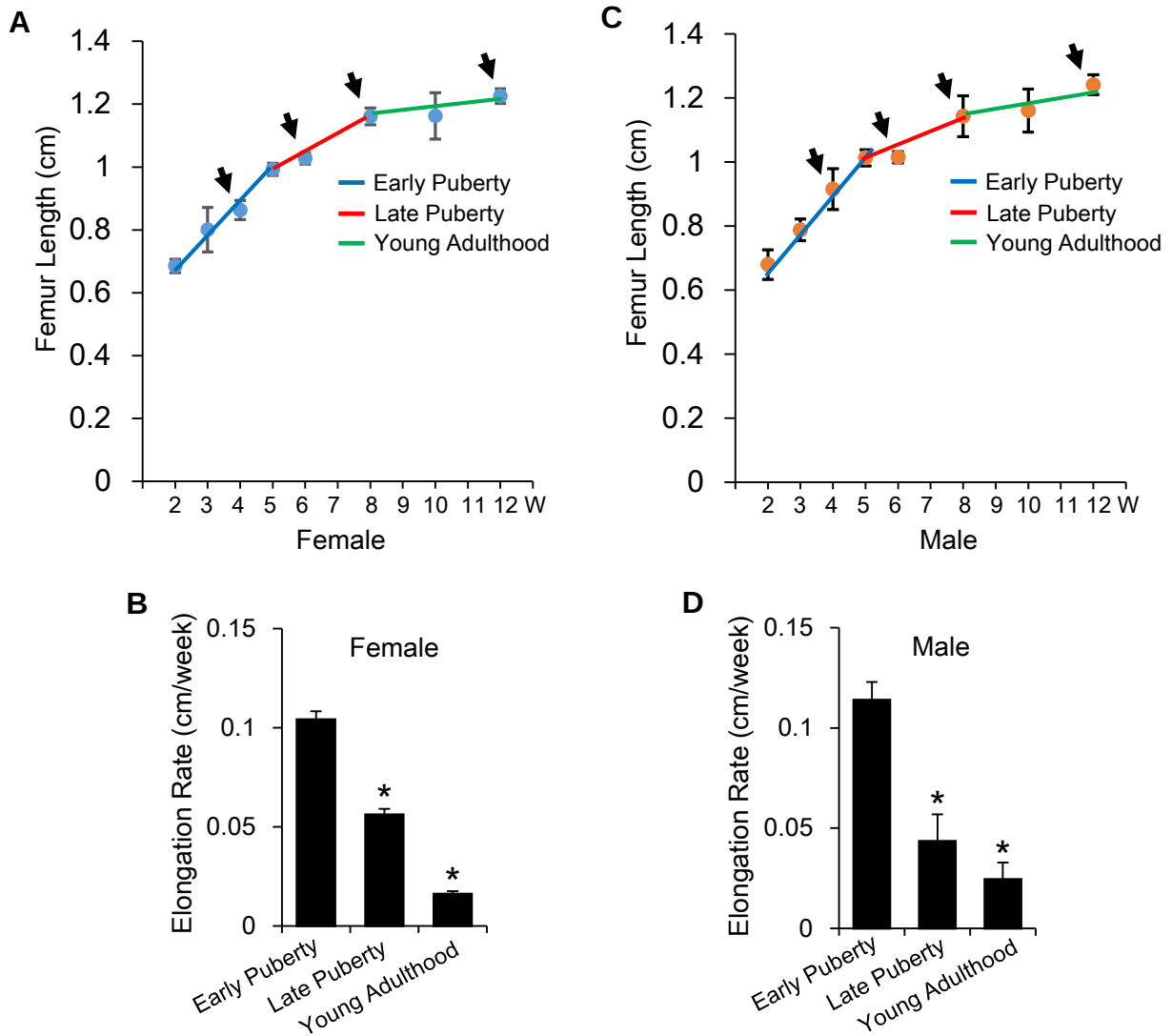
