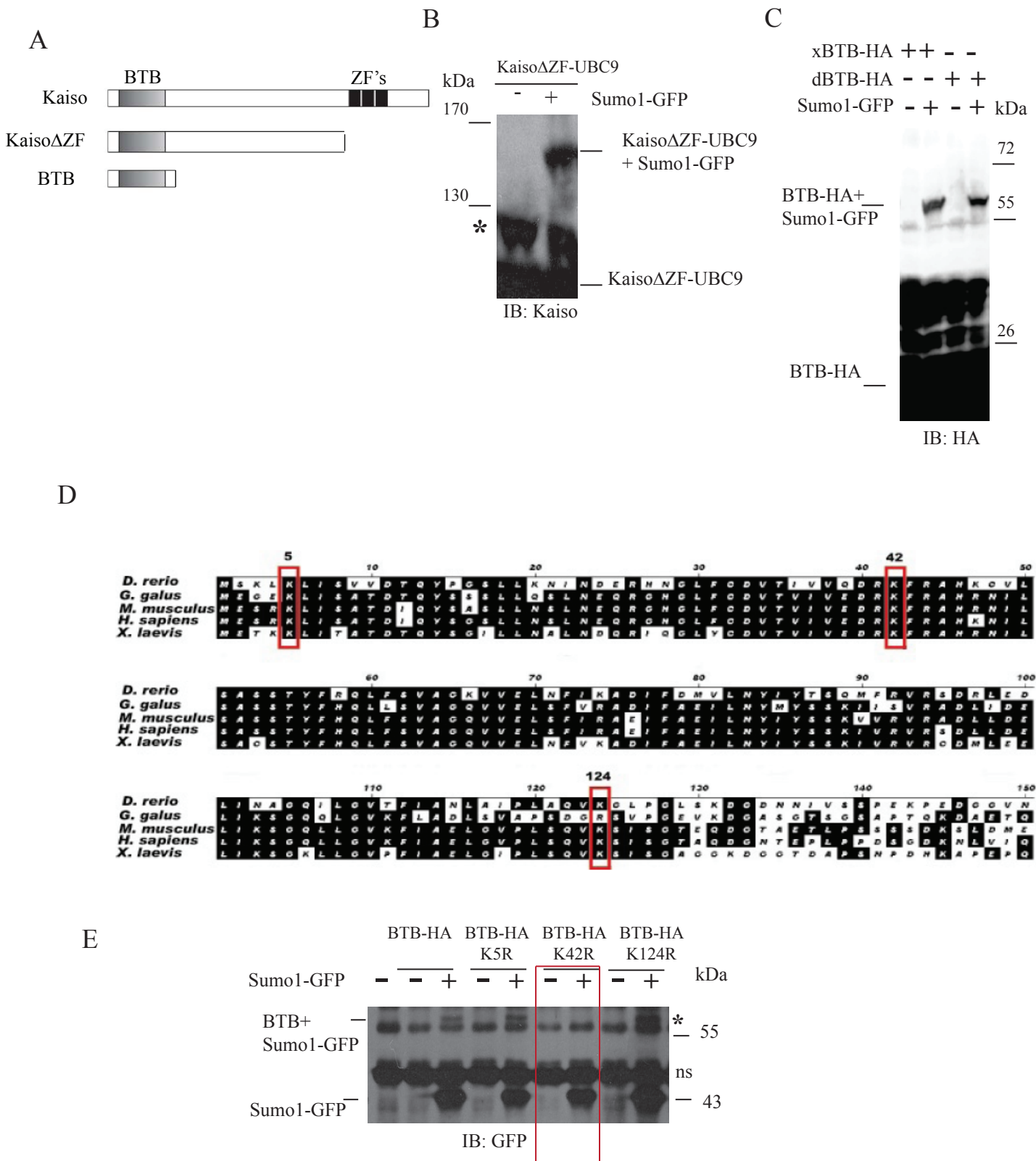
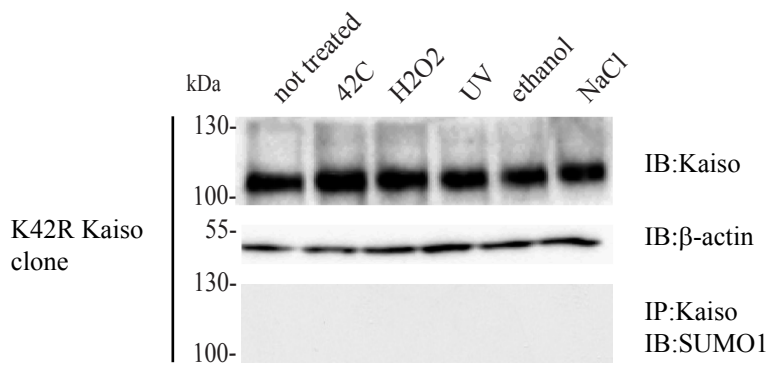


