Supplementary Figures.

Fig. S1. $p18^{INK4c}$ is a stable protein. Cultures of proliferating HDFs (TIG3 and Hs68) were treated with cycloheximide, to block protein synthesis, and cell lysates prepared at different time points were fractionated by SDS-PAGE and immunoblotted for the indicated proteins. Whereas the levels of cyclin D1, which is known to have a short half-life, declined dramatically within the first 3h, there was no significant reduction in $p18^{INK4c}$ levels during the duration of the experiment (15h). β-tubulin was used as a stable loading control. Note that different strains of HDF express different basal levels of $p18^{INK4c}$.

Fig. S2. Both *INK4c* transcripts are down-regulated by RAS and activated by E2F1. A. Diagram of the two transcripts generated from the *INK4c* locus and the PCR primers used to discriminate between them. B. RT-PCR detection of the long and short *INK4c* transcripts in HDFs (Hs68) expressing ER:E2F1 or infected with $H\text{-}RAS^{G12V}$ retrovirus. GAPDH was used as a loading control.

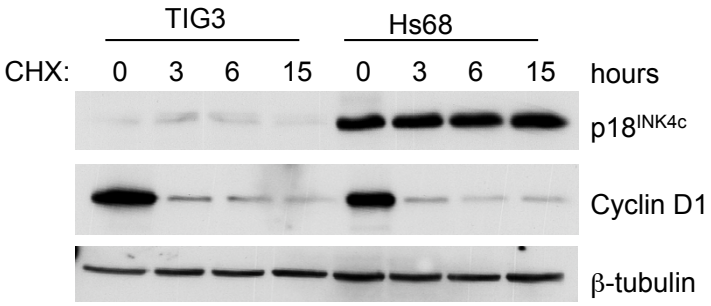
Fig. S3. ChIP for H3K27me3 at the *INK4c* and *INK4a* loci. A and B. Diagrams of the human *INK4c* and *INK4a* loci with exons depicted as boxes and coding domains shaded. The location of the transcriptional start sites (arrows) and primer sets (P1 etc) used for ChIP analyses are indicated. C. ChIP for H3K27me3 at the *INK4c* locus in IMR90 ER:RAS cells, with or without addition of OHT. D. Equivalent analyses for H3K27me3 at the *INK4a-ARF* locus using primer sets P2, P6 and P7.

Fig. S4. *INK4c* expression is regulated by E2F1 but is not affected by cell cycle arrest. A. HDFs (Hs68) were treated with hydroxyurea (HU), nocodazole (NO), or RO3306 (RO) for 24 h to block the cell cycle in different phases and the levels of *CCNE1*, *B-MYB* and *INK4c* RNA were assessed by qRT-PCR. The signals were normalized to the untreated controls. B. Immunoblot of $p18^{INK4c}$, cyclin E and β-tubulin in the cells described in A.

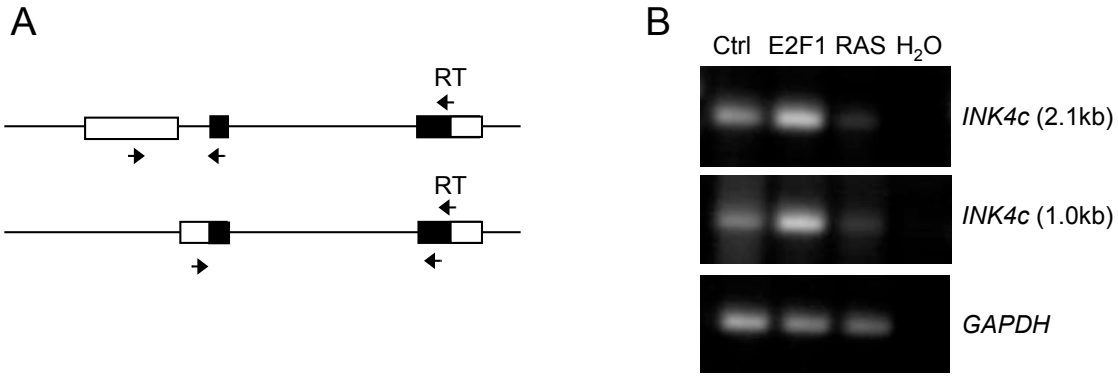
C. HDFs (Hs68) expressing an ER:E2F1 fusion protein were treated with OHT for 24 h and the levels of *CCNE1*, *B-MYB* and *INK4c* RNA were assessed by qRT-PCR. The signals were normalized to the untreated controls. D. Lysates from the cells described in panel C were fractionated by SDS-PAGE and immunoblotted for $p18^{INK4c}$, cyclin E and E2F1 as indicated.

