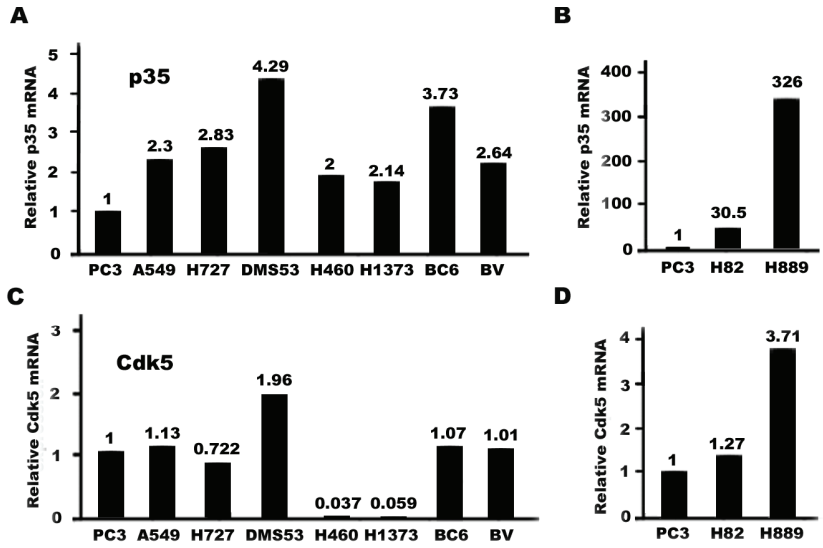
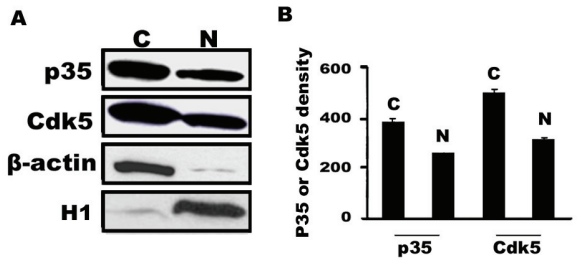
Supplemental Material/Figure Legends for Supplemental Figures



Supplemental Figure1

Figure 1S- Expression of p35 and Cdk5 in human lung cancer cell lines.

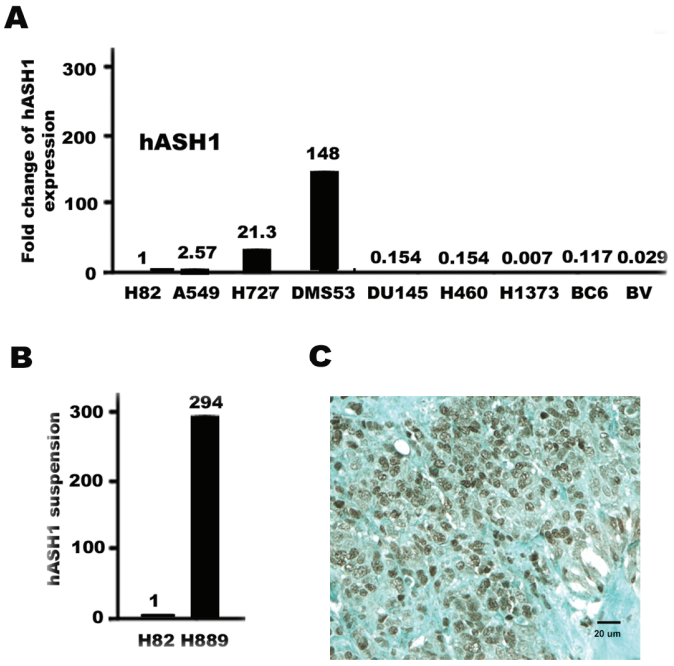
Measurements by qRT-PCR.(A) Relative expression of p35 mRNA in five human lung cancer cell lines, two immortalized bronchial epithelial lines. The results were compared to the human prostate cancer cell line PC3 (normalized as 1). All these cultures grew as adherent cells on the dish. (B) Relative p35 mRNA in two SCLC cell lines growing as suspension cultures compared to PC3 (normalized as 1). (C) Relative expression of Cdk5 mRNA in the same panel of cells (adherent cultures) as in A. (D) Relative Cdk5 mRNA in the same cell lines (suspension cultures) as in D.



