

Figure S1. Expression of Cbx7 protein in Cbx7 knock-out mice.

Western blot performed using antibodies recognizing the 5' region of the CBX7 protein. Proteins were extracted from *Cbx7*^{+/+}, *Cbx7*^{+/-} and *Cbx7*^{-/-} MEFs and kidney tissues. A band corresponding to the endogenous Cbx7 protein is observed in wt and heterozygous mice, but not in knock-out samples. No smaller band corresponding to a potential *Cbx7* gene transcript, was detected in the *Cbx7*^{+/-} and *Cbx7*^{-/-} cells and tissues. MWM = Molecular Weight Marker.

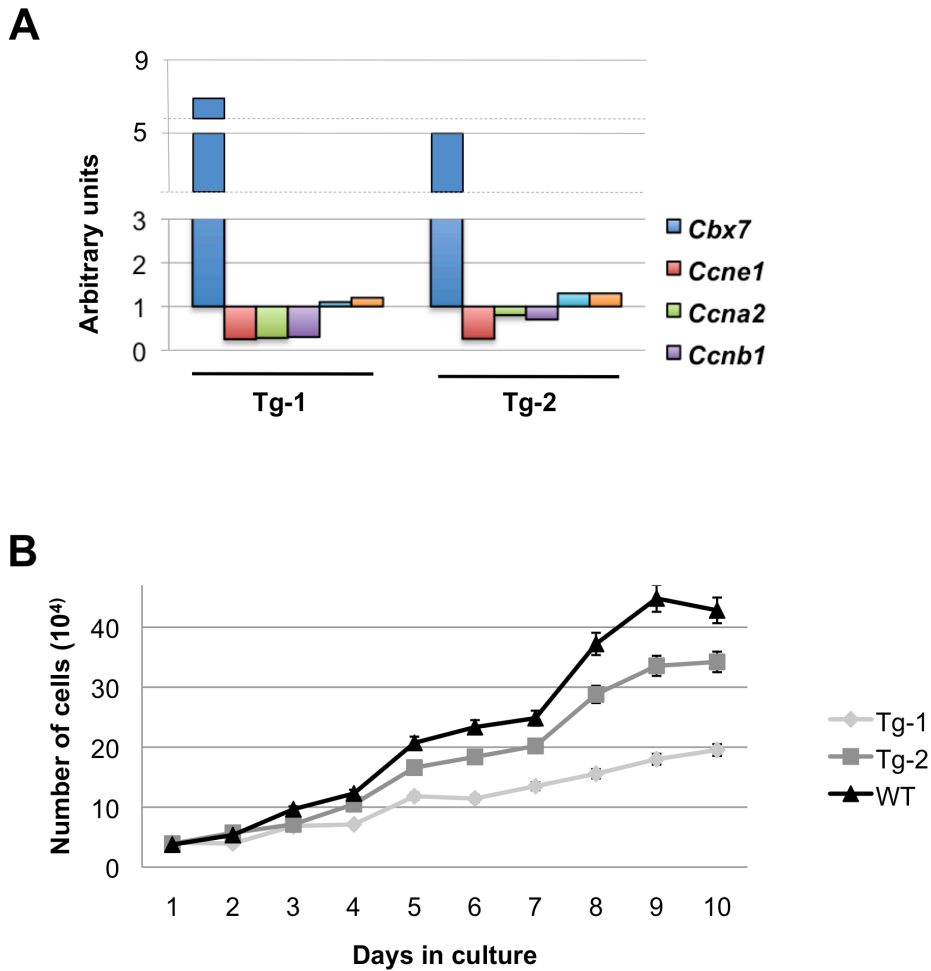


Figure S2. Growth analysis and expression of cell cycle and senescence regulators in MEFs derived from Cbx7-transgenic mice.

A) Expression of cell cycle and senescence regulators was analyzed by qRT-PCR in MEFs derived from Cbx7-transgenic (Tg-1 and Tg-2) and wild-type (wt) embryos at 12.5 dpc. The relative expression between transgenic and wt MEFs, assuming that the value of wt samples is equal to 1, are reported.

B) MEFs were plated at passage 4 and counted daily for 10 days to extrapolate growth curves. The average of three independent experiments and standard deviation are shown for each time point.