Supplementary figure 1

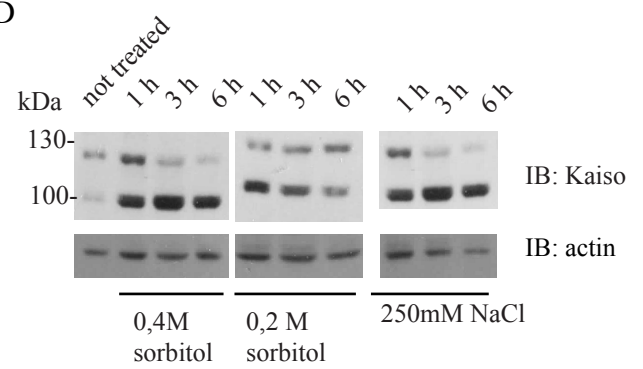


Supplementary figure 1. Sumoylation of Kaiso is evolutionary conserved. A, Schematic structure of Kaiso protein and its deletion mutants. B, Hypersumoylation of N-terminal part of Kaiso (1-492aa) that was fused to Ubc9 in presence of exogenous SUMO1-GFP as detected by western blot analysis with Kaiso rabbit pAntibodies. C, expression vectors that code for HA tagged BTB/POZ domain of Kaiso of xenopus (xBTB-HA) or danio rerio (dBTB-HA) origin were cotransfected with or without Sumo1-GFP and western blotted with HA Ab's. D, Alignment of BTB/POZ domains of Kaiso from different species. Most conserved lysines are in red rectangles. E, HA-tagged BTB domains that harbored single amino acid substitutions: K5R, K42R and K124R, were transfected to HEK293 cell line. Western blot of obtained lysates with GFP Ab's revealed sumoylated BTB/POZ domain. Corresponding band is marked with asterisk. Only BTB/POZ domain with K42R mutation cannot be sumoylated.