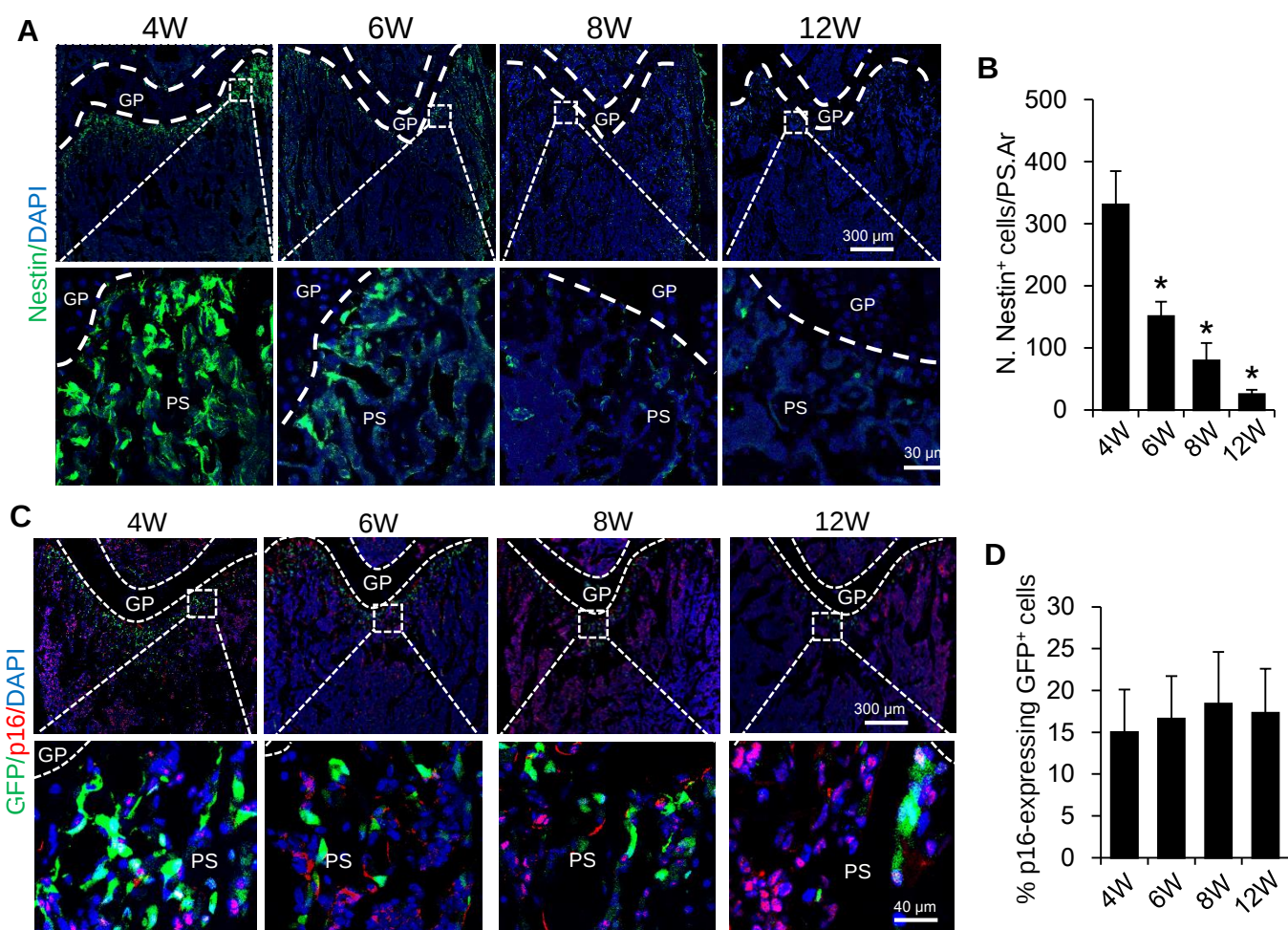




