**SUPPLEMENTAL INFORMATION**

**SUPPLEMENTAL MATERIALS AND METHODS**

**Analysis of global changes in protein expression upon HLX1 knockdown.** To evaluate the changes in protein levels caused by knockdown of HLX1, we took advantage of SILAC, which allows for quantitative mass spectrometry comparisons between complex samples. To this end, IMR-90 cells at passage 12 were infected with pRS or pRS-shHLX1.2 retroviruses and one day after infection, cells were grown in cell culture media with 'Light' (L, pRS-shHLX1.2 infected cells) or 'Heavy' (H, pRS-infected cells) isotope containing amino acids. 3 days after switching to H or L medium, cells were selected with puromycin for 4 additional days and cell pellets collected for analysis 6 days after selection. Specifically, for the 'Heavy' sample, IMR-90 cells were grown in Heavy SILAC DMEM (Pierce), supplemented with 10 % dialyzed FBS (Biosera, East Sussex, UK), containing $^{13}C(6)^{15}N(2)$ labeled lysine and $^{13}C(6)^{15}N(4)$ labeled arginine (CK gas, Hampshire, UK). Cell pellets were lysed using RIPA buffer and protein extracts subjected to SDS-PAGE. In total, 19 gel pieces were subjected to overnight in-gel trypsin digestion. The peptide extracts were subjected to a second round of trypsin digestion to minimize the number of missed cleavages. Peptide mixtures were analyzed using an UltiMate 3000 Rapid Separation LC, coupled to a LTQ-Orbitrap-Velos mass spectrometer (ThermoFisher Scientific). Separation was performed in a 3 h gradient using a 50 cm Acclaim pepmap c18 column. Protein identification and quantification was performed using Maxquant v1.2.0.13, with the embedded Andromeda 1.2.0.0 search engine and the IPI_Human_v3.37 database. The initial minimum ratio H/L count was set to 1, but to generate the data presented in Fig 3A and Table S1, the stringency was increased to a minimum ratio H/L count of 3 and a minimum unique peptide count of 2 per protein group. Note that Fig 3A and Table S1 show the log of the intensity of the ratio between the shHLX1.2 cells and the control pRS-infected cells (therefore the L/H ratio).

**High Content Analysis.** High Content Analysis (HCA) was performed as described elsewhere (Banito et al, 2009; Barradas et al, 2009; Bishop et al, 2010). Briefly, cells were seeded in 96-

well plates (Nunc) and visualized by IF. Two fluorescence images corresponding to DAPI and primary antibody/Alexa Fluor 488-secondary antibody were acquired for each field using the IN Cell Analyzer 1000 automated epifluorescence microscope at 10x magnification (GE Healthcare). HCA was performed using the IN Cell Investigator software (v1.7; GE Healthcare). For the analysis, DAPI staining of the nuclei was used to identify cells. The nuclei were then segmented using top-hat segmentation, specifying a minimum nucleus area of 100 $\mu m^2$. To define the cell segmentation, a collar segmentation routine was used with a ratio of 1 µm. To determine the cellular expression of the analyzed protein, the average intensity of pixels in the reference channel (Alexa Fluor 488) within the specified nuclear region (Object Nuclear Intensity) was measured. Each cell was assigned a nuclear intensity value for the specific protein expression and that value was used to set up a threshold filter, which determined positive and negative expressing cells. The threshold filter used a histogram for data visualization. In order to set the filter cut-off, expression in control cells was measured to define the negative population, followed by the analysis of the positive control. Once the cut-off was set up, analysis of the experiment was carried out. As a result, the software classified each cell as either positive or negative for the expression of the analyzed protein. The mean of the nuclear intensity was also routinely analyzed and equivalent results were obtained. The antibodies used for the analysis were tested with adequate controls (shRNAs or siRNAs) to test their specificity and if possible, alternative antibodies were tested in control experiments to confirm the results obtained.

**Analysis of homeodomain DNA-binding similarity.** Correlations between Homeobox DNA binding profiles were performed, as described in (Berger et al, 2008). Assessment of the significance of correlation within the 7 genes that we identified in Fig 8A as able to prevent the induction of the *INK4a* promoter (DLX3, HOXA9, HOXB13, HOXC13, HOXD3 and HOXD8 in addition to HLX1) was performed by comparing the correlation within these 7 genes to that within 1,000,000 random Homeobox gene sets of equal size. Significance was therefore

calculated empirically, as the proportion of random gene sets having higher mean correlation than the set under investigation.

## SUPPLEMENTAL REFERENCES

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## SUPPLEMENTAL TABLES

**Supplemental Table S1. Global changes in protein levels upon HLX1 knockdown analyzed by SILAC.**

This table is included as an independent Excel file (SupplemetalTableS1.xlsx).

**Supplemental Table S2. Details of siRNAs used in this study.**

This table is included in this document (page 11).

**Supplemental Table S3. Details of oligonucleotide primers and TaqMan probes used in this study.**

This table is included in this document (page 12).

**Supplemental Table S4. Oligonucleotides used for DNA precipitation assays.**

This table is included in this document (page 13).

**SUPPLEMENTAL FIGURE LEGENDS**

**Sup Fig S1. A screen for transcription regulators extending cellular lifespan identifies the Homeobox protein HLX1.**

(A, B) We cloned the indicated genes (HEY1, MSX2, NAB2, HLX1, SPDEF, TGIF and TSC22D4) into retroviral vectors and infected IMR-90 cells (passage 17) with either these vectors or appropriate controls (vector, hTERT or shp53). IMR-90 cells were then subjected to serial passage and the effect on cell growth analyzed by colony formation assays and crystal violet staining (A) or by plotting growth curves (B). The colony formation assay shown in (A) corresponds to passage 24. (C) WI-38 human fibroblasts were infected with the indicated retroviral vectors and growth curves were performed after selection.

**Sup Fig S2. Expression of point mutants of the HLX1 homeodomain causes premature senescence.**

(A) Cartoon depicting the domain structure of HLX1 (left). Domains were identified using Ensembl. Marked in color are a Glutamine-rich region (Gln), the Homeobox DNA-binding domain (homeodomain, Homeo) and a Serine-rich region (Ser). The two conserved residues in the homeodomain (A322 and T328) targeted to generate the mutants are highlighted. HLX1 wt and the homeodomain point mutants (A322T and T328M) are expressed to comparable levels, as shown by IB (right). (B) IMR-90 cells (passage 17) were infected with retroviral vectors expressing HLX1 wt, A322T, T328M or the indicated controls and growth curves were performed after selection. (C, D). At the time point indicated by an arrow in panel B, cells were seeded to perform $p16^{INK4a}$ IF. The percentage of $p16^{INK4a}$ positive cells is shown in (C) and representative images are shown in (D). Analysis was performed at passage 24.

**Sup Fig S3. Knockdown of HLX1 using retroviral or lentiviral-mediated shRNA delivery.**

(A) Cartoon depicting the human HLX1 mRNA (accession number NM_021958.3), summarizing the positions targeted by the different shRNAs and siRNAs used in this study. Sequences targeted by the 4 siRNAs and shRNAs are different. (B) IMR-90 cells were infected with a

lentiviral control vector (pLKO) or a lentiviral vector targeting HLX1 (pLKO-shHLX1.2). The efficiency of the knockdown achieved by the pLKO-shHLX1.2 vector in IMR-90 cells was assessed by IB (left) and qRT-PCR (second from left). The numbers under the blots correspond to quantification of HLX1 signal normalized to $\alpha$-tubulin. The effect of knocking down HLX1 on BrdU incorporation was measured using IF (second from right). HLX1 effect on INK4a transcription was monitored by qRT-PCR (right). (C) IMR-90 cells were infected with retroviral shRNA vectors targeting HLX1 or $p16^{INK4a}$ and subjected to $p16^{INK4a}$ IF after selection. Representative images (left) and a quantification of the percentage of $p16^{INK4a}$ positive cells (right) are presented (passage 14).

**Sup Fig S4. HLX1 knockdown by siRNA transfection causes growth arrest and induces $p16^{INK4a}$.**

(A) HPrEC, NHK or IMR-90 cells were transfected with the indicated siRNAs and 5 days later, the number of cells was counted in an equal number of fields using HCA software. (B, C) HPrEC, NHK or IMR-90 cells were transfected with the indicated siRNAs and 5 days later subjected to $p16^{INK4a}$ IF. Representative images are presented in (B) and the percentage of $p16^{INK4a}$ positive cells is presented in (C).

**Sup Fig S5. HLX1 expression represses $p16^{INK4a}$, but does not affect the levels of p53 or $p21^{CIP1}$ in IMR-90 cells.**

Representative images of DAPI (top) and either p53 (A), $p21^{CIP1}$ (B) or $p16^{INK4a}$ (C) IF (bottom) in IMR-90 cells infected with the indicated vectors. The images correspond to the data presented in Fig 3C.

**Sup Fig S6. E7 rescues the growth arrest caused by HLX1 depletion.**

IMR-90 cells were infected with a control retrovirus (vector) or a retrovirus expressing the oncoviral protein E7, which targets RB and other pocket proteins for degradation. IF against RB was conducted (left) and quantification shows the effect of E7 expression over RB levels (centre). Expression of E7 significantly prevents the arrest caused by HLX1 depletion as measured by BrdU incorporation (right).

**Sup Fig S7. HLX1 regulates PRC target genes.**

(A, B) GSEA of gene sets associated with HDAC targets (Senese et al, 2007) and PRC2 targets (Ben-Porath et al, 2008) are found enriched in transcriptional profiles of IMR-90 cells in which HLX1 expression has been knocked down. (C, D) Additional gene sets extracted from the Ben Porath et al. and Bracken et al. studies (Ben-Porath et al, 2008; Bracken et al, 2006) are enriched in transcriptional profiles of IMR-90 cells in which HLX1 expression has been knocked down. (E) GSEA of gene sets associated with HDAC targets (Senese et al, 2007) and EZH2 targets (Nuytten et al, 2008) were found enriched in transcriptional profiles of URE mouse leukemic cells in which HLX1 expression was knocked down (Kawahara et al, 2012). (F) Enrichment for PRC2 targets gene sets in transcriptional profiles of IMR-90 cells in which CBX7 expression has been knocked down. NES, normalized enrichment score; FDR, false discovery rate.

**Sup Fig S8. *In situ* PLA shows that HLX1 is associated with HDAC1 and the PRC2 complex.**

(A) *In situ* PLA in HEK 293T cells transfected with HLX1 and FLAG-SUZ12 or HLX1 and FLAG-HDAC1. Antibodies against FLAG and HLX1 were used to detect the protein complexes. (B) *In situ* PLA in IMR-90 cells infected with FLAG-HLX1. Antibodies against FLAG and rabbit antibodies recognizing HDAC1, SUZ12 or RING1A were used to detect the protein complexes. (C) *In situ* PLA in IMR-90 cells infected with the indicated lentiviruses. Antibodies against HDAC1 and HLX1 were used to detect the protein complexes. Representative images (left) and a quantification of the percentage of cells displaying an interaction (right) are shown. Cells presenting 10 or more nuclear spots were scored as positive. F, FLAG; H, HLX1; F+H, FLAG and HLX1. Blue: DAPI; Red: PLA signal.