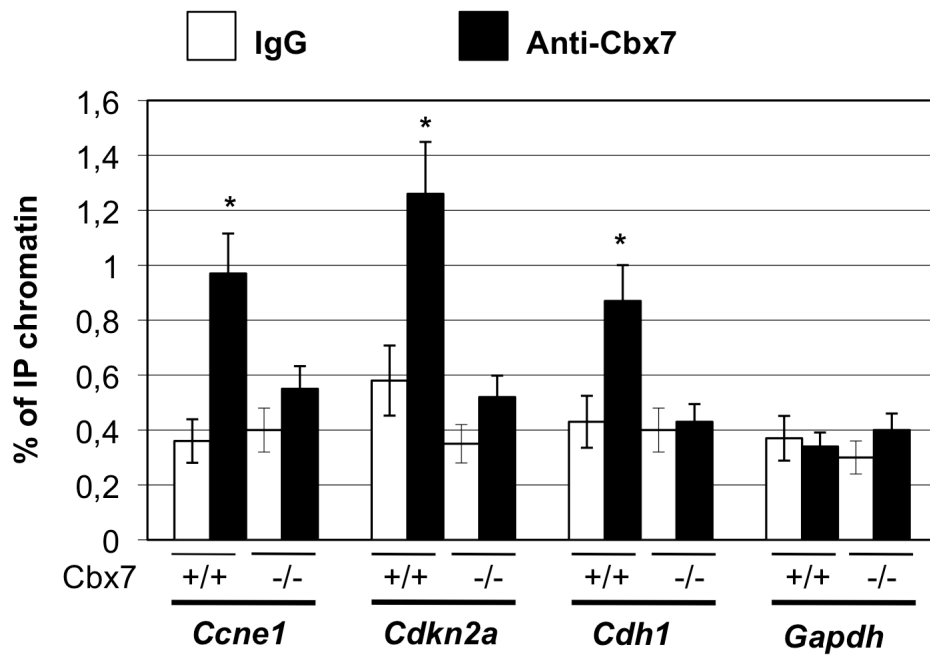


Figure S3. ChIP assay on mouse lung tissues.

ChIP assay, revealed by qRT-PCR, on normal lung tissue showing the binding of endogenous Cbx7 protein to *Ccne1*, *Cdkn2a*, *Cdh1* and *Gapdh* promoter regions. The average value of three independent experiment \pm SD are reported. *, $P < 0,05$

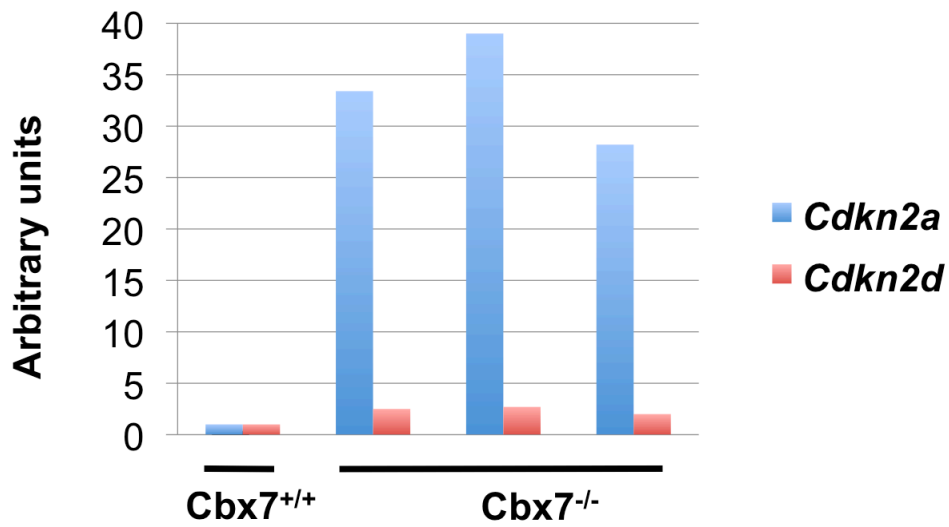


Figura S4. *Cdkn2a* and *Cdkn2d* gene expression in lung adenocarcinoma samples from Cbx7-ko mice

qRT-PCR analysis of *Cdkn2a* and *Cdkn2d* in lung carcinoma samples from 3 representative Cbx7^{-/-} mice. The relative expression between pathological and normal samples from wt mice (mean of 2), assuming that the value of the normal sample is equal to 1, is reported.