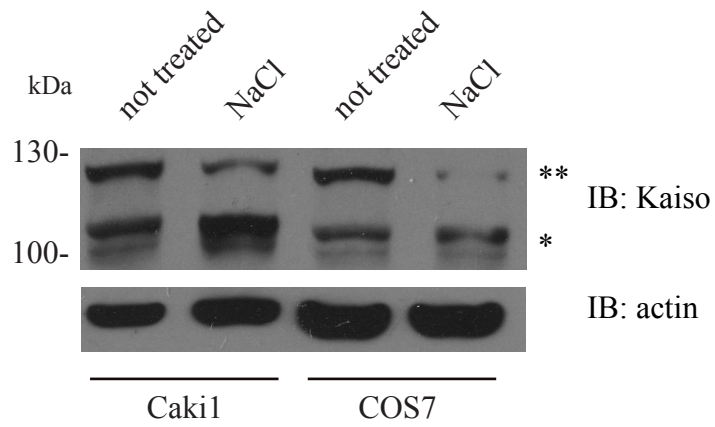
A



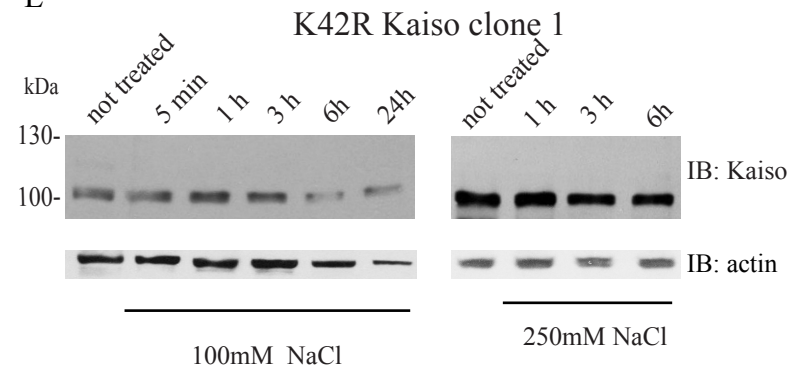
D



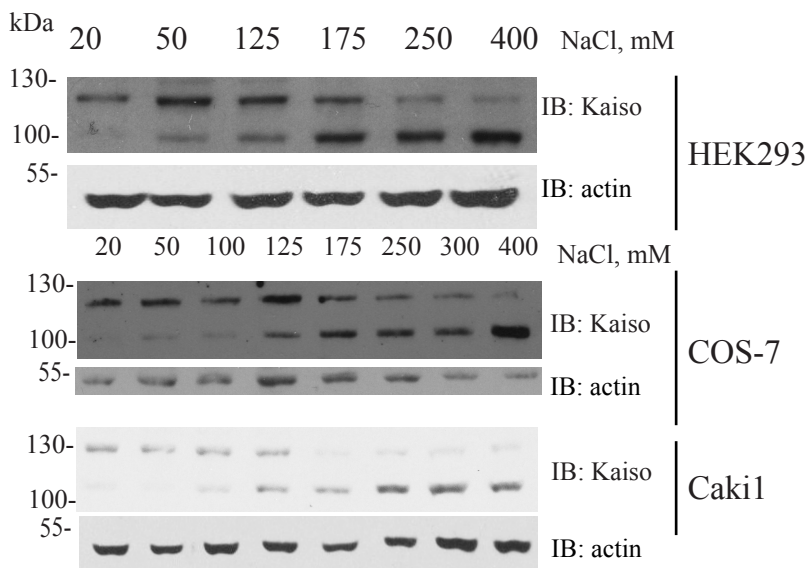
B



E



C



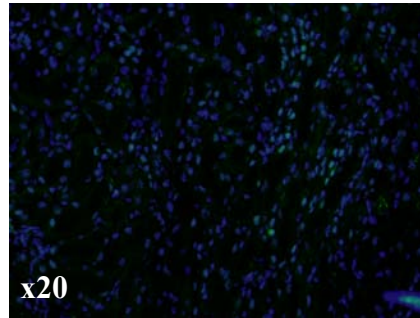
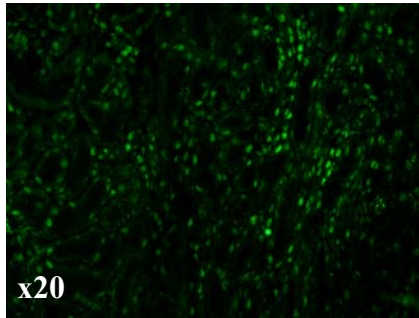
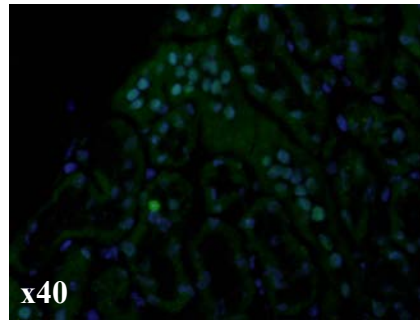
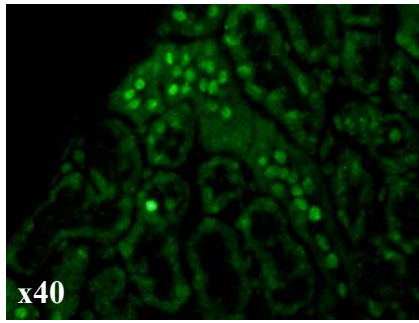
Supplementary figure 2. Hyperosmotic-dependent deSUMOylation of Kaiso. Cells were lysed in loading buffer, analyzed by SDS-PAGE and Western blotting by probing with anti-Kaiso antibodies: A- K42R Kaiso clone 1 was treated as indicated in figure, B- Caki1 and COS7 cells before and after 5min 400mM NaCl addition.

Kaiso is deSUMOylated in both cell lines; *- nonmodified Kaiso, ** - modified Kaiso.

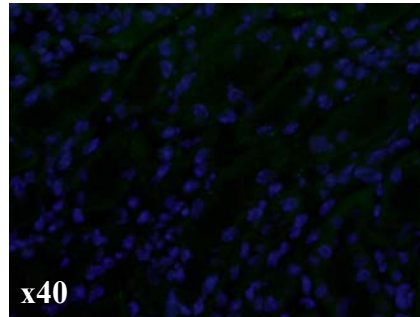
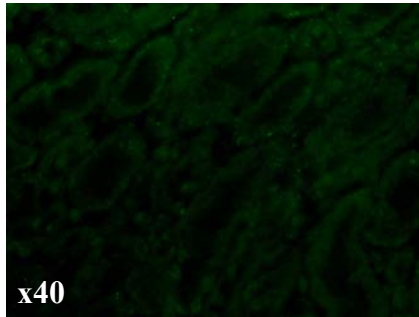
C-HEK293, Caki1, COS7 were incubated with increasing NaCl concentrations 5 minutes, D-time course treatment HEK293 cells with sorbitol during indicated times, E-time course treatment K42R kaiso clone cells with 100mM and 250 mM NaCl during indicated times