Supplementary Figure 1. Mouse Femur Length in Different Ages.

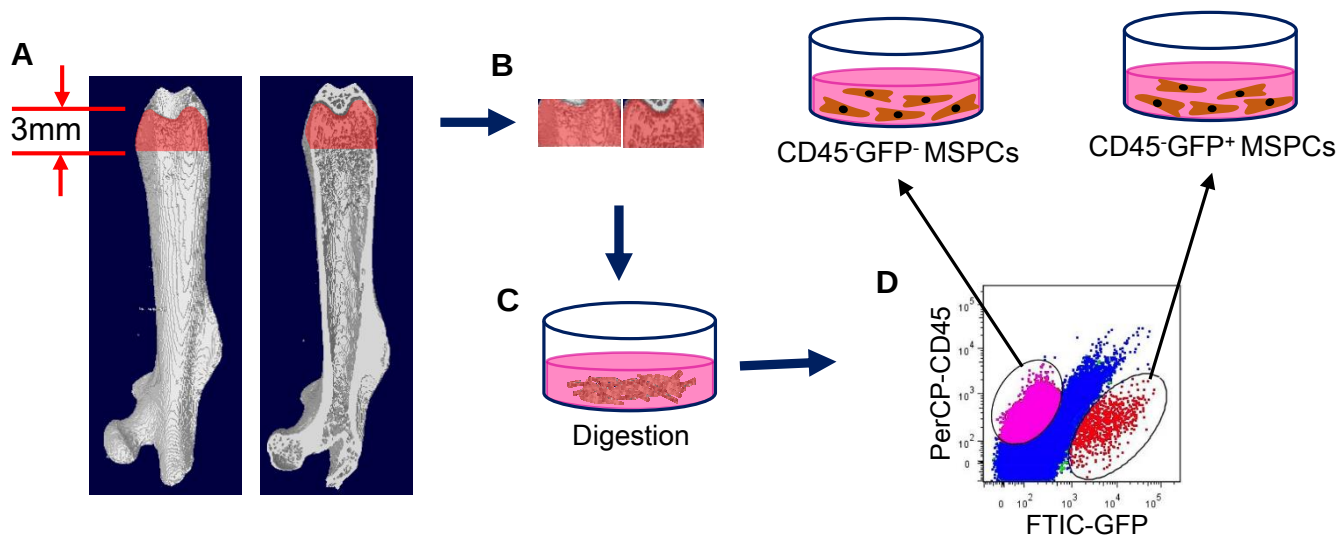
Femur lengths of 2-,3-,4-,5-,6-,8-,10-,12-week-old female (**A**) and male (**C**) *C57BL/6J* mice were measured using X-ray. Black arrows represent the designed time points for further analysis of cellular senescence in primary spongiosa of long bone; the blue lines represent early puberty period; the red lines represent late puberty period; the green lines represent young adulthood. The elongation rates of femora in early puberty, late puberty, and young adulthood were calculated from female (**B**) and male (**D**) *C57BL/6J* mice. Five mice per group. Data are represented as mean $\pm$ s.e.m. * $p < 0.05$ versus early puberty as determined by ANOVA.



Supplementary Figure 2.

Nestin⁺ Cells in Primary Spongiosa of Long Bone Gradually Diminished During Late Puberty.