**Sup Fig S9. HLX1 does not immunoprecipitate with PRC1 components.**

HEK 293T cells were transfected with plasmids expressing HLX1 and a FLAG-tagged version of BMI1, RING1A or HPH2 as indicated. Total lysates or FLAG immunoprecipitates were prepared and immunoblots conducted using antibodies recognizing HLX1 or the FLAG epitope. Specific

bands are indicated with an arrow, while non-specific bands are denoted by *. WB, western blot; IP, immunoprecipitation. These experiments were performed simultaneously with the experiments presented in Fig 6B-C which show an interaction between HLX1 and PRC2 components and between HLX1 and HDAC1.

**Sup Fig S10. SUZ12 and HDAC1 mediate the regulation of $p16^{INK4a}$ by HLX1.**

(A, B) IF showing the effects of siRNAs targeting SUZ12 and HDAC1 corresponding to those used in the experiment presented in Fig 7A-C. (C) Representative $p16^{INK4a}$ IF pictures corresponding to the data presented in Fig 7C.

**Sup Fig S11. CBX7 depletion results in reduced PRC occupancy of the *INK4a* promoter.**

(A) IMR-90 cells (passage 12-13) were infected with a control lentiviral vector (pLKO) or a vector knocking down CBX7 (pLKO-shCBX7.5) and CBX7 and INK4a mRNA levels were analyzed by qRT-PCR. (B) ChIP analysis showing the effect of CBX7 depletion on occupancy of the *INK4a* promoter (primer set 6). Occupancy of the *INK4a* promoter by CBX7, RING1B, SUZ12 and levels of H3K27me3 were analyzed at passage 14-15.

**Sup Fig S12. $HLX1^{T328M}$ mutant fails to recruit PRC to the *INK4a* locus.**

(A, B) The mutant and wt versions of HLX1 co-immunoprecipitate with SUZ12 (A) and HDAC1 (B). HEK 293T cells were transfected with plasmids expressing HLX1 and a FLAG-tagged version of SUZ12 (A) or HDAC1 (B) as indicated. Total lysates or FLAG immunoprecipitates were prepared and immunoblots conducted using antibodies recognizing HLX1 or the FLAG epitope. Specific bands are noted by an arrow and IgG bands by an asterisk. WB, western blot; IP, immunoprecipitation. (C) DNA pulldown assays showing the reduced binding of HLX1 T328M to a probe corresponding to the ~ -550 position. (D, E) IMR-90 cells were infected at passage 17 with an empty vector (vector) or retroviruses expressing HLX1 wt ot HLX1 T328M. Their effect over $p16^{INK4a}$ expression was measured by qRT-PCR (D). (E) ChIP analysis showing the effect of HLX1 wt and HLX1 T328M on occupancy of the *INK4a* promoter (primer

set 6). The occupancy of the locus by HLX1, RING1B, SUZ12 and levels of H3K27me3 was analyzed at passage 24.

**Sup Fig S13. Knockdown of selected homeodomain-containing proteins in HPrEC.**

(A) HPrEC were transfected with the indicated siRNAs and 5 days later, cells were collected, RNA prepared and the levels of the specific target genes (listed at the top of the graph) measured by qRT-PCR. The graph shows the relative mRNA expression compared with scrambled siRNA transfected cells. (B) HPrEC were transfected with the indicated siRNAs and cell numbers determined 5 days later using HCA software. As a reference, a gray background represents the cell number in HPrEC transfected with scrambled control siRNAs. (C) Plot denoting the correlation between $10^6$ random combinations of 7 homeodomain-containing proteins, as described in (Berger et al, 2008). A red line marks the correlation between the 7 homeodomain-containing proteins that we identified as $p16^{INK4a}$ regulators (HLX1, DLX3, HOXA9, HOXB13, HOXC13, HOXD3 and HOXD8). Correlation was 0.82, p= 0.038.

**Sup Fig S14. HOXA9 expression regulates senescence via PRC2- and HDAC1-dependent INK4a repression.**

(A) HOXA9 expression in IMR-90 cells extends cellular lifespan. Crystal violet staining of IMR-90 cells infected with a control vector or expressing HOXA9. (B) *In situ* PLA in IMR-90 cells infected with FLAG-HOXA9. Antibodies against FLAG and the indicated proteins were used to detect the protein complexes. Cells presenting 10 or more nuclear spots were scored as positive. The percentage of cells displaying an interaction is shown. (C) HEK 293T cells were trasnsfected with a vector expressing a FLAG-tagged version of HOXA9, as indicated. Total lysates, FLAG or HA immunoprecipitates were prepared and immunoblots conducted using FLAG or HDAC1 antibodies. WB, western blot; IP, immunoprecipitation.

**Sup Fig S15. Relation between HLX1 and HOXA9 on $p16^{INK4a}$ regulation.**

(A) IMR-90 cells were infected at passage 15 with a control retrovirus (vector) or retroviruses expressing HOXA9 (HOXA9). The effect of knocking down HLX1 expression over $p16^{INK4a}$ levels was measured using quantitative IF at passage 24-27 (left). IMR-90 cells were infected with a

control retrovirus (vector) or retroviruses expressing HLX1 (HLX1). The effect of knocking down HOXA9 expression over $p16^{INK4a}$ levels was measured using quantitative IF (right). Average of the nuclear intensity of $p16^{INK4a}$ is shown. a.u, arbitrary units. (B, C) HLX1 interacts with HOXA9. (B) HEK 293T cells were transfected with plasmids expressing HLX1 and a FLAG-tagged version of HOXA9. Total lysates or FLAG immunoprecipitates were prepared and immunoblots conducted using antibodies recognizing HLX1 or the FLAG epitope. WB, western blot; IP, immunoprecipitation. (C) *In situ* PLA in IMR-90 cells. Antibodies against HOXA9 and the indicated proteins were used to detect the protein complexes. Cells presenting 10 or more nuclear spots were scored as positive. The percentage of cells displaying an interaction is shown.

**Sup Fig S16. Expression of HLX1 and other Homeobox genes tested does not decrease during senescence.**

(A) qRT-PCR analysis showing the expression of *HLX1* and *INK4a* during serial passage of IMR-90 (replicative senescence) and upon treatment with 100 nM 4OHT of IMR-90 ER:RAS cells (OIS). (B) The expression of the six Homeobox genes tested does not change or increases during replicative senescence. (C) qRT-PCR analysis of the levels of PRC2 components (EZH2, EED and SUZ12) during replicative senescence and OIS.

**Supplemental Table S2. Details of siRNAs used in this study.**

| Targeted gene | siRNA | Catalogue no. | Target sequence |
|---|---|---|---|
| PPIB | siGLO | D-001630-02-05 | GGAAAGACTGTTCCAAAAA |
| - | AllStars | SI03650318 | *not provided* |
| GFP | Ctrl_GFP_2 | SI04380467 | AAGCAGCACGACTTCTTCAAG |
| CBX7 | Hs_CBX7_7 | SI04155312 | CGCCGTGGAGAGCATCCGGAA |
| INK4a | Hs_CDKN2A_15 | SI02664403 | TACCGTAAATGTCCATTTATA |
| SUZ12 | Hs_SUZ12_5 | SI03132885 | AAGGATGTAAGTTGTCCAATA |
| | Hs_SUZ12_6 | SI03246817 | TAGCATAATGTCAATAGATAA |
| | Hs_SUZ12_7 | SI04156537 | AAGGATATTTGTATCAGGGTT |
| HDAC1 | Hs_HDAC1_3 | SI00070623 | CCACAGCGATGACTACATTAA |
| | Hs_HDAC1_4 | SI00070630 | CGGGATTGATGACGAGTCCTA |
| | Hs_HDAC1_6 | SI02663472 | CACCCGGAGGAAAGTCTGTTA |
| DLX3 | Hs_DLX3_6 | SI03073063 | CAGTGATTCCATGGCCTGCAA |
| | Hs_DLX3_7 | SI03090745 | CTCCTGGTATCACGCACAGAA |
| HLX1 | Hs_HLX_2 | SI00439299 | AAGGTTTGAGATTCAGAAGTA |
| | Hs_HLX_5 | SI03228169 | TAGGACGCCTTAAATTTGTAA |
| HOXA9 | Hs_HOXA9_6 | SI04239165 | CTACCTAGGGTTTATGCTTAA |
| | Hs_HOXA9_8 | SI04346370 | CCCATCGATCCCAATAACCCA |
| HOXB13 | Hs_HOXB13_5 | SI04133304 | TCCGGGATATCCGGGAACCTA |
| | Hs_HOXB13_7 | SI04367902 | CTCCATGGAGCCCGGCAATTA |
| HOXC13 | Hs_HOXC13_4 | SI00117971 | CAGGAGCGAGGTGTCTCCCTA |
| | Hs_HOXC13_5 | SI03098011 | CTGGCCGGAGAGCCTTATGTA |
| HOXD3 | Hs_HOXD3_6 | SI04209520 | CTCGCCATAAATCAGCCGCAA |
| | Hs_HOXD3_8 | SI04315318 | AAGAGTCTCGACAGAACTCCA |
| HOXD8 | Hs_HOXD8_5 | SI04258709 | AGCCGAAGGCCTGACAAATTA |
| | Hs_HOXD8_6 | SI04286387 | AGAGGCCGAGCTGGTACAATA |

**Supplemental Table S3. Details of oligonucleotide primers and TaqMan probes used in this study.**

**Primers used for qRT-PCR**

| Target | Forward primer | Reverse primer |
|---|---|---|
| Human ANKRD1 | GGCCACTATGAGTGCGCGGAG | TCCATCGGCGTCTTCCCAGC |
| Human AREG | GCGAAGGACCAATGAGAGCCCC | TGGTCCCCAGAAAATGGTTCACGC |
| Human BMP2 | GGACATTCGGTCCTTGCGCCA | GGTCGACCTTTAGGAGACCGC |
| Human CLDN1 | GGCTGTCATTGGGGGTGCGATA | GGCAGAGAGAAGCAGCAGCCC |
| Human CTSS | AGTTCCCTGAGAGTTCCCAGCC | GGGCCCCCACAGCACTGAAAG |
| Human CYP1B1 | ACTGACATCTTCGGCGCCAGC | CAAGGCAGACGGTCCCTCCC |
| Human FER1L6 | GTGGTTCATGAGCCCCTTTA | CTATGAGCCCACAACGATCC |
| Human GDF15 | GGATACTCACGCCAGAAGTGC | AGGGCGGCCCGAGAGATACG |
| Human IL8 | GAGTGGACCACACTGCGCCA | TCCACAACCCTCTGCACCCAG |
| Human INK4A | CGGTCGGAGGCCGATCCAG | GCGCCGTGGAGCAGCAGCAGCT |
| Human KYNU | TGGGAGCCAAACACTCCATTGGG | CGCAATGCGCTGCACTGTGT |
| Human PAPPA | CTGGCTGAACCCCACACGGG | GGAGGTGCAGCAATCCCCACC |
| Human PRDMI | GGTACACACGGGAGAAAAGC | GCAGTTTCAGGTGCACAAAC |
| Human RPS14 | TCACCGCCCTACACATCAAACT | CTGCGAGTGCTGTCAGAGG |
| Human SSP1 | GGACATCACCTCACACATGG | GTGGGTTTCAGCACTCTGGT |
| Human UIMC1 | GCTTCTCCAGATGCCGGGGCT | CACATCCTTCTTCTCCAGGTTCCGA |

