



Suppl. Fig. 7. Hyperosmotic shock induces LIMK1 activation through the p38-MK2 pathway.

(A) Hyperosmotic shock induces LIMK1 activation. MSS31 cells were incubated in a medium containing 0.3 M NaCl for 0-30 min. Endogenous LIMK1 was immunoprecipitated and subjected to an *in vitro* kinase assay. The bottom panel indicates the relative kinase activities of LIMK1, as means \pm SD of five independent experiments. Cell lysates were analyzed by immunoblotting with anti-P-p38 and anti-p38 antibodies. (B) SB203580 inhibits hyperosmotic shock-induced LIMK1 activation. MSS31 cells were pretreated with 1 μ M SB203580 for 10 min, then treated with or without 0.3 M NaCl for 15 min. LIMK1 was immunoprecipitated and subjected to an *in vitro* kinase assay. (C) Ser-323 phosphorylation is required for osmotic shock-induced LIMK1 activation. MSS31 cells transfected with Myc-LIMK1 mutants were incubated with or without 0.3 M NaCl for 15 min. Myc-LIMK1 mutants were precipitated with anti-Myc antibody and subjected to an *in vitro* kinase assay. The bottom panel indicates the relative kinase activities, as means \pm SD of five independent experiments. *, $p < 0.001$.