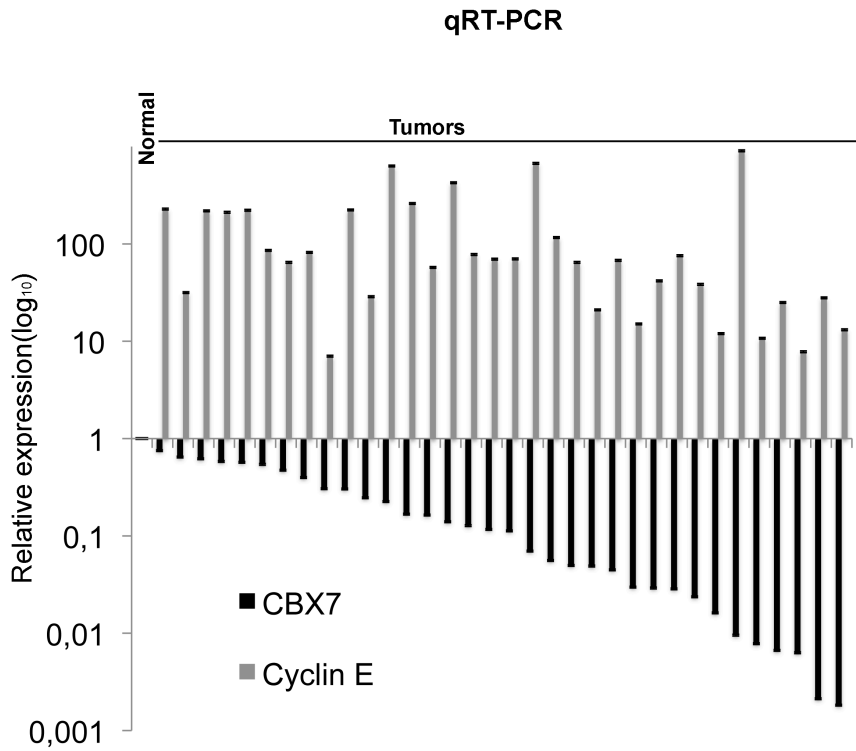


Figure S5. *CBX7* and *CCNE1* gene expression in human lung tumour samples.

CBX7 and *CCNE1* gene expression in a panel of 34 human lung tumour samples was analysed by qRT-PCR. The relative expression between tumour and normal samples (mean of 5), assuming that the value of normal sample is equal to 1, is reported.

