

Figure 9: Effects of cyclic uniaxial stretch (10%, 1 Hz) and/or catalase (1200 U/ml) on the activity of Notch3, as assessed by translocation of Notch3 from the membrane to the nucleus. Cells that had been incubated in the absence and presence of catalase (1200 U/ml, 4 hrs) were then exposed to cyclic uniaxial stretch (10%, 1 Hz) or left non-stretched for 1 hr. Samples were processed to separate nuclear, cytosolic and membrane fractions as described in the Methods. Effectiveness of the fractionation was assessed by analyzing the expression by Western blot of Lamin A/C (for nuclei), GAPDH (for cytosol) and EGFR (for membranes). Notch3 expression was also assessed by Western blot (Notch3 PIC, predominantly intracellular component). Notch3 PIC was spliced between cytosol and membrane fractions to match the order of Lamin A/C, GAPDH and EGFR, and white spaces were inserted between images of these two fractions. Membrane and Nuclear Notch3 was expressed as a percentage change in the levels in non-stretched 'Control' cells (normalized to EGFR and Lamin A/C expression, respectively), and are presented as means  $\pm$  SEM, n = 3. \*, denotes statistically significant difference from non-stretched 'Control' cells (\*\*, P < 0.01). #, denotes statistically significant difference from untreated stretched 'Stretch' cells (#, P < 0.05; # #, P < 0.01; not shown for comparisons to non-stretched cells).