**TaqMan probes used for qRT-PCR**

| Target | Probe |
|---|---|
| Human GAPDH | 4333764F |
| Human TBP | 4333769F |
| Human CBX7 | Hs00545603_m1 |
| Human DLX3 | Hs00270938_m1 |
| Human EZH2 | Hs01016789_m1 |
| Human EED | Hs00537777_m1 |
| Human HLX1 | Hs00172035_m1 |
| Human HOXA9 | Hs00365956_m1 |
| Human HOXB13 | Hs00197189_m1 |
| Human HOXC13 | Hs00600868_m1 |
| Human HOXD3 | Hs00232506_m1 |
| Human HOXD8 | Hs00251905_m1 |
| Human SUZ12 | Hs00248742_m1 |

**Primers used for ChIP**

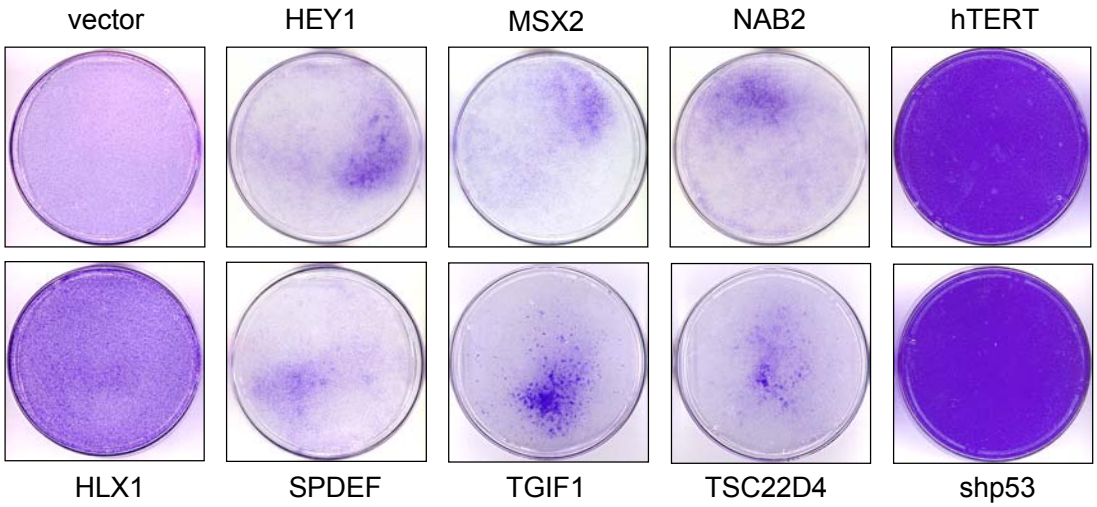
| Primers | Relative Location | Forward primer | Reverse primer |
|---|---|---|---|
| PS1 | *INK4b*, exon 1 | GGAACCTAGATCGCCGATGTAG | TGTTTTACGCGTGGAATGCAC |
| PS2 | *ARF*, exon 1 | GTGGGTCCCAGTCTGCAGTTA | CCTTTGGCACCAGAGGTGAG |
| PS3 | 4.5 kb downstream *ARF* start site | GGAGCGATGTGATCCGTTATC | TGAAATCCCAATCGTCTTCCAC |
| PS4 | 15 kb downstream *ARF* start site | GCACTTGCCCTTCCAGGTATA | TGATAGTTCAAGGCCCTATGCC |
| PS5 | 1 kb upstream *INK4a* promoter | CTCAAAGCGGATAATTCAAGAGC | AAGCCTTAAGAACAGTGCCACAC |
| PS6 | 0.2 kb upstream *INK4a* promoter | ACCCCGATTCAATTTGGCAG | AAAAAGAAATCCGCCCCCG |
| PS7 | 3' end *INK4a*, exon 1 | AGAGGGTCTGCAGCGG | TCGAAGCGCTACCTGATTCC |
| PS8 | 0.2 kb downstream *INK4a*, exon 1 | GCCAAGGAAGAGGAATGAGGAG | CCTTCAGATCTTCTCAGCATTCG |
| PS12 | 9.0 kb downstream *INK4a*, exon 1 | ACAGGGCGGCTCAAACAATAG | ATGTAAGGACACAGTGGGAAGGGG |
| *CCND1* | 0.1 kb downstream *CCND1* promoter | CACGGACTACAGGGGAGTTTTG | TTTCCACTTCGCAGCACAGGAG |

**Supplemental Table S4. Oligonucleotides used for DNA pulldown assays.**

| Probe | Forward oligonucleotide | Reverse oligonucleotide |
|---|---|---|
| A | GGTATTAGCTTAGGATGTGTGGCACTGTTCTTAAGGCTTA | TAAGCCTTAAGAACAGTGCCACACATCCTAAGCTAATACC |
| B | GCACTGTTCTTAAGGCTTATATGT**ATTA**ATACATCATTTA | TAAATGATGTATTAATACATATAAGCCTTAAGAACAGTGC |
| B.m1 | GCACTGTTCTTAAGGCGGGGGTGT**ATTA**ATACATCATTTA | TAAATGATGTATTAATACACCCCCGCCTTAAGAACAGTGC |
| B.m2 | GCACTGTTCTTAAGGCTTATATGTATGGGGGCATCATTTA | TAAATGATGCCCCCATACATATAAGCCTTAAGAACAGTGC |
| C | TATGT**ATTA**ATACATCATTTAAACTCACAACAACCCCTAT | ATAGGGGTTGTTGTGAGTTTAAATGATGTATTAATACATA |
| C.m | TATGTATGGGGGCATCATTTAAACTCACAACAACCCCTAT | ATAGGGGTTGTTGTGAGTTTAAATGATGCCCCCATACATA |
| D | AACTCACAACAACCCCTATAAAGCAGGGGGCACTCATATT | AATATGAGTGCCCCCTGCTTTATAGGGGTTGTTGTGAGTT |
| E | AGTCCGTCCTTCCAATGACTCCCTCCCCATTTTCCTATCT | AGATAGGAAAATGGGGAGGGAGTCATTGGAAGGACGGACT |
| F | CCCTCCCCATTTTCCTATCTGCCTACAGGCAGAATTCTCC | GGAGAATTCTGCCTGTAGGCAGATAGGAAAATGGGGAGGG |
| G | GCCTACAGGCAGAATTCTCCCCCGTCCGT**ATTA**AATAAAC | GTTTATTTAATACGGACGGGGGAGAATTCTGCCTGTAGGC |
| G.m | GCCTACAGGCAGAATTCTCCCCCGTCCGGGGGGAATAAAC | GTTTATTCCCCCCGGACGGGGGAGAATTCTGCCTGTAGGC |
| H | CCCGTCCGT**ATTA**AATAAACCTCATCTTTTCAGAGTCTGC | GCAGACTCTGAAAAGATGAGGTTTATTTAATACGGACGGG |
| H.m | CCCGTCCGGGGGGAATAAACCTCATCTTTTCAGAGTCTGC | GCAGACTCTGAAAAGATGAGGTTTATTCCCCCCGGACGGG |
| I | CTCATCTTTTCAGAGTCTGCTCTTATACCAGGCAATGTAC | GTACATTGCCTGGTATAAGAGCAGACTCTGAAAAGATGAG |

Underlined: mutated sequences
Bold: core ATTA Homeobox-binding motif on the forward oligo.

A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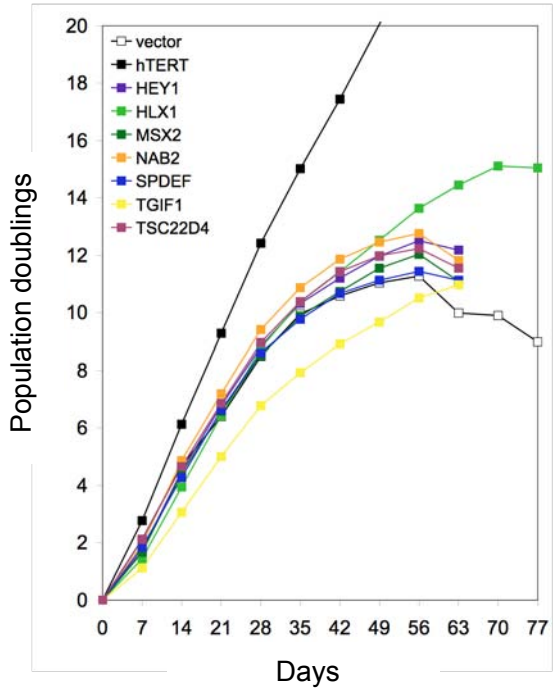
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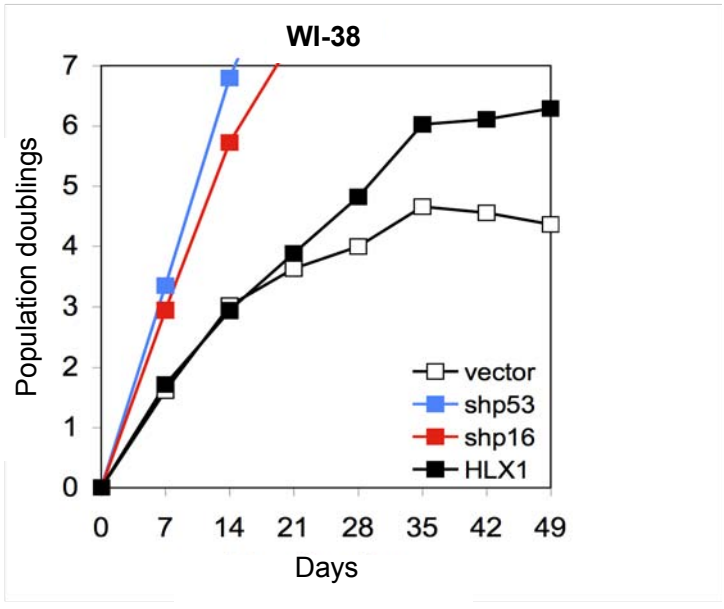
C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Population doublings
Days
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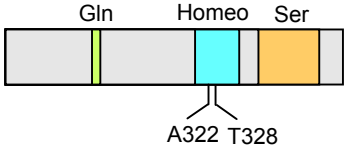
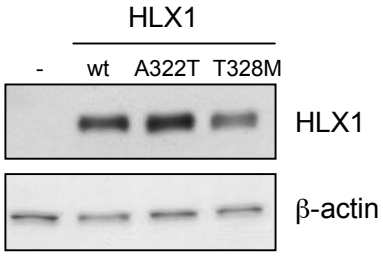
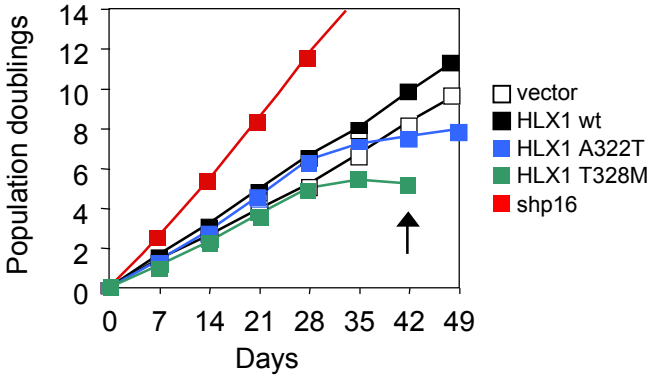
0 1 2 3 4 5 6 7