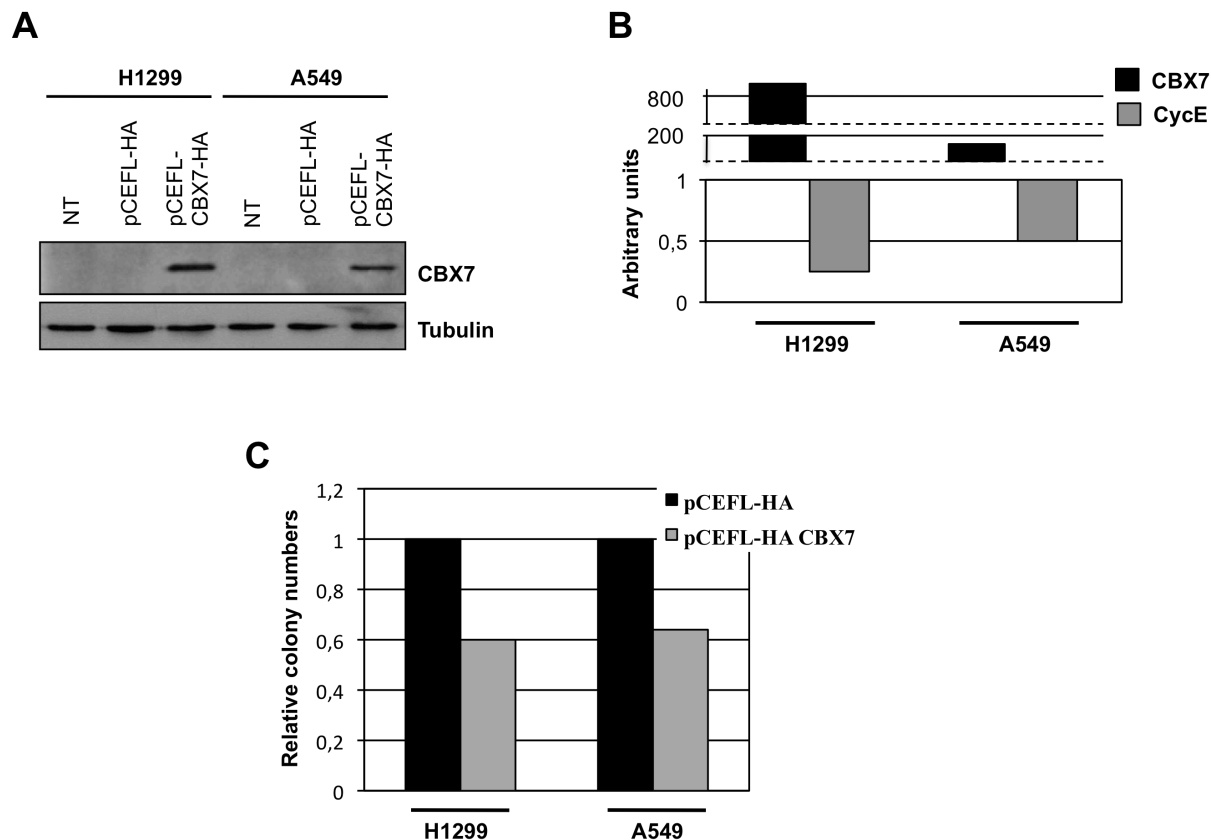


Figure S6. Restoration of CBX7 expression in lung carcinoma cell lines.

A) Western blot analysis of CBX7 expression in H1299 and A549 lung carcinoma cells non transfected (NT) or transfected with a vector expressing CBX7 (pCEFL-HA CBX7) or the empty vector (pCEFL-HA). Tubulin expression was evaluated as a loading control.

B) Expression levels, measured by qRT-PCR, of *CBX7* and *CCNE1* (CycE) in cells transfected as in A. The relative expression levels between CBX7- and empty vector-transfected cells, assuming that the value of the control is equal to 1, are reported.

C) Colony-forming assay performed on A549 and H1299 cells transfected with a vector expressing CBX7 (pCEFL-HA CBX7) or the empty vector (pCEFL-HA). The relative values, normalized *versus* pCEFL-HA-transfected cells, are shown.