Supplemental figure 2

Figure 2S – Subcellular localization of p35 and Cdk5 in lung cancer cells

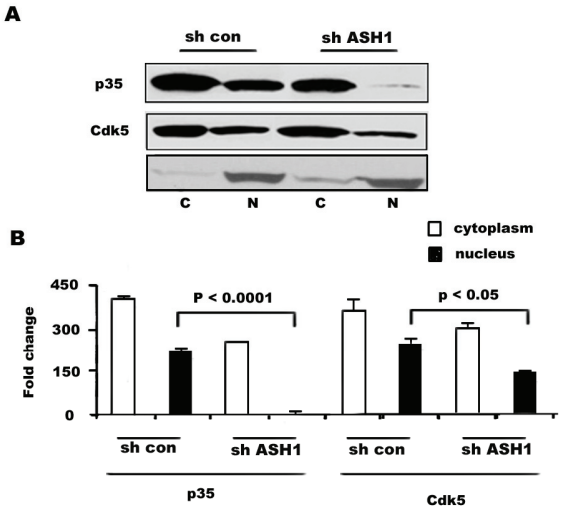
(A) Western blot of protein lysates from H727 cell line following cell fractionation. Expression of p35 and Cdk5 is shown in both cytoplasmic (C) and nuclear (N) compartments. Markers included β-actin for cytoplasmic and histone H1 for nuclear compartment indicating the purity of fractions (B) Quantification by densitometry. While p35 and Cdk5 were found in both compartments, the expression was higher in the cytoplasm.



Supplemental Figure 3

Figure 3S – Expression of hASH1 in human lung cancer.

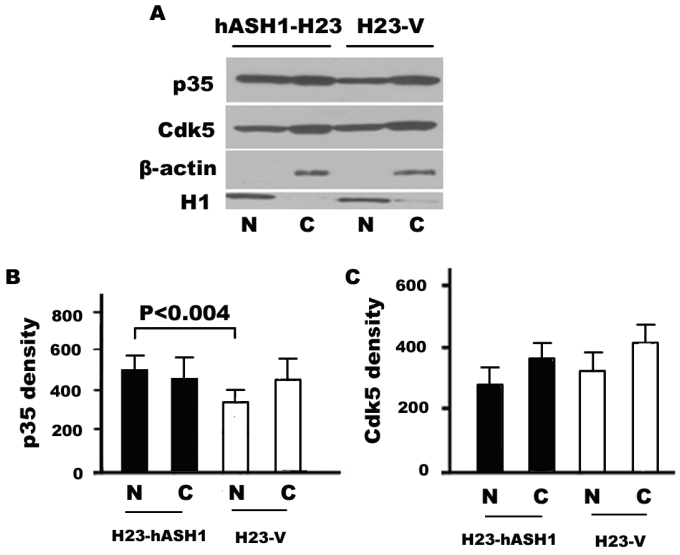
(A) Expression of hASH1 mRNA by qRT-PCR in five human lung cancer and two immortalized bronchial epithelial cell liness with adherent growth pattern. Also included is human prostate cancer cell line DU145. Results were compared with the variant SCLC cell line H82 (normalized as 1), previously shown to be negative (or have very low levels) for hASH1. Two cell lines, pulmonary carcinoid H727 and SCLC DMS53 cells, reveal high levels of hASH1 mRNA. (B) High level of hASH1 expression in the classic SCLC cell line H889 growing as suspension culture compared to the variant SCLC cell line H82 (normalized as 1). (C) Photomicrograph of hASH1 expression in a SCLC tumor specimen. Most cells showed nuclear immunoprecipitate (immunoperoxidase stain, bar=20µm).



Supplemental figure 4

Figure 4S – Silencing of hASH1 reduces nuclear p35 in H727 cells.