A

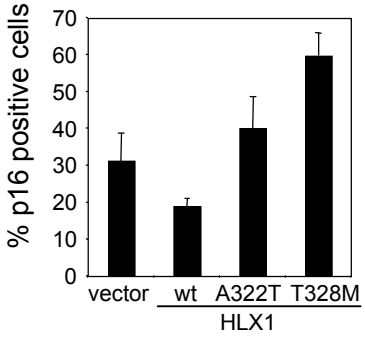


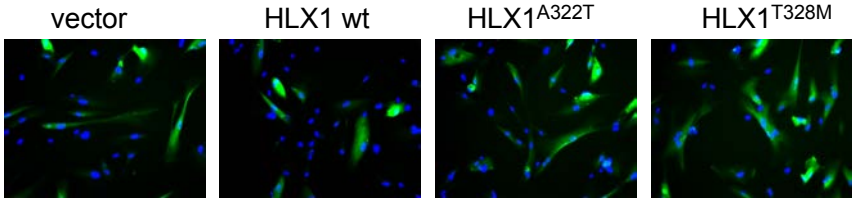

B



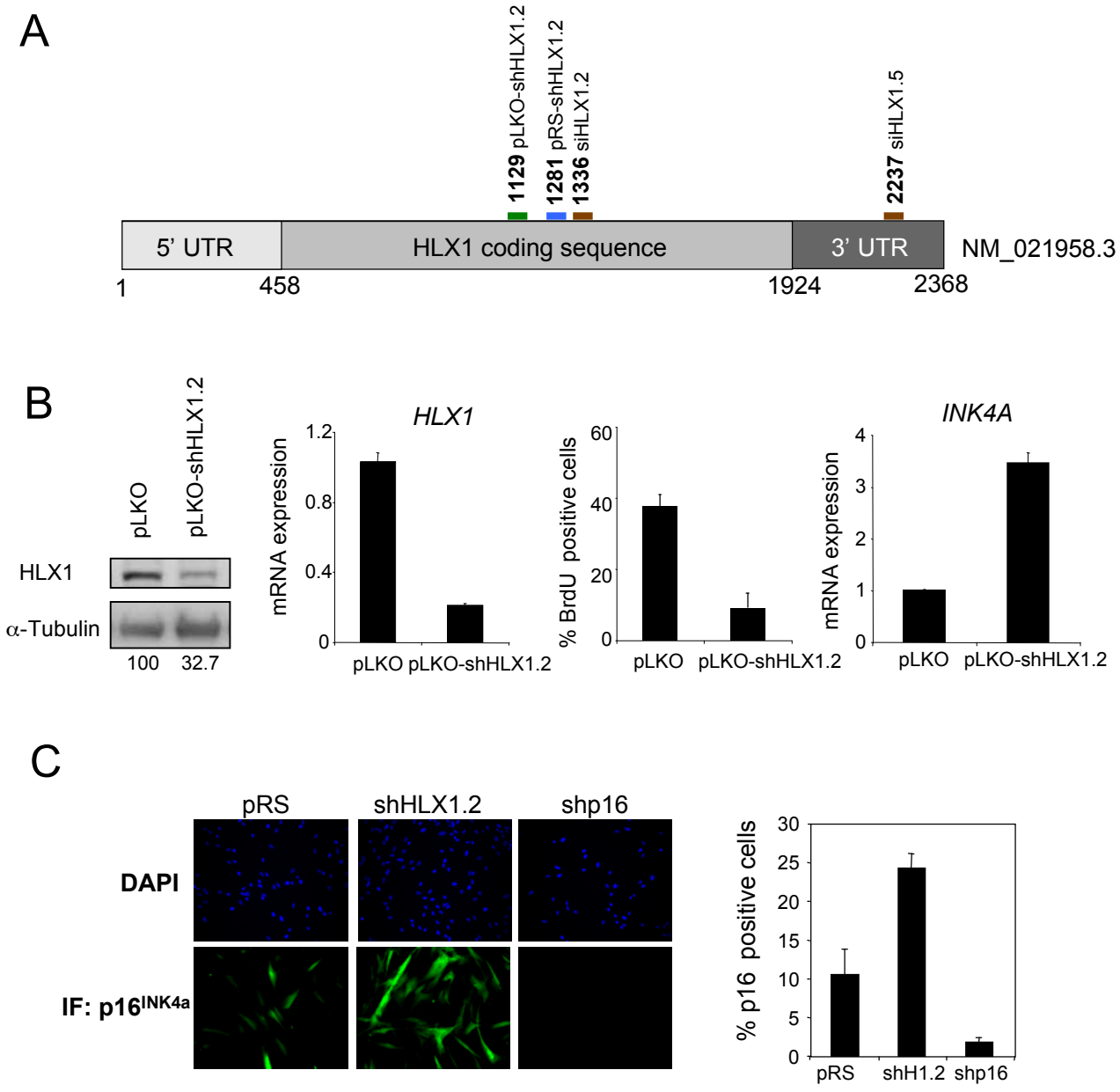

C



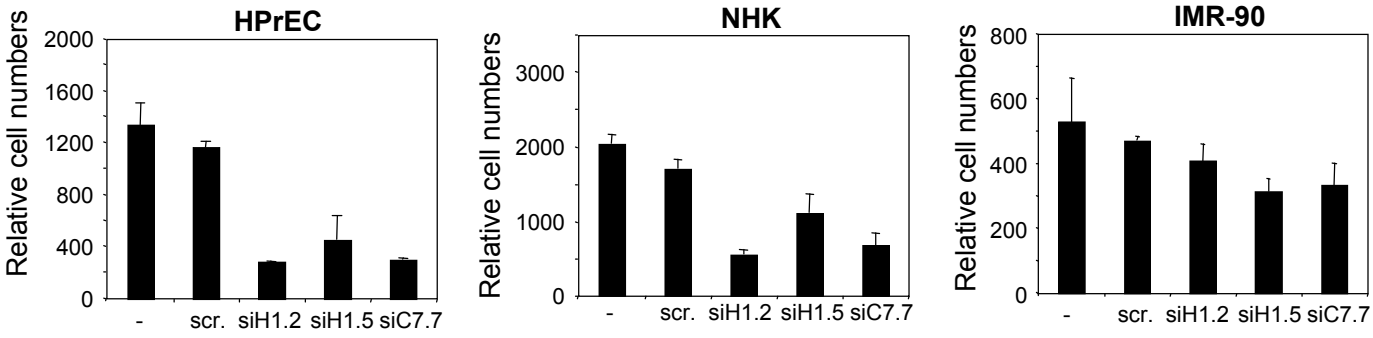

D



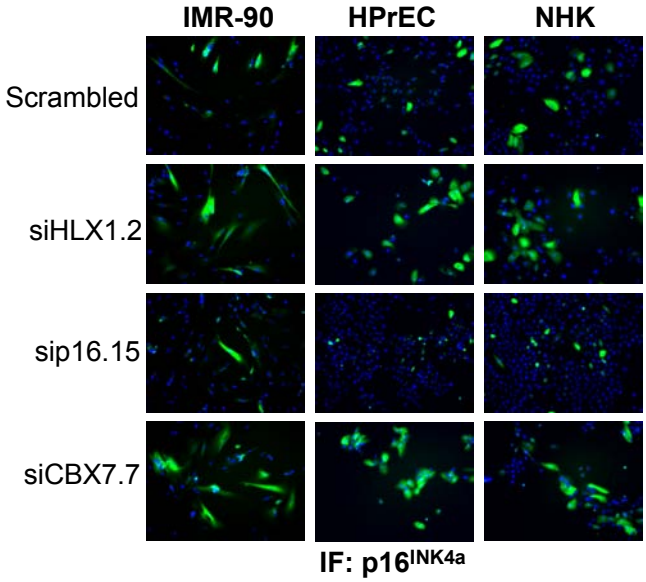

**IF: p16$^{INK4a}$**


A
1129 pLKO-shHLX1.2
1281 pRS-shHLX1.2
1336 siHLX1.2
2237 siHLX1.5
5' UTR
HLX1 coding sequence
3' UTR
NM_021958.3
1
458
1924
2368
B
pLKO
pLKO-shHLX1.2
HLX1
α-Tubulin
100
32.7
HLX1
mRNA expression
1.2
0.8
0.4
0
pLKO
pLKO-shHLX1.2
% BrdU positive cells
60
40
20
0
pLKO
pLKO-shHLX1.2
INK4A
mRNA expression
4
3
2
1
0
pLKO
pLKO-shHLX1.2
C
pRS
shHLX1.2
shp16
DAPI
IF: p16INK4a
% p16 positive cells
30
25
20
15
10
5
0
pRS
shH1.2
shp16

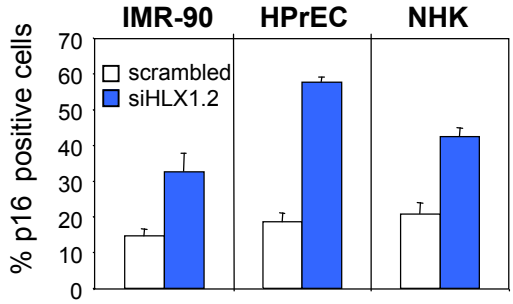
A


HPrEC
Relative cell numbers
2000
1600
1200
800
400
0
-
scr.
siH1.2
siH1.5
siC7.7
NHK
Relative cell numbers
3000
2000
1000
0
-
scr.
siH1.2
siH1.5
siC7.7
IMR-90
Relative cell numbers
800
600
400
200
0
-
scr.
siH1.2
siH1.5
siC7.7