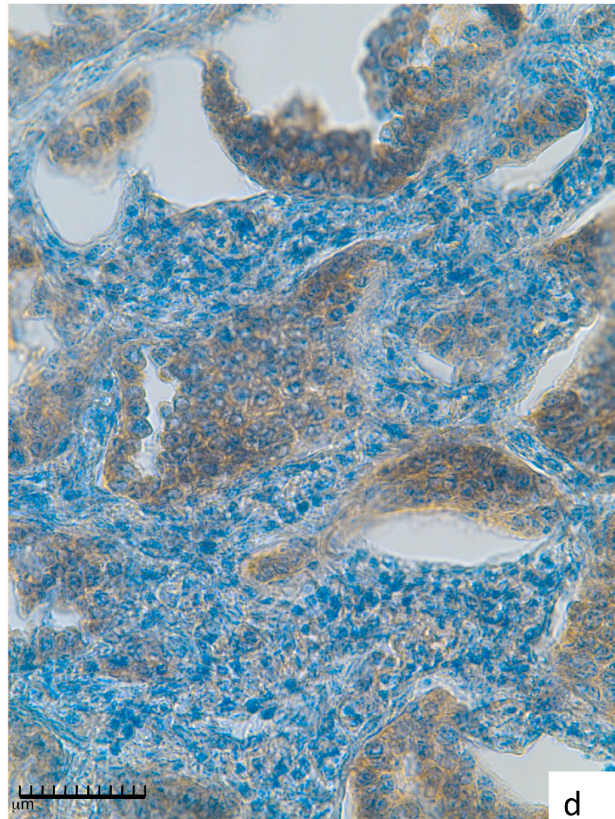
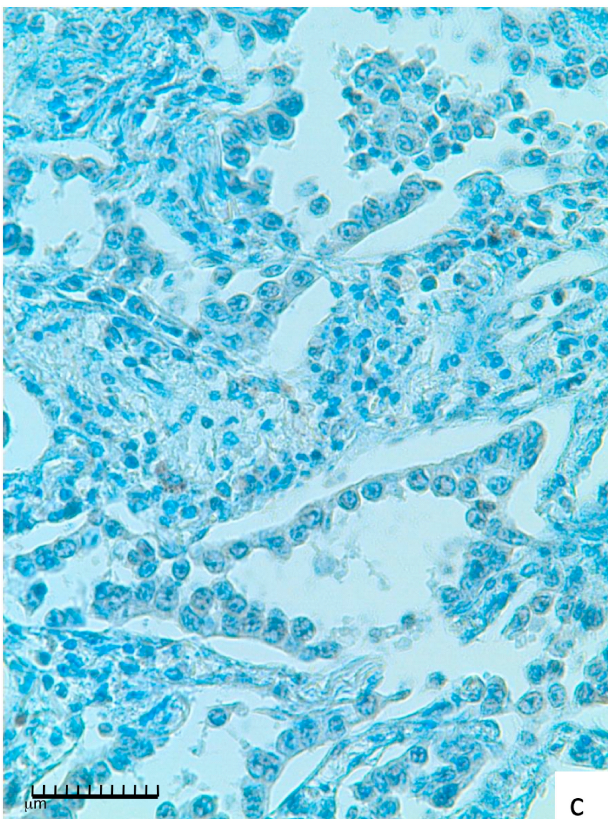
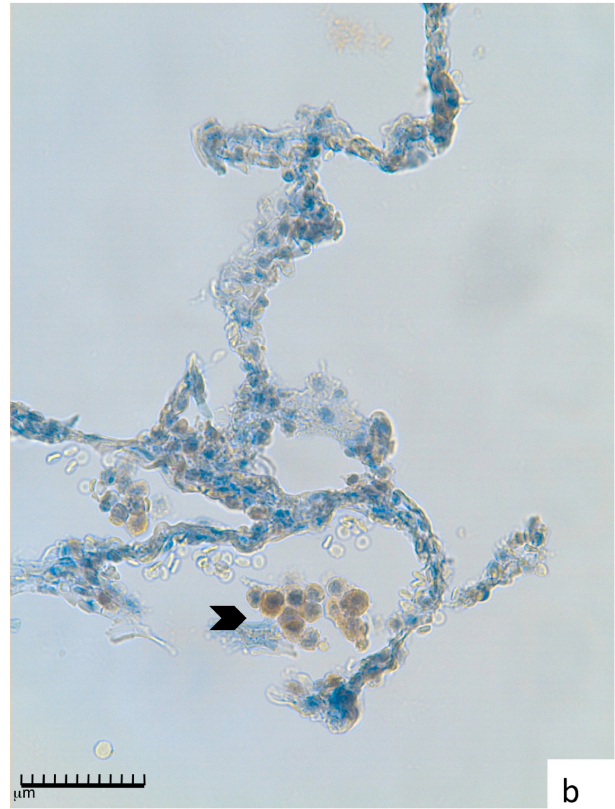
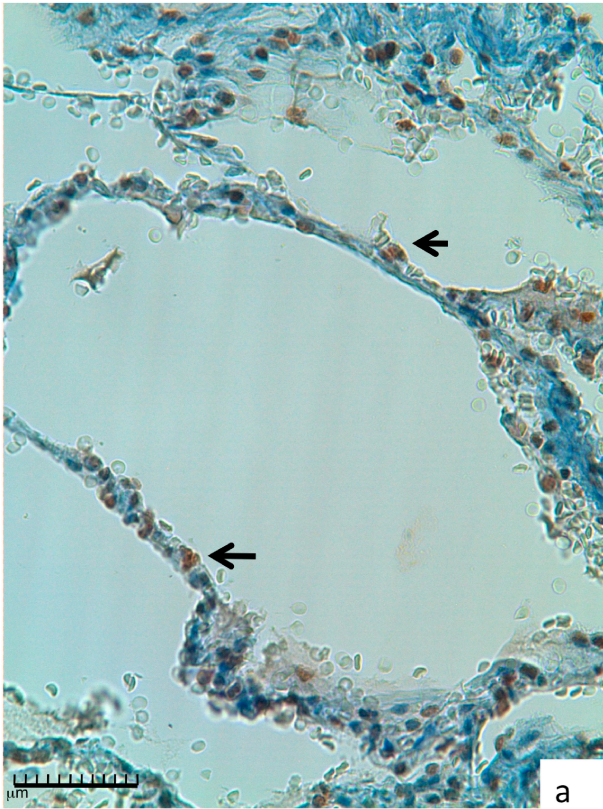