Fig. S5. Substitution of $p18^{INK4c}$ by $p16^{INK4a}$ at senescence. Lysates from young and senescent fibroblasts were subjected to gel filtration to separate the ternary cyclin D-CDK-CIP/KIP complexes (~150-200 kDa) from CDK-INK4 complexes (50kDa) and free INK4 proteins (15-19kDa). The fractions were either analyzed directly and immunoblotted for cyclin D1, CDK4, CDK6, $p21^{CIP1}$ and $p16^{INK4a}$ (panel A) or immunoprecipitated with a rabbit antiserum against CDK6 prior to immunoblotting for cyclin D1, CDK6, $p16^{INK4a}$ and $p18^{INK4c}$ as indicated (panel B). Note that the data in panel A formed part of a previously published figure (Ruas *et al*. Molecular and Cellular Biology 27: 4273-4282, 2007)

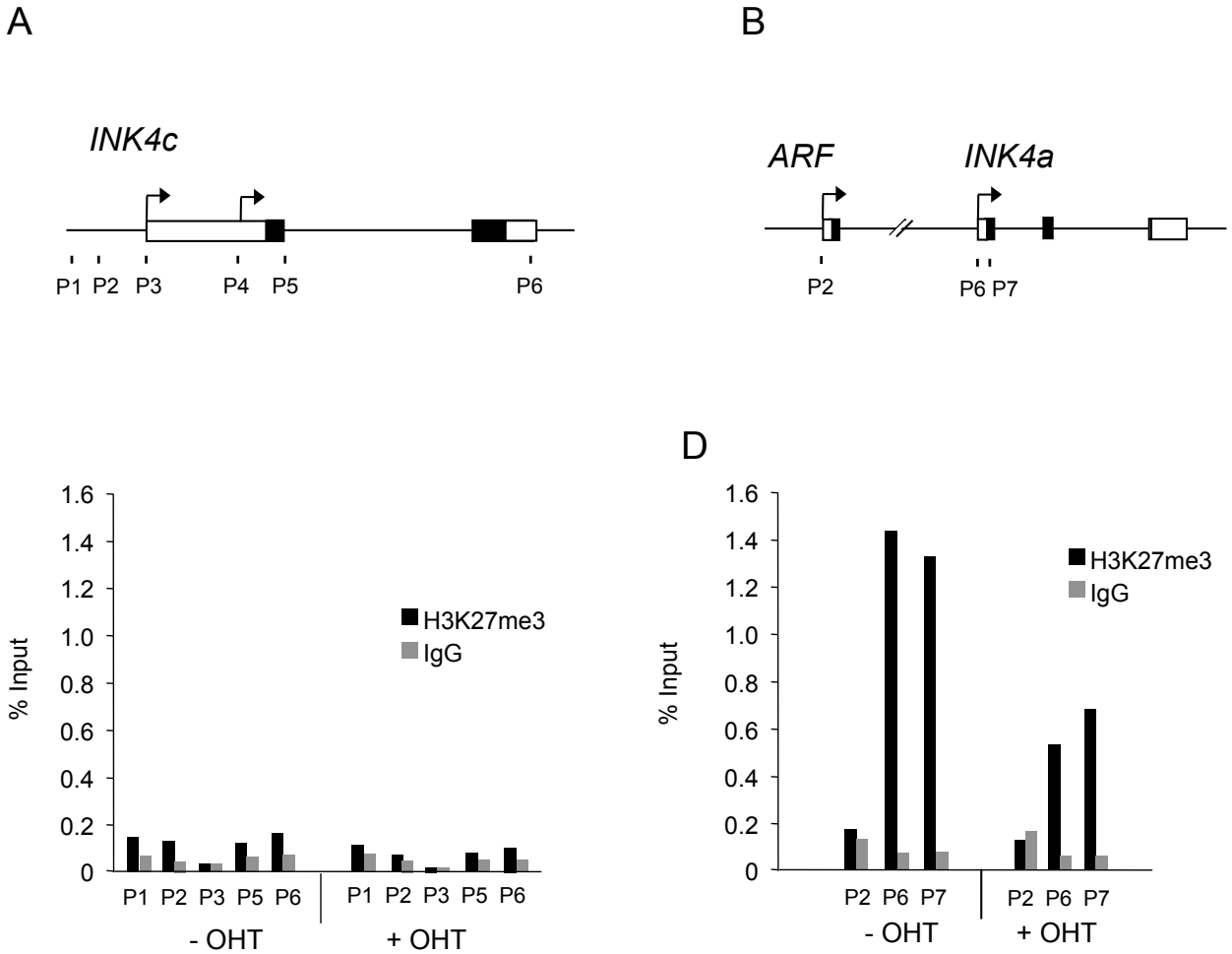
Gagrica et al Supplementary Figure S1



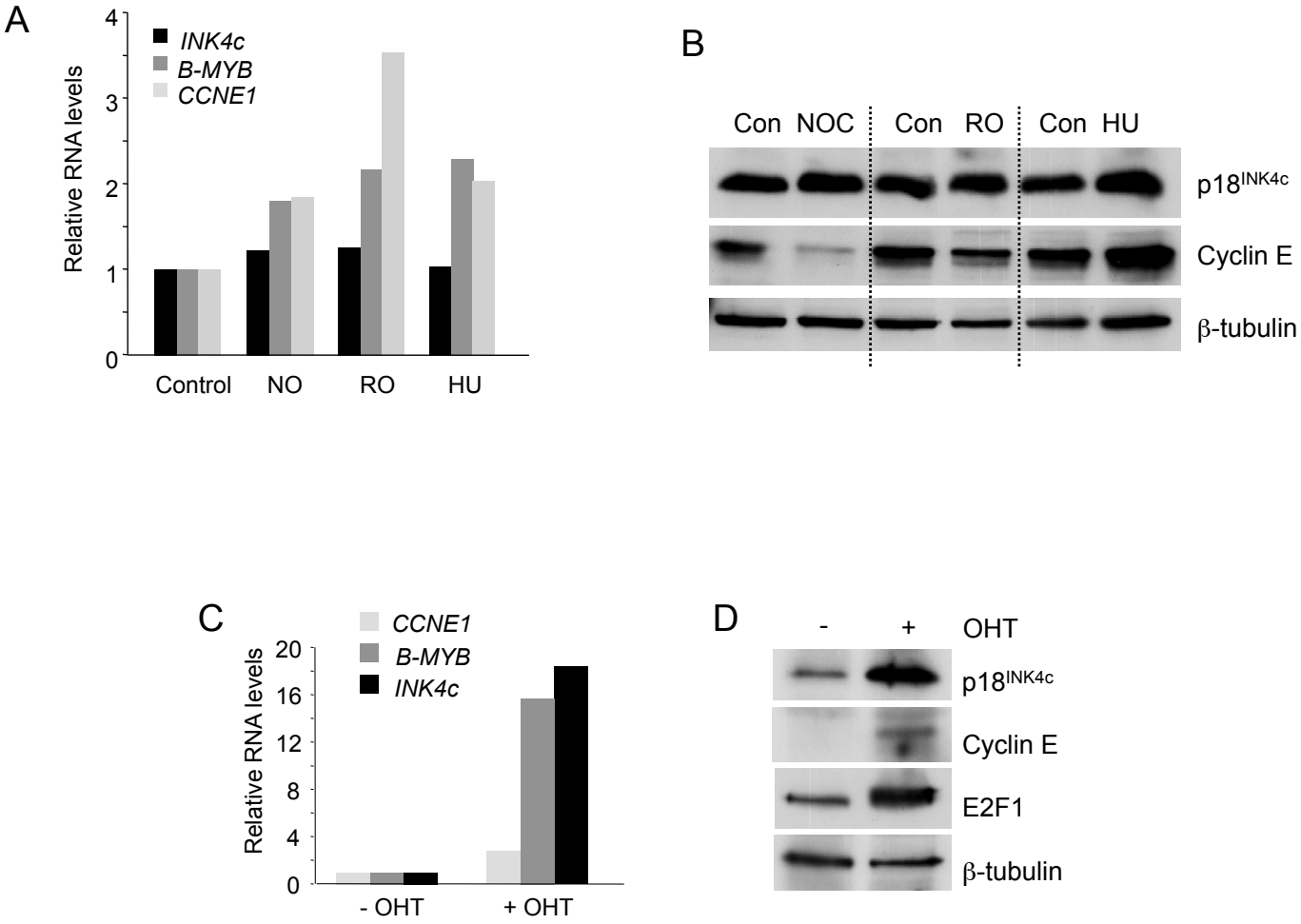
Gagrica et al Supplementary Figure S2



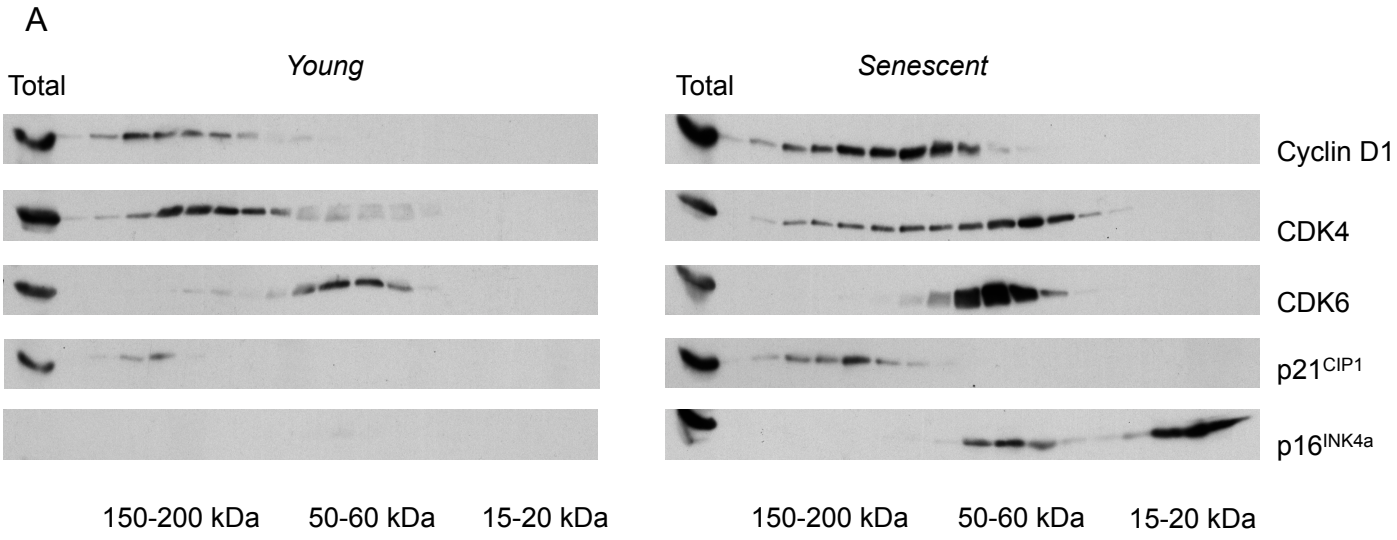
Gagrica et al Supplementary Figure S3

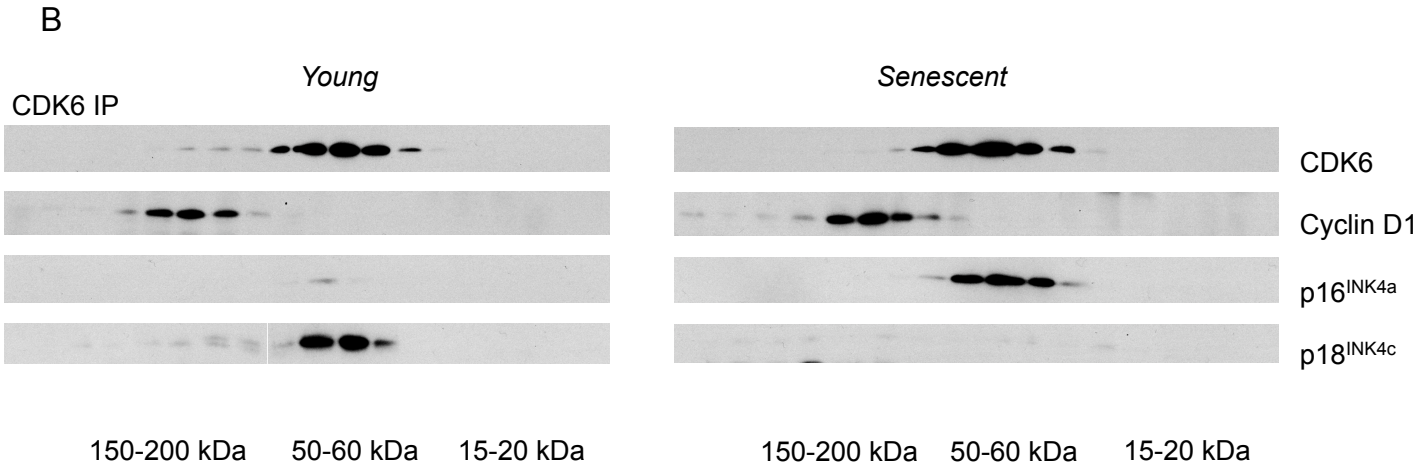


Gagrica et al Supplementary Figure S4



Gagrica et al Supplementary Figure S5





Supplementary Table 1 Oligonucleotide primers used for qRT-PCR.

Primer	Direction	Sequence
P18INK4c-1	Fwd	5' AGTTCCTGGTGAAGCACACG