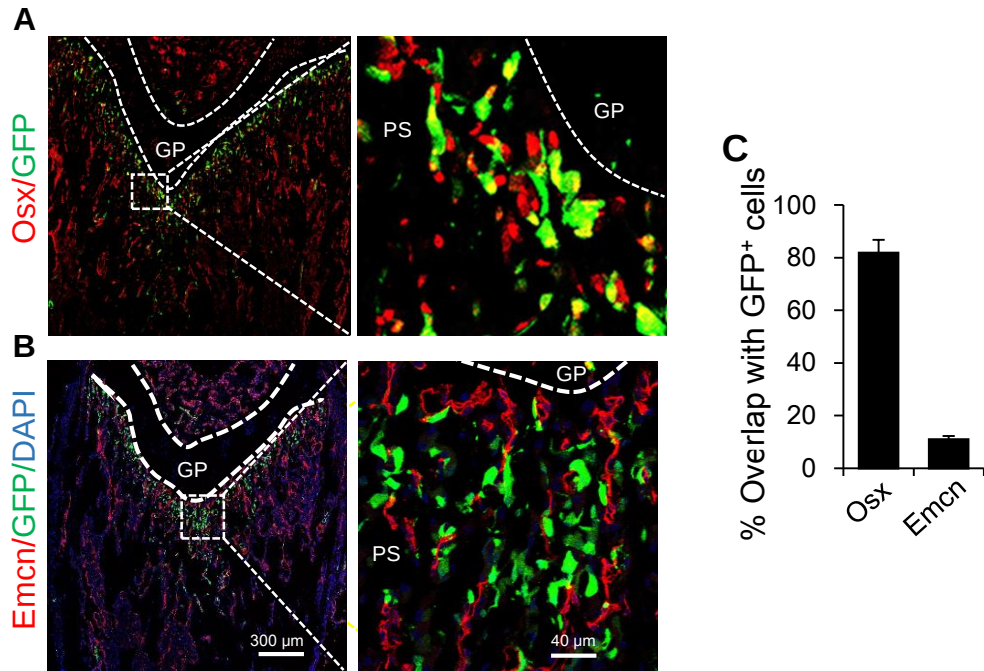
Representative images of nestin immunofluorescence staining (**A**, nestin-positive signal is in green) and quantitative analysis (**B**) of nestin⁺ cells in primary spongiosa of femur sections from 4-, 6-, 8-, 12-week-old male *C57BL/6J* mice. Images in upper panels in (**A**) are lower power with boxes outlining the area of higher power in lower panels. DAPI stains nuclei blue. Numbers of nestin⁺ cells per mm² tissue area in primary spongiosa (N. Nestin⁺ cells/PS.Ar) (**B**). Double-immunofluorescence images of femoral metaphysis sections from 4, 6, 8, 12-week-old male *Nestin-GFP* mice using antibodies against GFP (green) and either p16 (red) (**C**). DAPI stains nuclei blue. Images in upper panels in (**C**) are lower power with boxes outlining the area of higher power in lower panels. Quantification of the percentage of GFP⁺ cells that express p16 (**D**) are shown. Five mice per group. Data are represented as mean $\pm$ s.e.m. GP, growth plate. PS, primary spongiosa. * $p < 0.05$ versus 4-week-old mice group as determined by ANOVA.



Supplementary Figure 3.

Steps to Isolate CD45-GFP⁻ and CD45-GFP⁺ Mesenchymal Stem/Progenitor Cells (MSPCs).