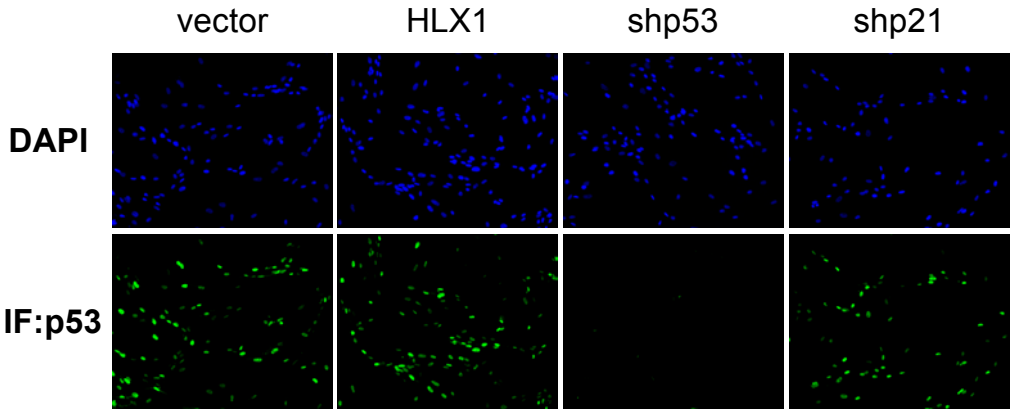
B


IMR-90
HPrEC
NHK
Scrambled
siHLX1.2
sip16.15
siCBX7.7
IF: p16INK4a

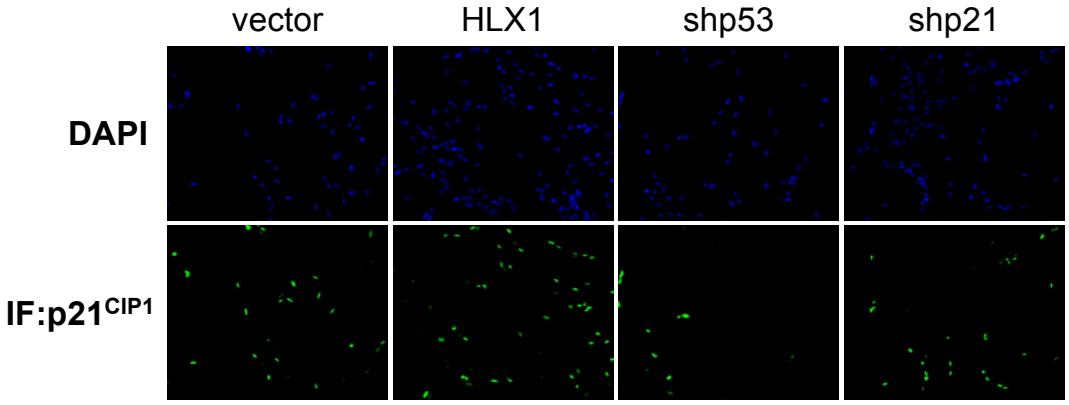
C


IMR-90
HPrEC
NHK
% p16 positive cells
70
60
50
40
30
20
10
0
scrambled
siHLX1.2

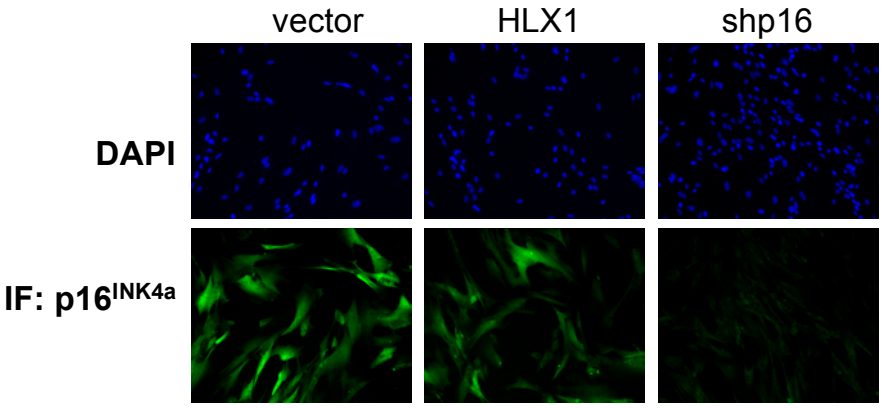
A

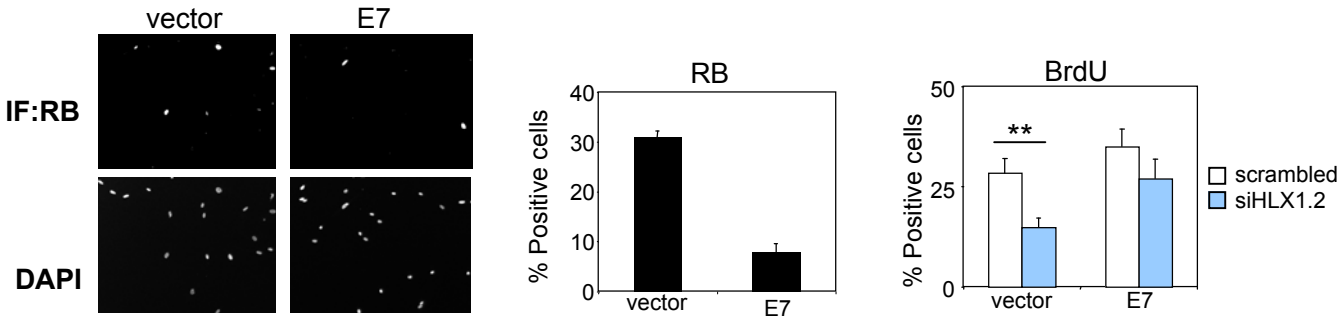

vector
HLX1
shp53
shp21
DAPI
IF:p53

B

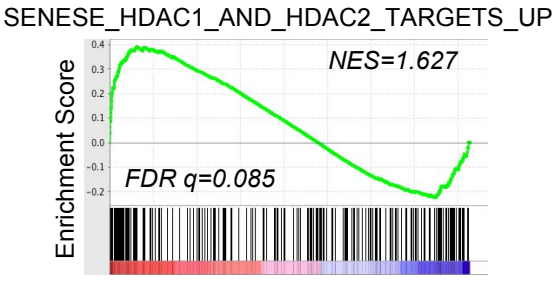

vector
HLX1
shp53
shp21
DAPI
IF:p21CIP1

C

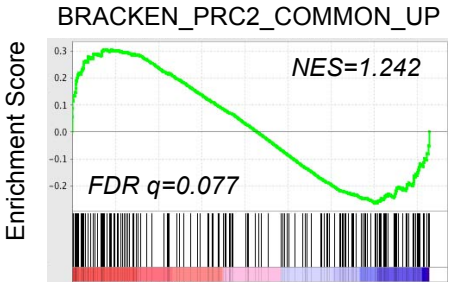

vector
HLX1
shp16
DAPI
IF: p16INK4a


vector
E7
IF:RB
DAPI
RB
% Positive cells
40
30
20
10
0
vector
E7
BrdU
% Positive cells
50
25
0
**
vector
E7
scrambled
siHLX1.2

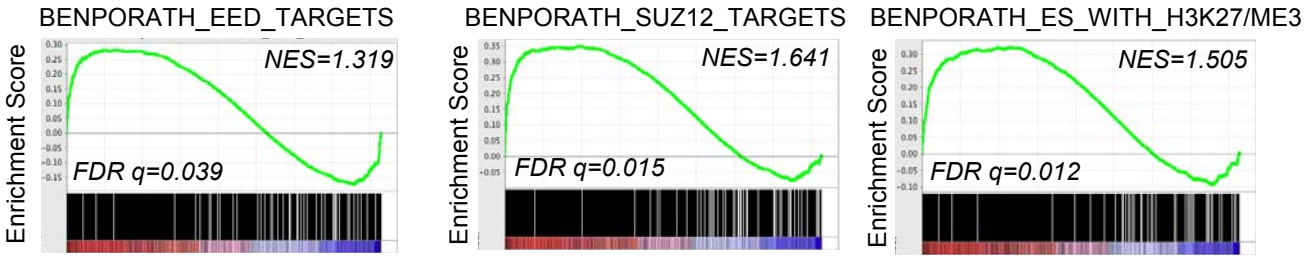
A


SENESE_HDAC1_AND_HDAC2_TARGETS_UP
Enrichment Score
NES=1.627
FDR q=0.085

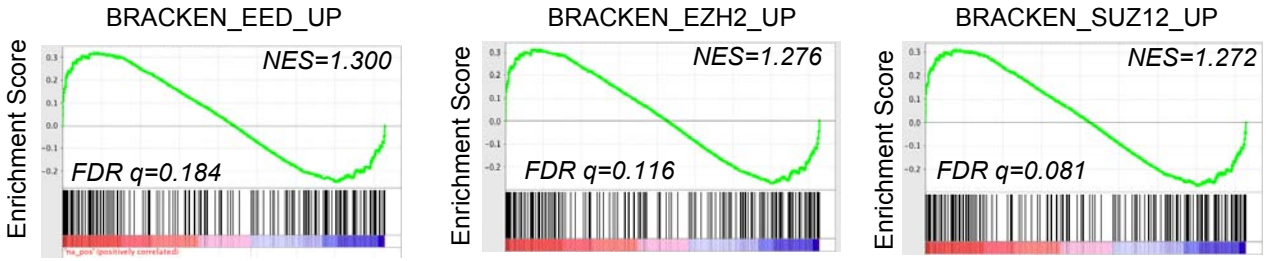
B


BRACKEN_PRC2_COMMON_UP
Enrichment Score
NES=1.242
FDR q=0.077

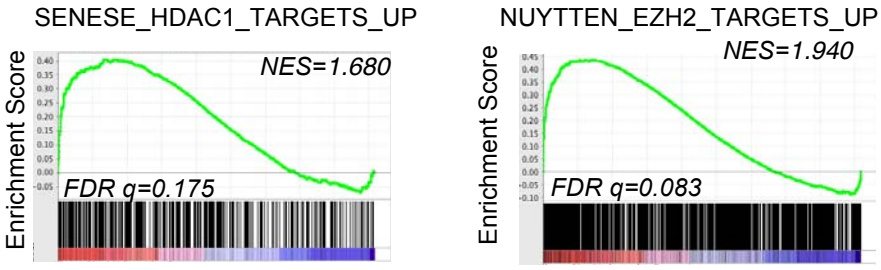
C


BENPORATH_EED_TARGETS
Enrichment Score
NES=1.319
FDR q=0.039
BENPORATH_SUZ12_TARGETS
Enrichment Score
NES=1.641
FDR q=0.015
BENPORATH_ES_WITH_H3K27/ME3
Enrichment Score
NES=1.505
FDR q=0.012

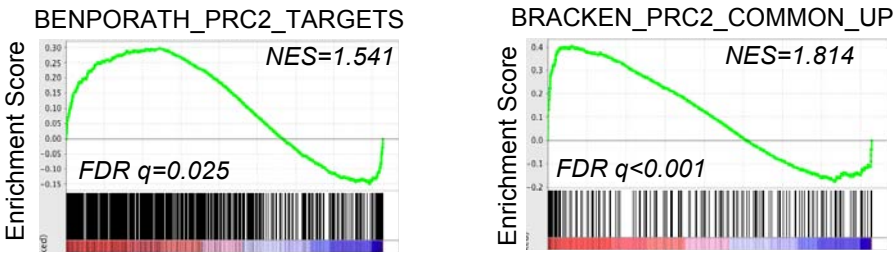
D


BRACKEN_EED_UP
Enrichment Score
NES=1.300
FDR q=0.184
BRACKEN_EZH2_UP
Enrichment Score
NES=1.276
FDR q=0.116
BRACKEN_SUZ12_UP
Enrichment Score
NES=1.272
FDR q=0.081

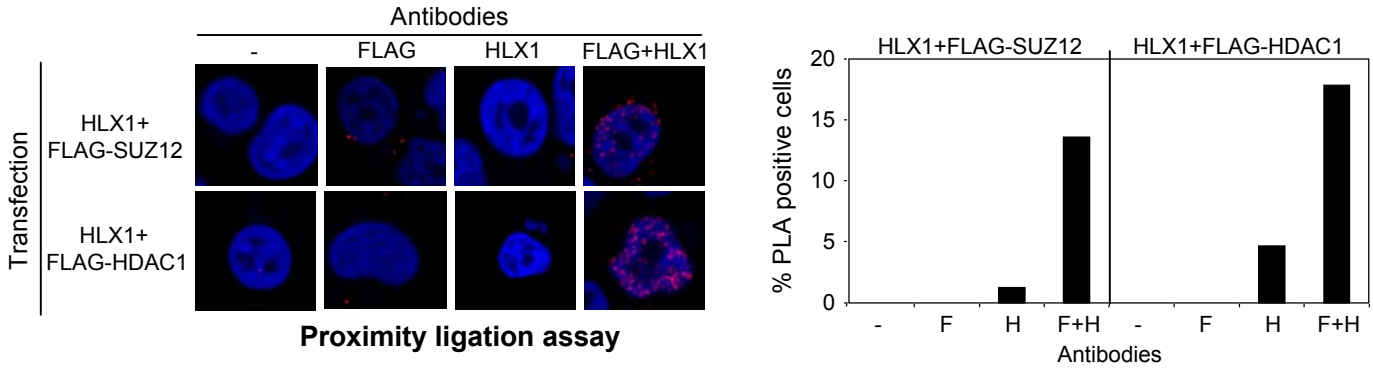
E


SENESE_HDAC1_TARGETS_UP
Enrichment Score
NES=1.680
FDR q=0.175
NUYTTEN_EZH2_TARGETS_UP
Enrichment Score
NES=1.940
FDR q=0.083