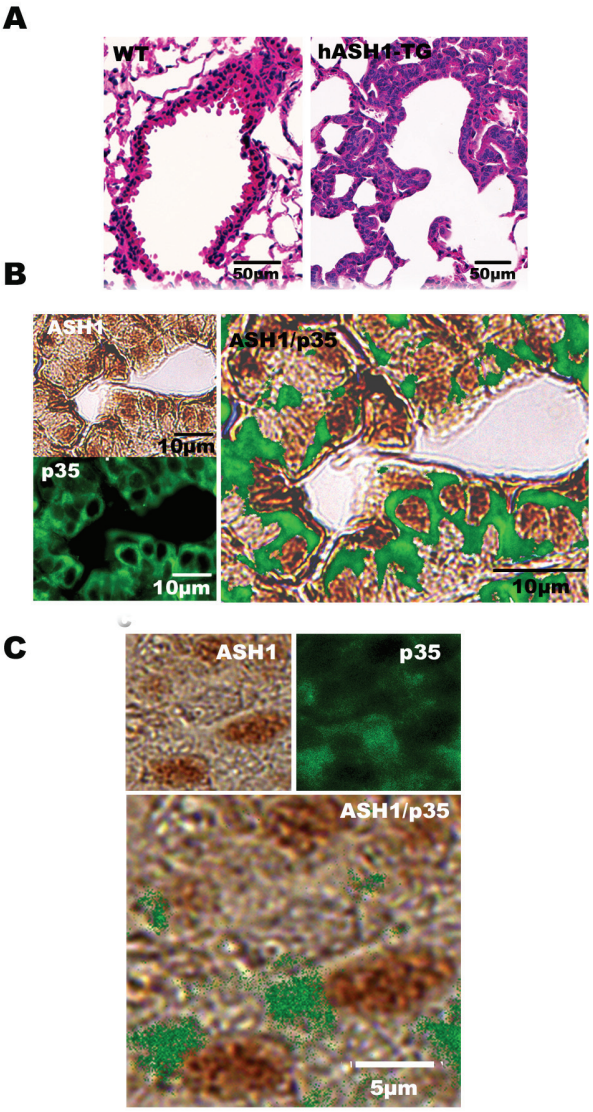
(A) Western blot analysis of p35 and Cdk5 expression following hASH1 shRNA (sh ASH1) transfection of H727 lung cancer cells. Control H727 cells (sh con) were transfected by scrambled RNA. N= nuclear and , C= cytoplasmic fractions were harvested for analysis. Histone H1 (H1) was used as a control for nuclear fractions. (B) Quantification of Western blot by densitometry revealed a significant reduction of nuclear p35 protein by hASH1 shRNA ($P < 0.0001$) compared with control cells. The reduction in nuclear Cdk5 protein was also significant ($P < 0.05$). The data present mean ± SD from at least three independent experiments.



Supplemental figure 5

Figure 5S – Subcellular localization of p35 and Cdk5 in H23 lung cancer cells overexpressing hASH1.

(A) Western blot of of p35 and Cdk5 expression in nuclear (=N) and cytoplasmic (=C) fractions of hASH1-H23 cells that overexpress hASH1 compare with H23-V control cells. The purity of fractions were verified by the expression of β-actin for cytoplasmic and histone H1 (H1) for nuclear fractions. (B) Quantification of the Western blot in A. There was a significant increase in nuclear p35 expression in hASH1-H23 cells compared to H23-V cells ($p < 0.004$). The data represent mean ± SD from three independent experiments.



Supplemental figure 6

Figure 6S – Co-localization of ASH1 with p35 in mouse models.

(A) Photomicrographs of normal lung and typical metaplastic changes in the lung of transgenic mice that constitutively express hASH1 in the airways. WT=wild type; hASH1 TG= CC10-hASH1 transgenic mouse (hematoxylin-eosin stain). Bar=50µm. (B) Photomicrographs of nuclear expression (brown) of hASH1 in the metaplastic epithelium of CC10-hASH1 transgenic mouse lung (left upper panel; immunoperoxidase stain). Same view of the expression of p35 (green; left lower panel, immunofluorescence). Co-localization of the nuclear expression of

hASH1 (brown) and p35 (green) by immunoperoxidase and –fluorescence, respectively, in the metaplastic lesions of CC10-hASH1 transgenic mouse lung (right panel). Bar = 10μm

(C) Photomicrographs of nuclear ASH1 (brown, immumoperoxidase, upper left) and p35 (green, immunofluorescence, upper right) in a mouse model of SCLC. Photomicrograph of co- localization of nuclear hASH1 (brown, immumoperoxidase) and p35 immunofluorescence (green) in a mouse model of SCLC (bottom). Bar=5μm.