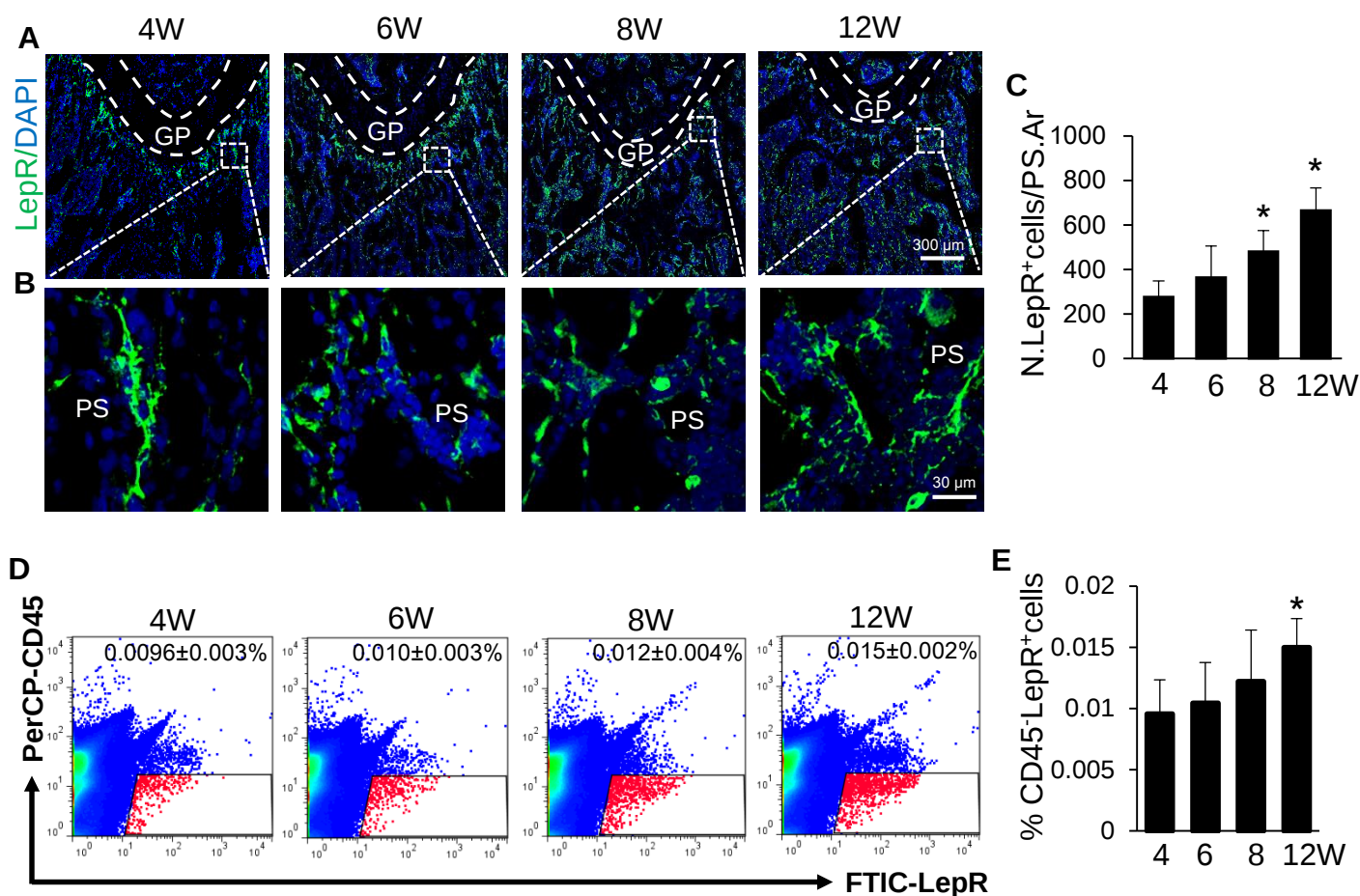
Femur of two month-old male *Nestin-GFP* mice were collected. The end of distal femur was removed at growth plate site, and the remaining bone were cut at 3 mm from the growth plate (A), and this piece of bone at the end was considered as metaphyseal region (B). To isolate cells from the metaphyses, the bone were then digested with a protease solution (2 mg/ml collagenase A and 2.5 mg/ml trypsin in PBS) for 20 min to remove the periosteum and periosteal progenitors (Step I). The bones were cut into small pieces and digested in the protease solution for another 1 h (C, Step II). Cells within the supernatant were collected for flow cytometry. CD45-GFP⁺ cells were then sorted according to side scatter and GFP-FITC fluorescence at $> 10^3 \log \text{FI-1}$ (GFP-FITC) fluorescence after negative selection of leucocyte common antigen CD45 at $< 10^3 \log \text{FI-3}$ (CD45-PerCP) fluorescence. CD45-GFP⁻ cells were then sorted according to side scatter and GFP-FITC fluorescence at $< 10^3 \log \text{FI-1}$ (GFP-FITC) fluorescence after negative selection of leucocyte common antigen CD45 at $< 10^3 \log \text{FI-3}$ (CD45-PerCP) fluorescence. The sorted cells were plated for expending (D). The adherent cells were considered as CD45-GFP⁺ and CD45-GFP⁻ MSPCs, which were used for further culture and analysis.



Supplementary Figure 4.

Nestin-GFP Labels Both Osteoprogenitor Cells and Vascular Endothelial Cells.

