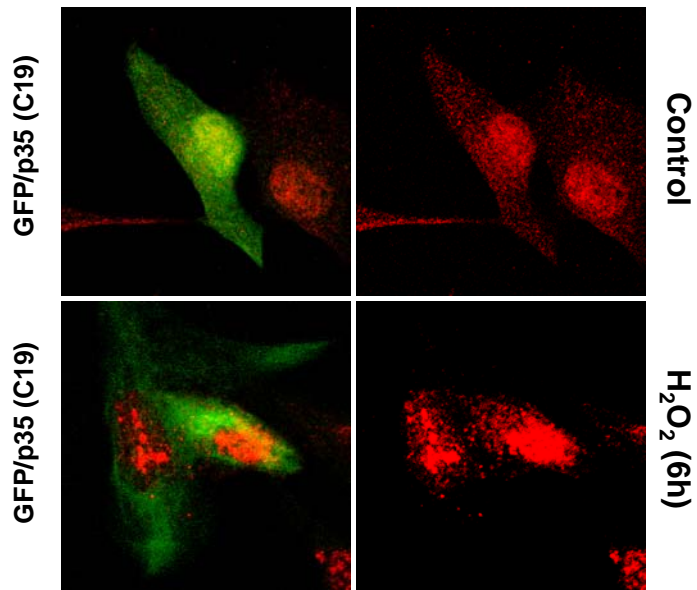
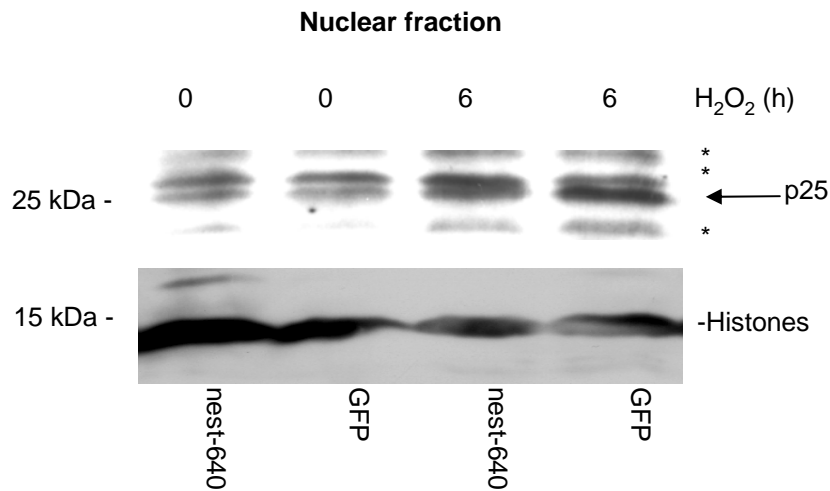


A



Supplementary figure 6 A. Immunofluorescence labeling of untreated and H₂O₂-treated ST15A cells cotransfected with GFP and p35. Cytoplasmic labeling of p35 faded out in H₂O₂-treated cells when co-expressed with GFP (compare with the results in Fig. 7E, showing that nest-640 and nest-1177 retained the cytoplasmic p35 signal).

B



Supplementary figure 6 B. In order to verify the nuclear localization of p25 a nuclear enrichment protocol was used to isolate nuclear fractions from control and H₂O₂-treated GFP and nest-640 expressing cells. P25 is present in the nuclear fractions after H₂O₂-treatment. Accumulation of p25 in the fraction is reduced in nestin overexpressing cells. Asterisk denotes unspecific bands. Successful nuclear fractionation was confirmed by immunoblotting with an anti-acetyl histone H4 antibody (Upstate).

ST15A cells were transfected with p35, Cdk5 and either nest-640 or GFP. To prepare nuclear enriched fractions cell pellets were carefully suspended in 800 µl of buffer containing 10 mM Tris, pH 7.4, 10 mM NaCl, 3 mM MgCl₂, 0.5 mM PMSF, 2 mM DTT and complete protease inhibitor cocktail (Roche) and incubated 10 minutes on ice. 0.3 % of NP-40 was added to each sample and the tubes were inverted a few times. Incubation was continued for an additional minute after which the samples were centrifugated 400xg, 4°C for 4 min. Pellets were carefully washed with buffer described above and centrifugated 400 x g, 4 °C for 4 min. After the last centrifugation pellets were suspended in 150 µl of buffer containing 20 mM Tris, pH 7.4, 40 mM Napyruvate, 5 mM MgCl₂, 50 mM NaF, 100 µM Na₃VO₄, 10 mM EDTA, 1% Triton-X 100, 1% SDS and protease inhibitors, sonicated briefly and centrifugated 20000 x g, 4 °C for 10 min. Supernatants were suspended on Laemmli sample buffer and analyzed on SDS-PAGE