P18INK4c-1	Rev	5' CCTGCATCAGGCTAACAAC
P18INK4c-A long	Fwd	5' GGACCCAGGACTATCCCTTC
P18INK4c-B long	Rev for RT	5' CTCGGGATTTCCAAGTTTCA
P18INK4c-D long	Rev	5' CCATTTTGTGCATTGACGTT
P18INK4c short	Fwd	5' CAGCCTGGTTAGGAGCAAAG
P18INK4c short	Rev	5' AGCAGTCTCCTGGCAATCTC
P16INK4a	Fwd	5' CGGTCGGAGGCCGATCCAG
P16INK4a	Rev	5' GCGCCGTGGAGCAGCAGCAGCT
Menin	Fwd	5' AGGGGCCAGACAGTCAATG
Menin	Rev	5' GTCAATGGAAGGGTTGATGG
E2F1	Fwd	5' AGCAGATGGTTATGGTGATCAAAG
E2F1	Rev	5' GGAGATCTGAAAGTTCTCCGAAGA
CCNE1	Fwd	5' GAGCCAGCCTTGGGACAATA
CCNE1	Rev	5' CGGTCATCATCTTCTTTGTCAGG
B-MYB	Fwd	5' CCACTTCCCTAACCGCACTG
B-MYB	Rev	5' GCCCCTTGACAAGGTCTGG
CDC6	Fwd	5' AGCACTGGATGTTTGCAGGAG
CDC6	Rev	5' GGGAATCAGAGGCTCAGAAGG
GAPDH	Fwd	5' TGATGACATCAAGAAGGTGGTGAAG
GAPDH	Rev	5' TCCTTGGAGGCCATGTGGGCCAT

Supplementary Table 2 Primers used for ChIP at the INK4c locus

Primer set	Direction	Sequence
PS1	Fwd	5' CTGCCAGATTCTTCCCAGTT
PS1	Rev	5' AAGCGGATCCTCAAGCAGTA
PS2	Fwd	5' ACTTCGGCAACCAAGAAATG
PS2	Rev	5' AAGGACTGGAAACTGCGAAA
PS3	Fwd	5' CTCTGCCGAGCCTCCTTAAAACT
PS3	Rev	5' TTTTCGCTGAAACAATTGCTGCT
PS5	Fwd	5' ACGTCAATGCACAAAATGGA
PS5	Rev	5' TAGAAACCCGGGTCACGTAG
PS6	Fwd	5' GCCAATCTCAACACCCAAGT
PS6	Rev	5' GGGTTCTAAAGGAGGGAAAATC