Figure S7. Immunohistochemical analysis on human lung samples.

a) Normal alveoli have intense immunoreactivity for CBX7 (arrows) whereas b) they are negative for Cyclin E expression (positive histiocytes providing a positive internal control, arrowhead). (63x, hematoxylin counterstained). Lung adenocarcinoma is negative for CBX7 (c) but strongly positive for Cyclin E (d) (63x, hematoxylin counterstained).

Primers used for qRT-PCR experiments:

The following primer sequences were used for the amplification of the indicated mouse genes:

Cbx7-F, 5'-ttgcatggcctacgagga-3'

Cbx7-R, 5'-tgggttcggacctctctt-3'

Cyclin E-F, 5'-ctgagagatgagcactttctgc-3'

Cyclin E-R, 5'-gagcttatagacttcgcacacct-3'

Hmgal-F, 5'-ggcagaccaagaaactgg-3'

Hmgal-R 5'-ggcactgcgagtggatgat-3'

p19-F 5'-gggtttcttggtgaagttcg-3'-

p19-R 5'-ttgccatcatcatcacct-3

p16-F 5'-cagattcgaactgcgagga-3'

p16-R 5'-accagcgtgtccaggaag-3'

p21-F 5'-cagatccacagcgatatcca-3'

p21-R 5' ggcacactttgctcctgtg-3

p53-F 5-acgcttctccgaagactgg-3'

p53-R 5- agggagctcgaggctgata

G6PD-F, 5'-gaaagcagagtgagcccttc-3'

G6PD-R 5'-cataggaattacgggcaaaga-3'

The following primer sequences were used for the amplification of the indicated human genes:

Cbx7-F, 5'-cgagtatctggtgaagtggaaa-3'

*Cbx7-R*5'-gggggtccaagatgtgct-3'

Cyclin E-F, 5'-ggccaaaatcgacaggac-3'

*Cyclin E -R*5'-gggtctgcacagactgcat-3'

G6PD-F, 5'-gatctaccgcatcgaccact-3'

G6PD-R, 5'-agatcctgttgcaaatctca-3'

Primers used for ChIP-ReChIP experiments:

H-Pp16 Fw 5'-cggctgggagcagggaggc-3';

H-Pp16 Rv 5'-gaatgtggcaccctgaagtcgc-3';

H-PGAPDH-Fw 5'cccaaagtctctctgttca; 3';

H-PGAPDH-Rv 5'-gtcttgaggcctgagctacg-3';

M-PCadh-BoxUP Fw 5'-gaaggctgctcttatccaca-3';

M-PCadh_BoxUP_Rv 5'-ggttggatgagttgttcttaggg-3';

M-PCylE Fw 5-tgaggcatgtgcatactctgaggt-3;

M-PCylE Rv 5- accgtttcagaaagcctct-3

M-PGAPDH Fw 5'-tgagtcctatcctgggaacctca-3'

M-PGAPDH Rv 5'-tttgaatgtgcacgcaccaagcg-3';

M-Pr-16-Fw 5'-ggattctctaggatttctcgggc-3'

M-Pr-P16-RW tcagtgaatttaggccagccgta-3'