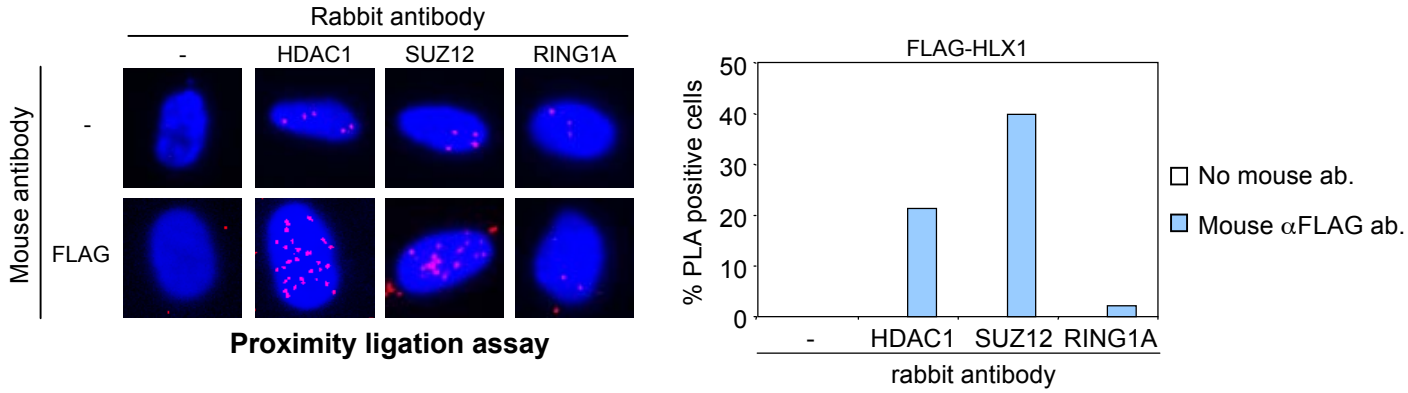
F


BENPORATH_PRC2_TARGETS
Enrichment Score
NES=1.541
FDR q=0.025
BRACKEN_PRC2_COMMON_UP
Enrichment Score
NES=1.814
FDR q<0.001

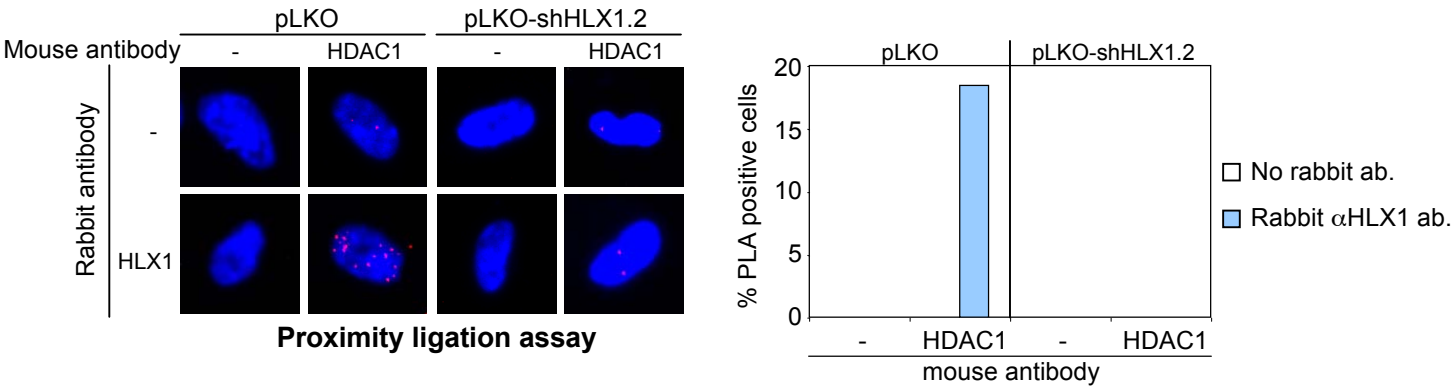
A

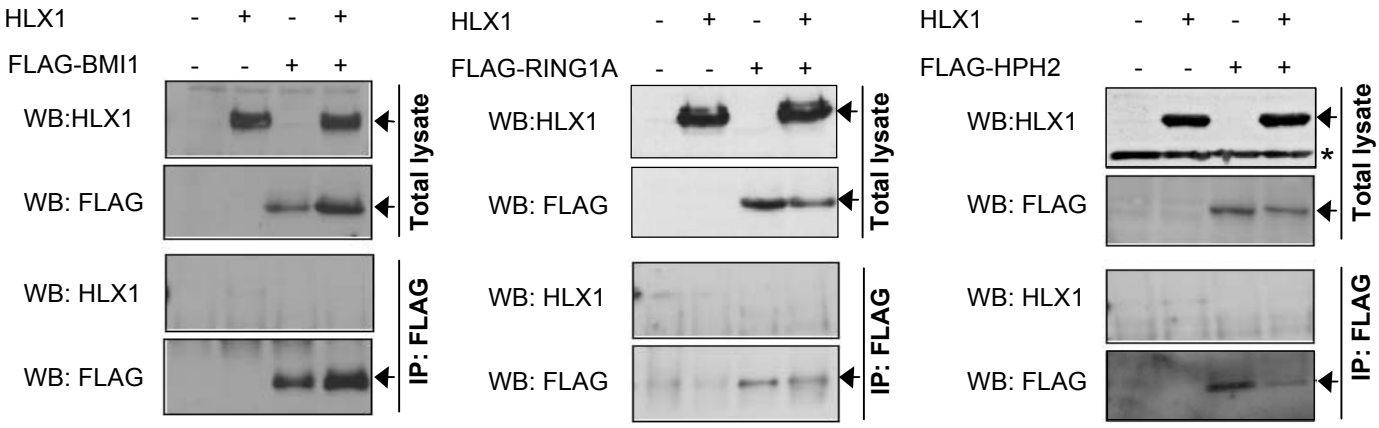

Antibodies
-
FLAG
HLX1
FLAG+HLX1
Transfection
HLX1+
FLAG-SUZ12
HLX1+
FLAG-HDAC1
Proximity ligation assay
HLX1+FLAG-SUZ12
HLX1+FLAG-HDAC1
% PLA positive cells
20
15
10
5
0
-
F
H
F+H
-
F
H
F+H
Antibodies

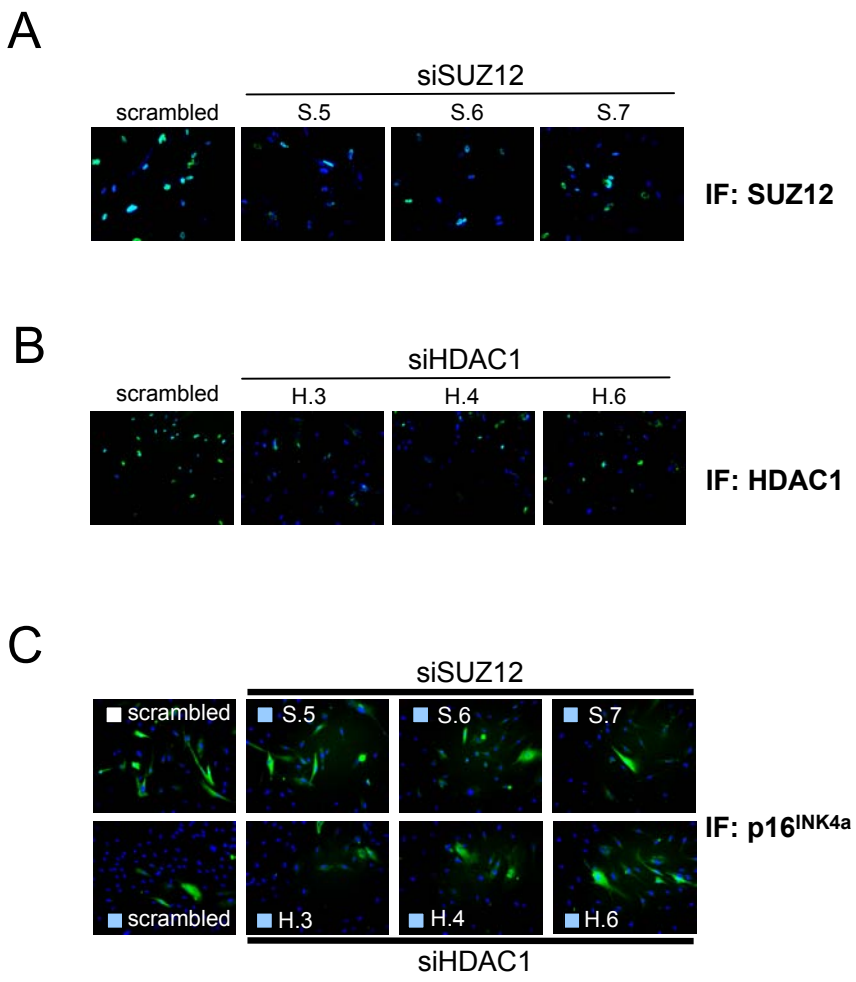
B

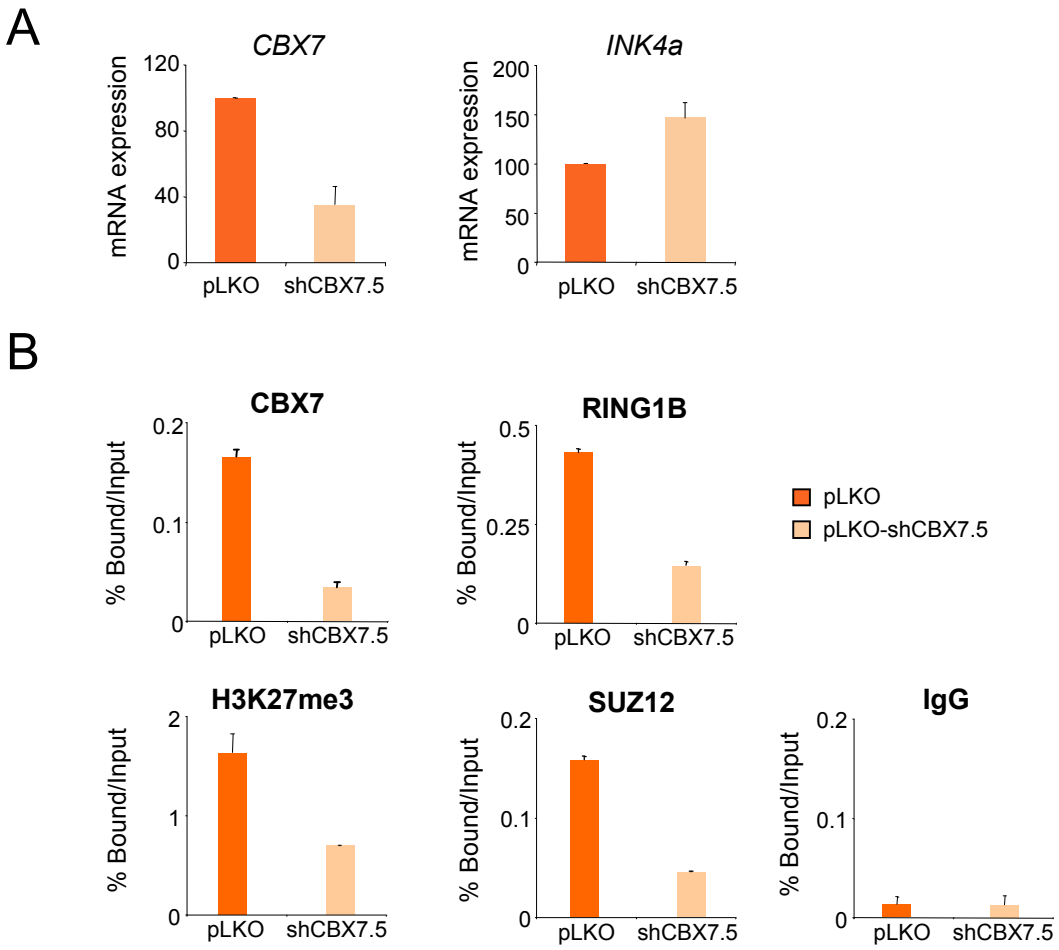

Rabbit antibody
-
HDAC1
SUZ12
RING1A
Mouse antibody
-
FLAG
Proximity ligation assay
FLAG-HLX1
% PLA positive cells
50
40
30
20
10
0
-
HDAC1
SUZ12
RING1A
rabbit antibody
No mouse ab.
Mouse αFLAG ab.

