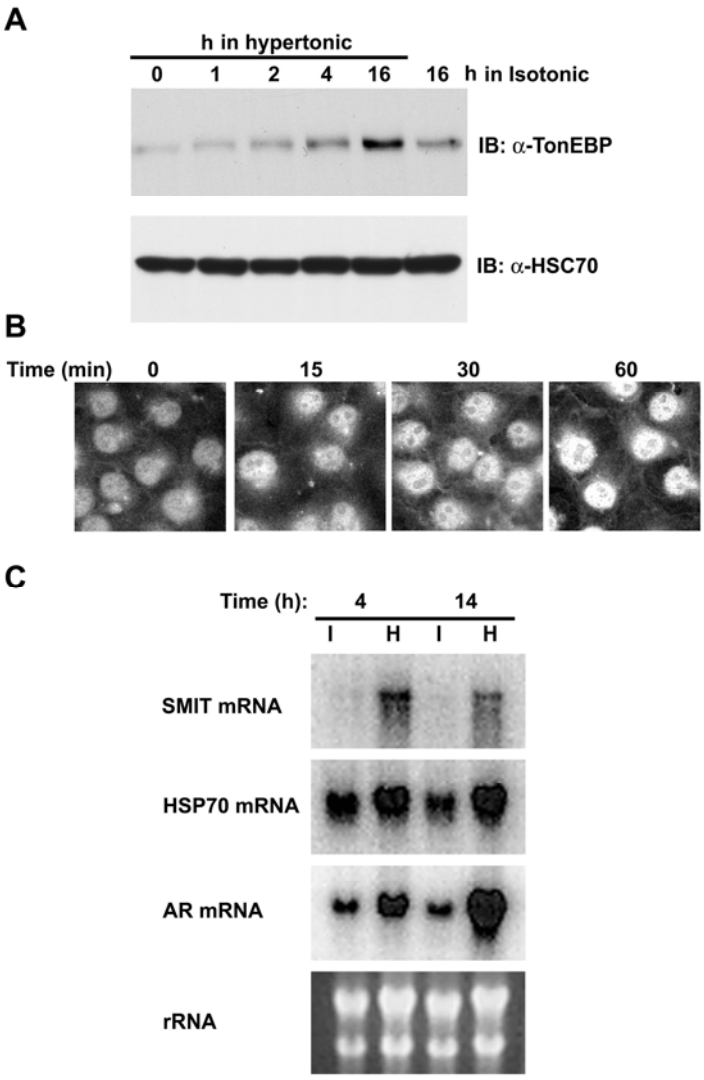
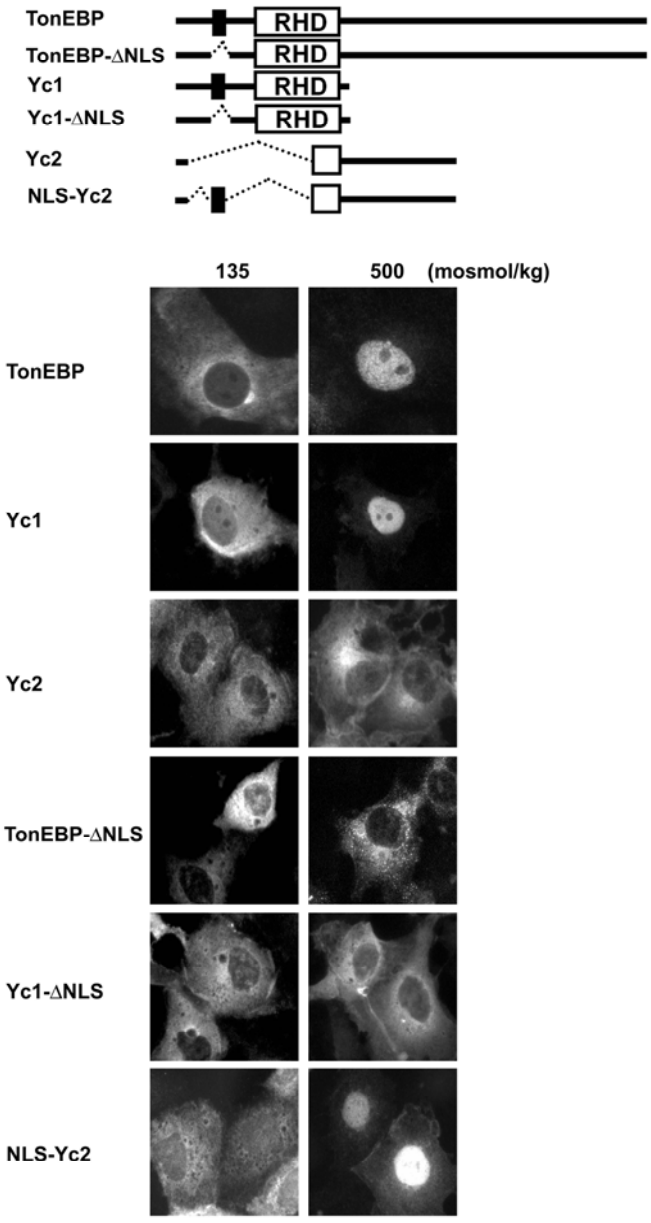


Supplemental Figure 1. Stimulation of TonEBP in response to hypertonicity in COS7 cells. (A) Induction of TonEBP. COS7 cells were switched to hypertonic medium (500 mosmol/kg H_2O, made by addition of NaCl) or kept in isotonic medium for up to 16 hours. Cell lysates were immunoblotted (IB) for TonEBP and HSC70. (B) Increased nuclear localization of TonEBP. COS7 cells were grown on glass coverslips and switched to the hypertonic medium for up to 60 minutes as indicated on top. TonEBP was visualized by immunofluorescence. All the pictures were taken at the same exposure for comparison. (C) Induction of TonEBP target genes. COS7 cells were switched to the hypertonic medium (H) for 4 or 14 hours or remained control isotonic medium (I). Northern analyses were performed to detect mRNA for SMIT (Na^+/*myo*-inositol cotransporter), HSP70 (heat shock protein 70), and AR (aldose reductase). A blot was stained with ethidium bromide to visualize rRNA bands (bottom).



Supplemental Figure 2. Nuclear localization signal (NLS) of TonEBP. (Top) Schematic representations of TonEBP and deletion constructs (TonEBP-ΔNLS, Yc1, Yc1-ΔNLS, Yc2, and NLS-Yc2). Filled box near the N-terminus represents NLS, amino acids 199-215. Open box labeled RHD represents the Rel-homology domain (DNA binding). (Bottom) Role of NLS in nucleocytoplasmic trafficking of TonEBP. COS7 cells were transfected with myc-tagged deletion constructs indicated and switched to 130 or 500 mosmol/kg for 30 minutes. Immunofluorescence detection of the myc epitope is shown. In each condition, representative distribution of myc epitope representing more than 90% of cells is shown.