A anti Kaiso abs' Alexa488

DAPI +Alexa 488



wt
kidney



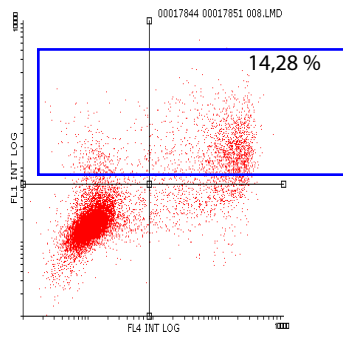
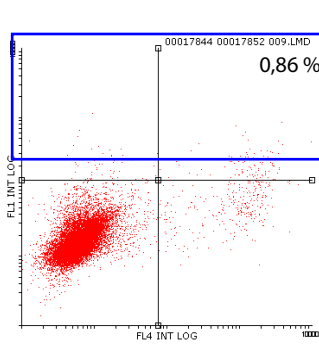
Kaiso KO
kidney

Supplementary figure 3 Kaiso expression in mouse kidney (wt and Kaiso KO as a control,) staining with Kaiso rabbit ab's.

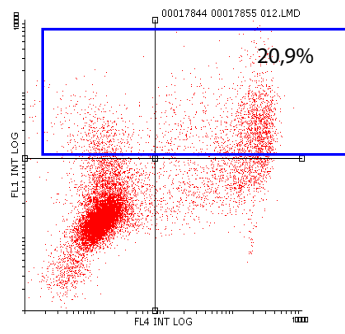
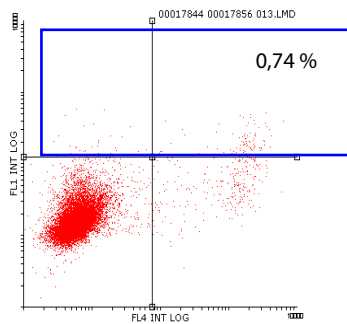
normal

+100mM NaCl 24 h

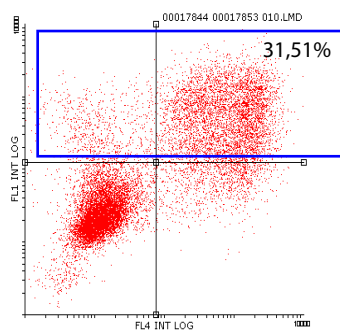
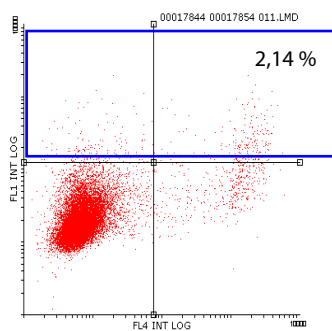
HEK293



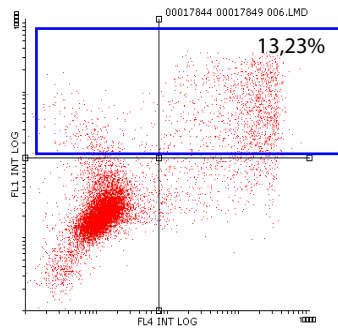
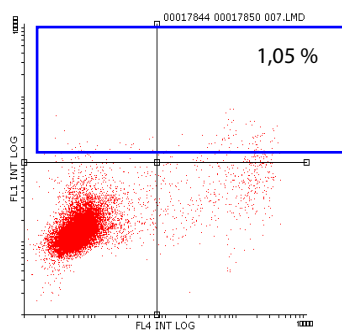
K42R
clone 1



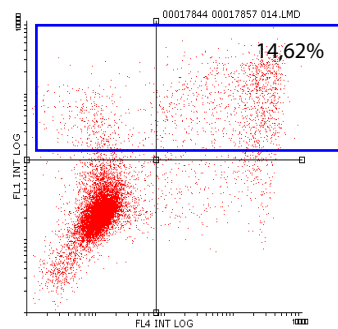
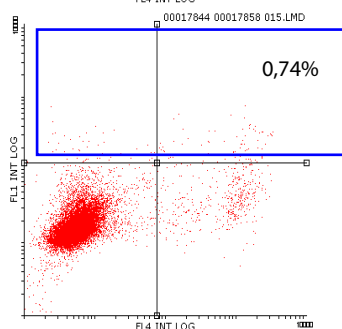
K42R
clone 2