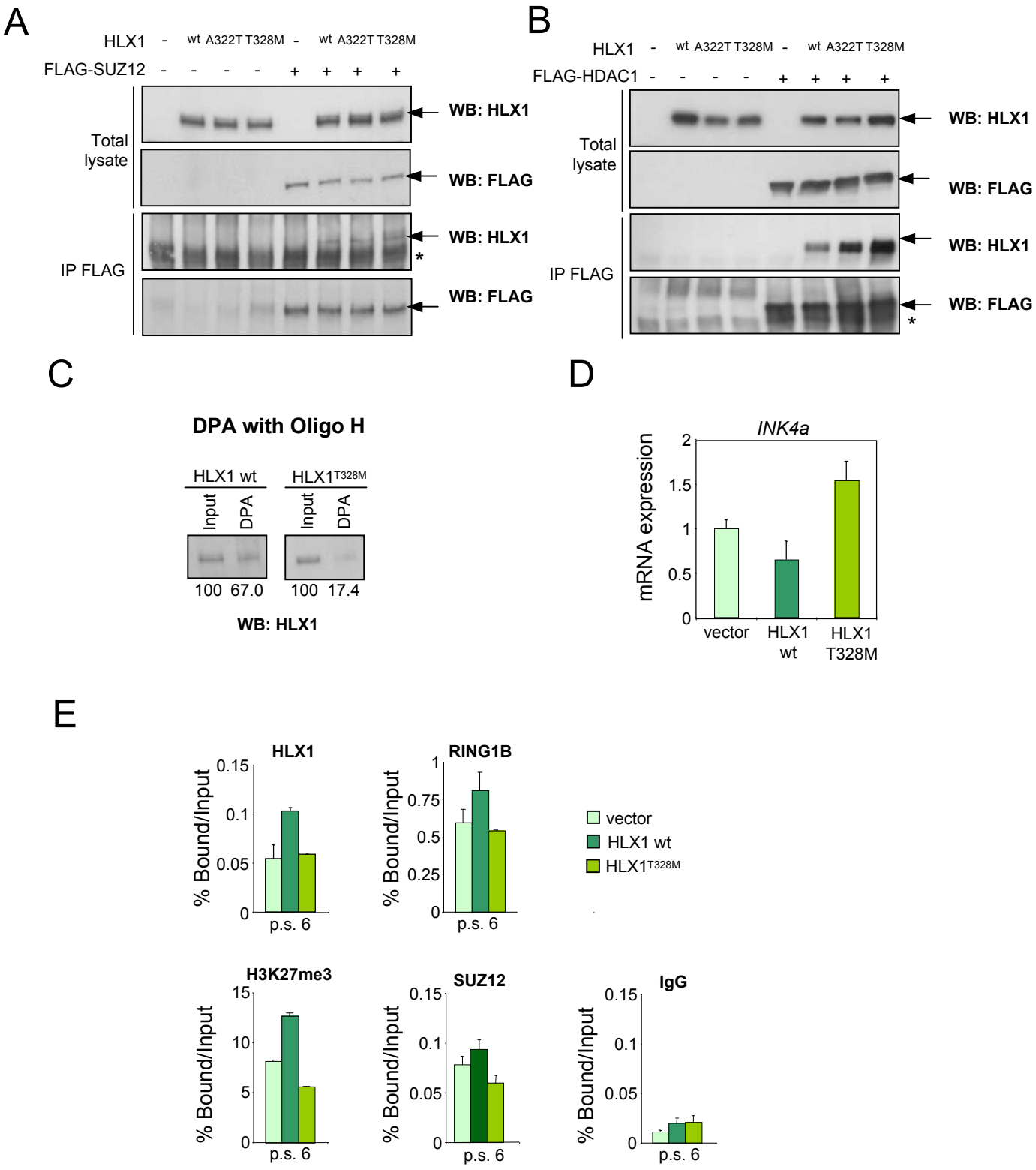
C


pLKO
pLKO-shHLX1.2
Mouse antibody
-
HDAC1
-
HDAC1
Rabbit antibody
-
HLX1
Proximity ligation assay
pLKO
pLKO-shHLX1.2
% PLA positive cells
20
15
10
5
0
-
HDAC1
-
HDAC1
mouse antibody
No rabbit ab.
Rabbit αHLX1 ab.

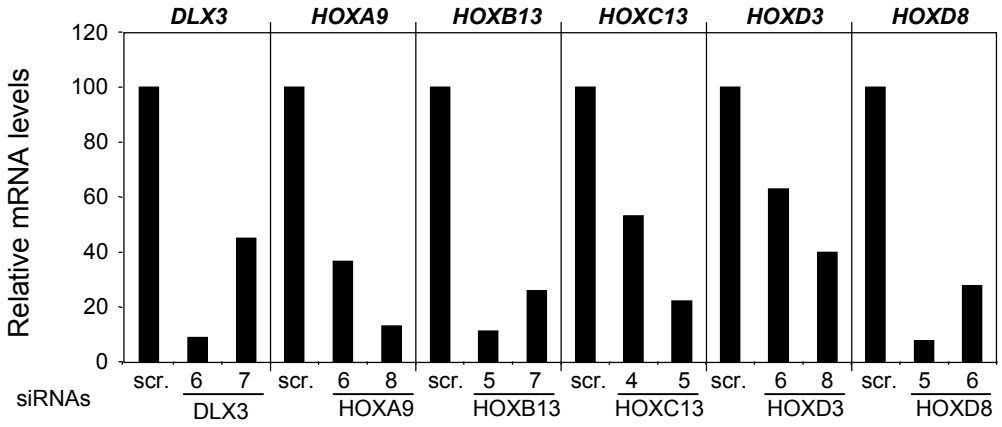

HLX1
- + - +
FLAG-BMI1
- - + +
WB:HLX1
WB: FLAG
Total lysate
WB: HLX1
WB: FLAG
IP: FLAG
HLX1
- + - +
FLAG-RING1A
- - + +
WB:HLX1
WB: FLAG
Total lysate
WB: HLX1
WB: FLAG
IP: FLAG
HLX1
- + - +
FLAG-HPH2
- - + +
WB:HLX1
*
WB: FLAG
Total lysate
WB: HLX1
WB: FLAG
IP: FLAG


A
siSUZ12
scrambled
S.5
S.6
S.7
IF: SUZ12
B
siHDAC1
scrambled
H.3
H.4
H.6
IF: HDAC1
C
siSUZ12
scrambled
S.5
S.6
S.7
scrambled
H.3
H.4
H.6
siHDAC1
IF: p16INK4a

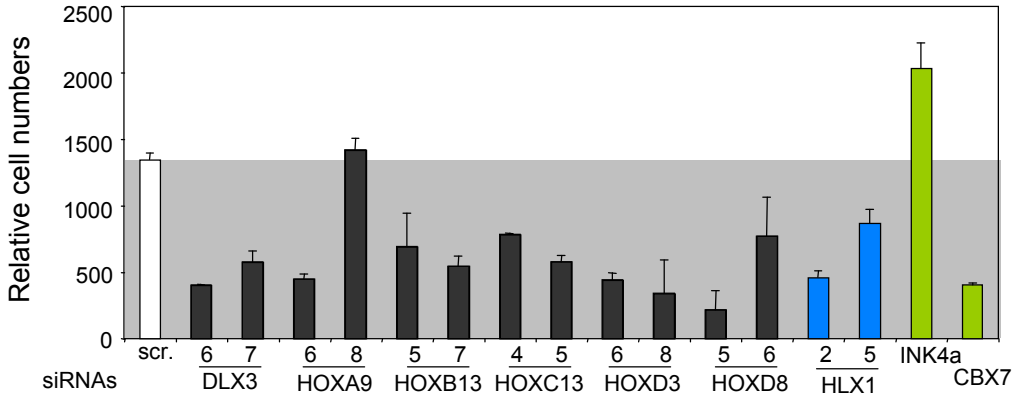

A
CBX7
mRNA expression
120
80
40
0
pLKO
shCBX7.5
INK4a
mRNA expression
200
150
100
50
0
pLKO
shCBX7.5
B
CBX7
% Bound/Input
0.2
0.1
0
pLKO
shCBX7.5
RING1B
% Bound/Input
0.5
0.25
0
pLKO
shCBX7.5
pLKO
pLKO-shCBX7.5
H3K27me3
% Bound/Input
2
1
0
pLKO
shCBX7.5
SUZ12
% Bound/Input
0.2
0.1
0
pLKO
shCBX7.5
IgG
% Bound/Input
0.2
0.1
0
pLKO
shCBX7.5


A
HLX1 - wt A322T T328M - wt A322T T328M
FLAG-SUZ12 - - - - + + + +
Total lysate
IP FLAG
WB: HLX1
WB: FLAG
WB: HLX1
WB: FLAG
*
B
HLX1 - wt A322T T328M - wt A322T T328M
FLAG-HDAC1 - - - - + + + +
Total lysate
IP FLAG
WB: HLX1
WB: FLAG
WB: HLX1
WB: FLAG
*
C
DPA with Oligo H
HLX1 wt
HLX1T328M
Input
DPA
Input
DPA
100 67.0
100 17.4
WB: HLX1
D
INK4a
mRNA expression
2
1.5
1
0.5
0
vector
HLX1 wt
HLX1 T328M
E
HLX1
RING1B
% Bound/Input
0.15
0.1
0.05
0
1
0.75
0.5
0.25
p.s. 6
vector
HLX1 wt
HLX1T328M
H3K27me3
SUZ12
IgG
15
10
5

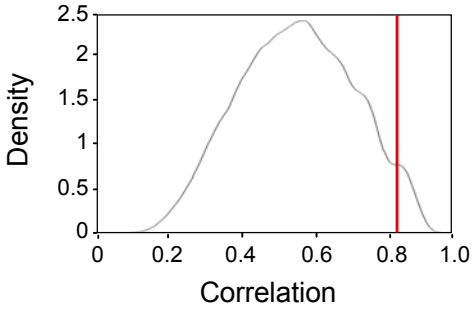
A


DLX3
HOXA9
HOXB13
HOXC13
HOXD3
HOXD8
Relative mRNA levels
120
100
80
60
40
20
0
siRNAs
scr. 6 7 scr. 6 8 scr. 5 7 scr. 4 5 scr. 6 8 scr. 5 6
DLX3
HOXA9
HOXB13
HOXC13
HOXD3
HOXD8

