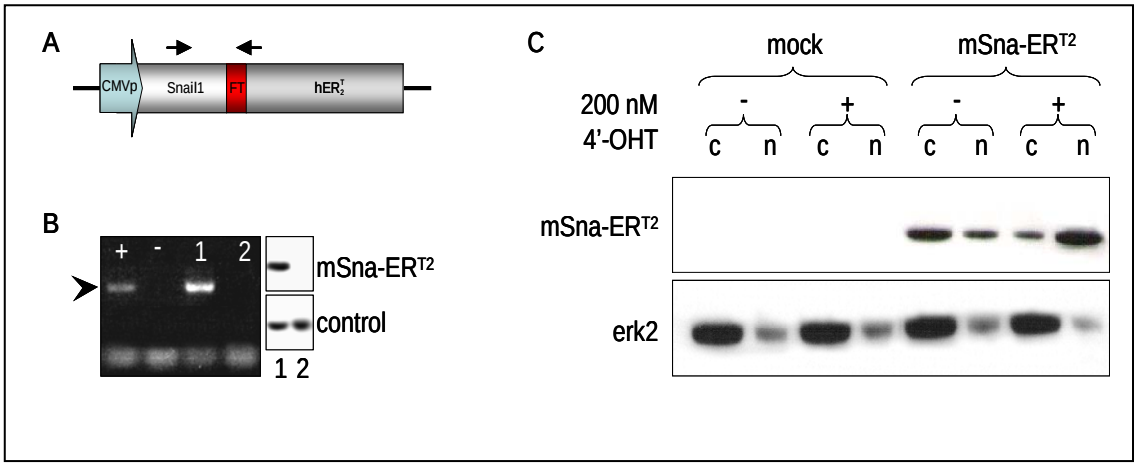

Analysis of transgenic mice expressing Snail inducible protein. (A) Schematic representation of the transgene construct. FT, Flag tag. (B) PCR of genomic DNA expression in Snail1-ER^{T2} mouse lines. +, plasmid positive control; -, negative control; 1, Snail1tg/tg; 2: wild type. Right panel, Western blot analysis of protein expression in caudal regions of 9.5 dpc embryos. (C) Nuclear translocation of the fusion protein in stable transfected cells upon treatment with 200 nM 4'-OHT. Immunoblotting of cytoplasmic total erk2 was used as a control for cytoplasmic content. n, nuclear extract; c, cytoplasmic extract.

Stable transfections and Western blots

Transfection of MDCK cells was carried out as described (Cano et al., 2000). Six independent clones were isolated from both pcDNA3-Snail-FT-ERT2 (mSna-ERT2) and control pcDNA3 transfections (mock). Cells were cultured in the absence or the presence of 200 nM 4'-OH-Tamoxifen for 48 h to activate the fusion protein.

Following treatment, the cells were scraped off the plates after washing with cold PBS, and lysed at 4ºC in hypotonic cytosolic lysis buffer (10 mM Hepes-K at pH 7.9, 10 mM KCl, 10 mM MgCl2, 0.5 mM DTT, 1mM NaF, 1 mM β-glycerophosphate, 5 mM NaPPi, 5 mg/ml leupeptin, 1 mM sodium o-vanadate and 1 mM PMSF). After isolating cytosolic proteins, the pellet was diluted in hypertonic nuclear lysis buffer (20 mM hepes-K at pH 7.9, 25% glycerol, 420 mM NaCl, 1.5 mM MgCl2, 0.5 mM EDTA, 0.5 mM DTT, 1mM NaF, 1 mM β-glycerophosphate, 5 mM NaPPi, 5 mg/ml leupeptin, 1 mM sodium o-vanadate and 1 mM PMSF) to isolate nuclear proteins. The presence of the Snail1-ER fusion protein was assed by Western blotting using a human estrogen receptor antibody (Santa Cruz; 1:100).