KO clone1
Kaiso KO
clone 1

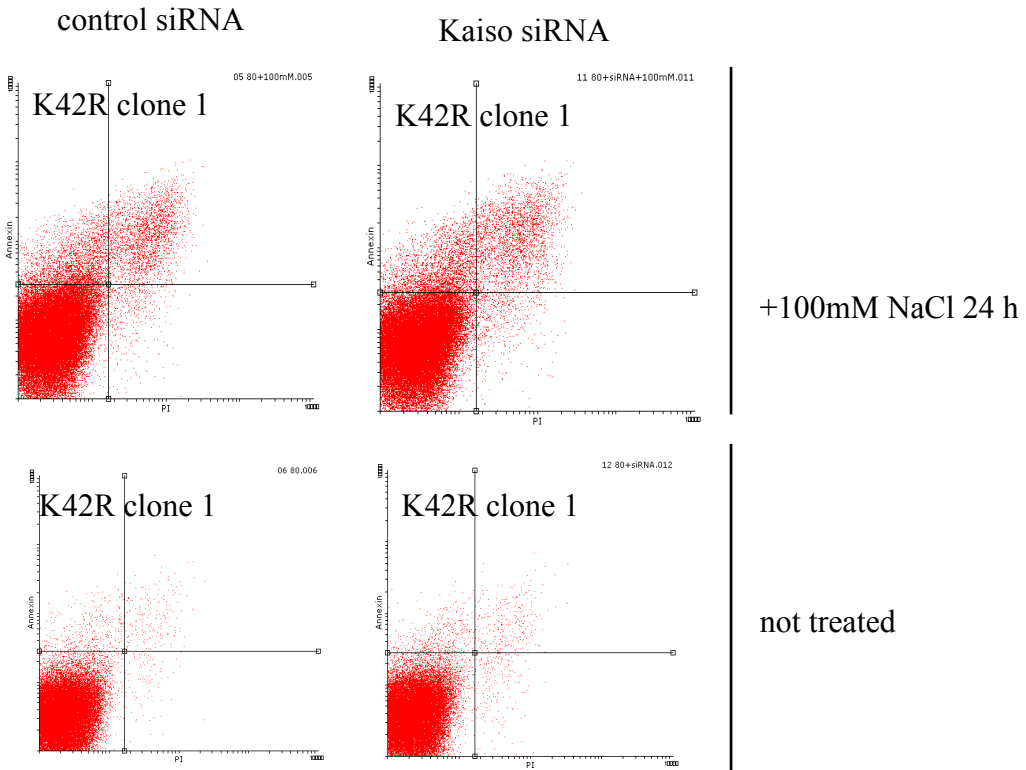
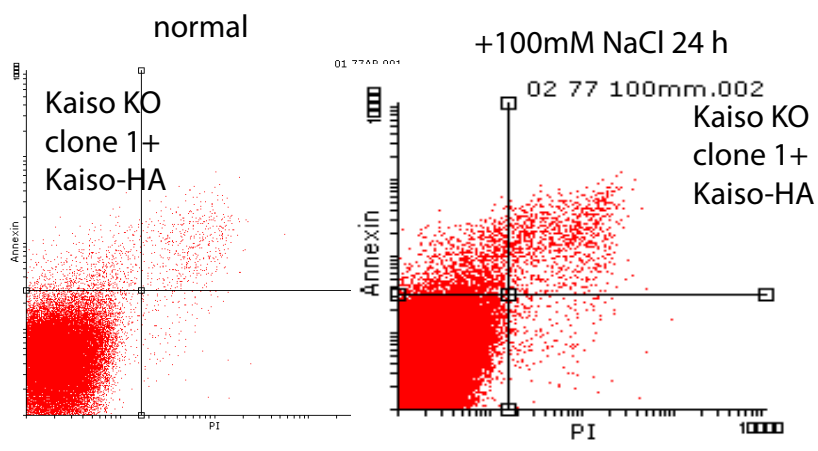


KO clone2



Kaiso KO
clone 2

Supplementary figure 4. K42R mutant Kaiso predisposed cells to apoptosis after hyperosmotic treatment. Flow cytometry analysis of cells stained with Annexin V -FITC and PI



Supplementary figure 5. Analysis of apoptosis in rescue experiments.
KO transfected with Kaiso-GFP, K42R clones transfected with control or Kaiso siRNA