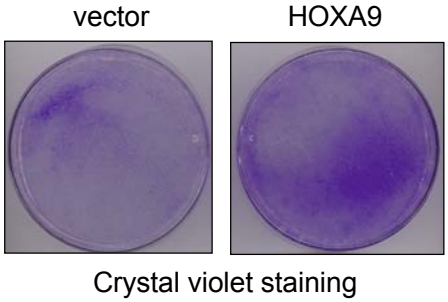
B


Relative cell numbers
2500
2000
1500
1000
500
0
siRNAs
scr. 6 7 6 8 5 7 4 5 6 8 5 6 2 5 INK4a CBX7
DLX3
HOXA9
HOXB13
HOXC13
HOXD3
HOXD8
HLX1

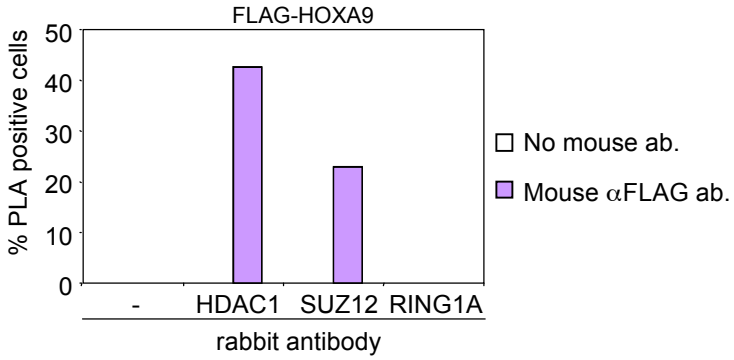
C


Density
2.5
2
1.5
1
0.5
0
0 0.2 0.4 0.6 0.8 1.0
Correlation

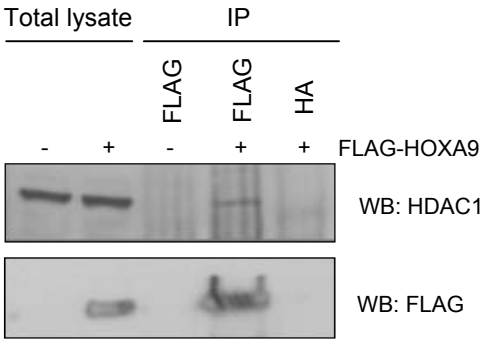
A


vector
HOXA9
Crystal violet staining

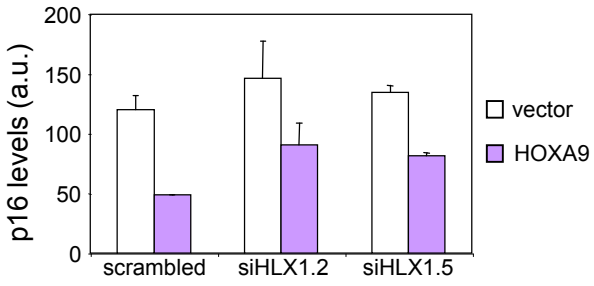
B

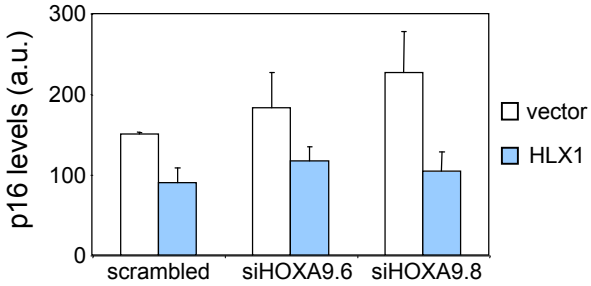

FLAG-HOXA9
% PLA positive cells
50
40
30
20
10
0
-
HDAC1
SUZ12
RING1A
rabbit antibody
No mouse ab.
Mouse αFLAG ab.

C

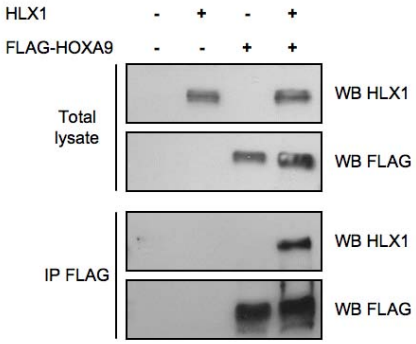

Total lysate
IP
FLAG
FLAG
HA
-
+
-
+
+
FLAG-HOXA9
WB: HDAC1
WB: FLAG

A

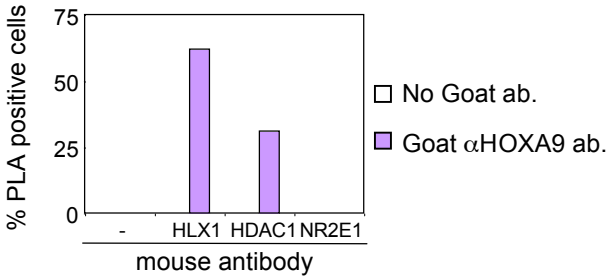

p16 levels (a.u.)
200
150
100
50
0
scrambled
siHLX1.2
siHLX1.5
vector
HOXA9


p16 levels (a.u.)
300
200
100
0
scrambled
siHOXA9.6
siHOXA9.8
vector
HLX1

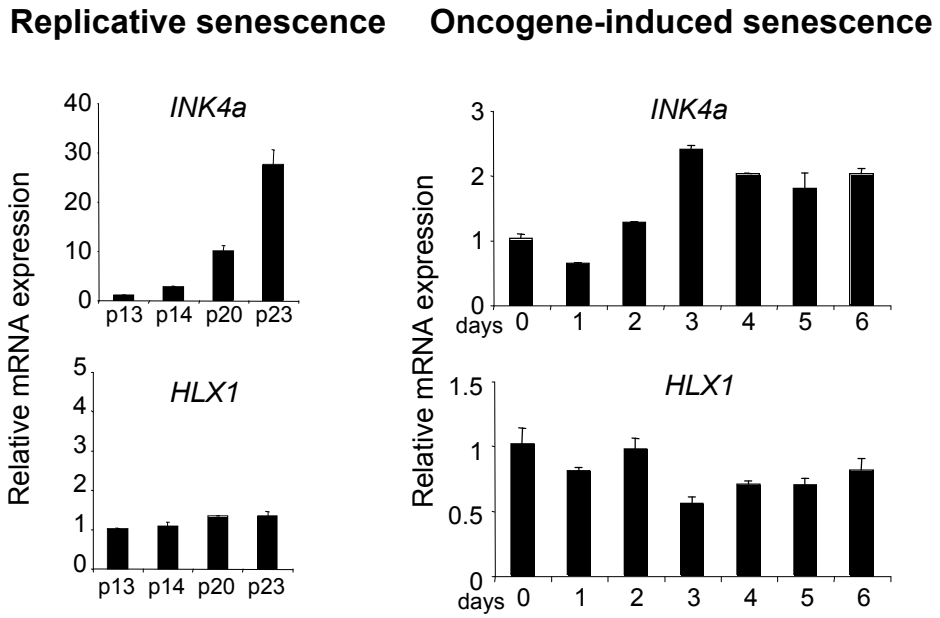
B


HLX1 - + - +
FLAG-HOXA9 - - + +
Total lysate
WB HLX1
WB FLAG
IP FLAG
WB HLX1
WB FLAG

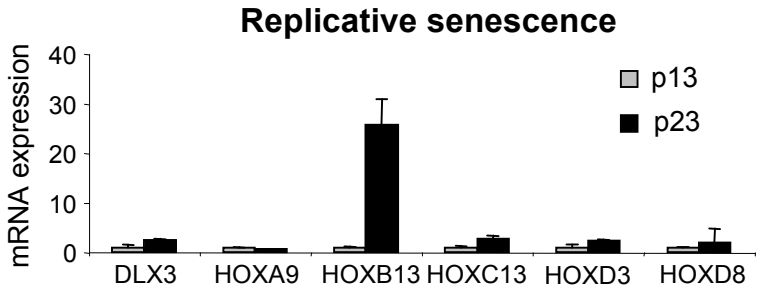
C


% PLA positive cells
75
50
25
0
-
HLX1
HDAC1
NR2E1
mouse antibody
No Goat ab.
Goat αHOXA9 ab.

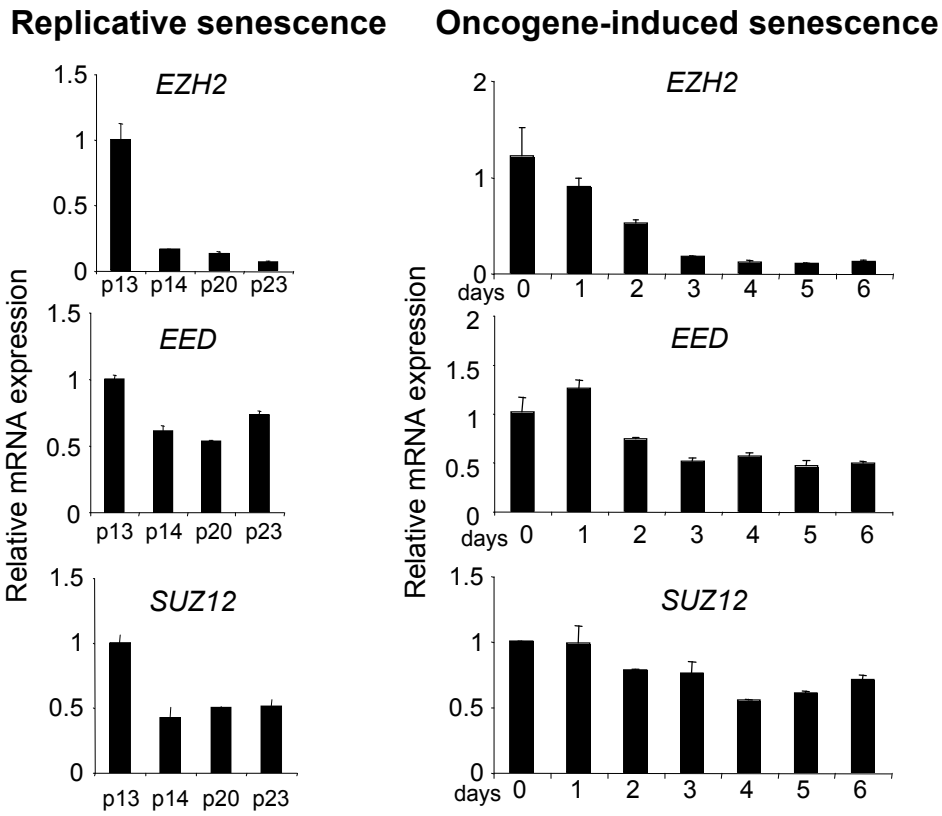
A


Replicative senescence
Oncogene-induced senescence
INK4a
HLX1
Relative mRNA expression
p13 p14 p20 p23
days 0 1 2 3 4 5 6

B


Replicative senescence
mRNA expression
p13
p23
DLX3 HOXA9 HOXB13 HOXC13 HOXD3 HOXD8

C


Replicative senescence
Oncogene-induced senescence
EZH2
EED
SUZ12
Relative mRNA expression
p13 p14 p20 p23
days 0 1 2 3 4 5 6