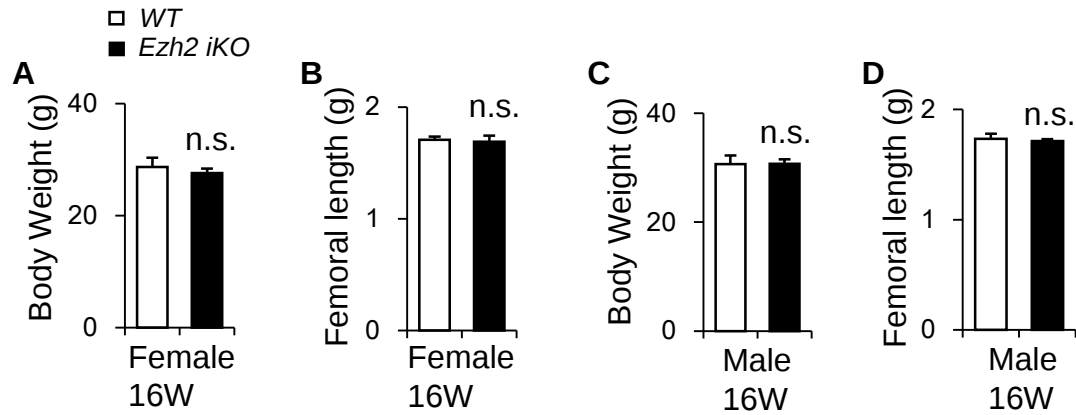
Double-immunofluorescence staining of metaphysis of femur sections from 4-week-old male *Nestin-GFP* mice was performed using antibodies against GFP (green) and either osterix (Osx) (red) (A) or endomucin (Emcn) (red) (B), respectively. Images in the left panes are lower power with boxes outlining the area of higher power in the right panels. DAPI stains nuclei blue. Quantification of the percentages of GFP⁺ cells that express Osx or Emcn, respectively (C). Five mice per group. Data are represented as mean $\pm$ s.e.m. GP, growth plate. PS, primary spongiosa.



Supplementary Figure 5.

LepR⁺ Cells in Primary Spongiosa of Long Bone Gradually Increase During Late Puberty.

Representative images of immunofluorescence staining (**A**, **B**) and quantitative analysis (**C**) of leptin receptor (LepR)⁺ cells in primary spongiosa of femur sections from 4, 6, 8, 12-week-old male *C57BL/6J* mice. Images in (**A**) are lower power with boxes outlining the area of higher power in (**B**). Numbers of LepR⁺ cells per mm² tissue area in primary spongiosa (N. LepR⁺ cells/PS.Ar) (**C**). Representative images of the flow cytometry analysis (**D**) and the percentage (**E**) of the LepR⁺ cells isolated from the metaphysis region. CD45⁻LepR⁺ cells were then analyzed according to side scatter and LepR-FITC fluorescence at $> 10^1$ log FI-1 (LepR-FITC) fluorescence after negative selection of leucocyte common antigen CD45 at $< 10^1$ log FI-3 (CD45-PerCP) fluorescence. Six mice per group. Data are represented as mean $\pm$ s.e.m. GP, growth plate. PS, primary spongiosa. * $p < 0.05$ vs. 4-week-old mice group as determined by ANOVA.



Supplementary Figure 6.

Mice with deletion of *Ezh2* in *Nestin*⁺ Cells Have Unchanged Body Weight and Femoral Length.

Three-week-old female (A, B) and male (C, D) *Nestin-Cre^{ERT2}::Ezh2^{fllox/fllox}* (*Ezh2* iKO) mice and *Nestin-Cre^{ERT2}* mice (WT) were injected with 3 doses of tamoxifen (every other day). The mice were humanely killed at 16 weeks of age. Body weight (A, C) and femora length (B, D) of the mice were measured. Six mice per group. Data are represented as mean $\pm$ s.e.m. n.s, not significant as determined by Student's *t*-tests.

Supplementary Table 1. Primers used for ChIP-PCR

Primers	Forward	Reverse	TSS	Ref
<i>p15^{INK4b}-1</i>	CCGCCTAGAGATCGAACTAGCC	CGCTTTTGCAATTGACTGAC	-595~ -481	1
<i>p15^{INK4b}-2</i>	CACCGAAGCTACTGGGTCTC	CTGTGGCAGAAATGGTCCTT	-107~ -15	1
<i>p15^{INK4b}-3</i>	ATGTTCTAAGAGGCTTTGTTTCCA	CATTTGTGCATAGGAGATCAGG	+1542-+1623	1
<i>p16^{INK4a}-1</i>	TCCGATCCTTTAGCGCTGTT	CCCGGACTACAGAAGAGATG	-252 ~ -184	2
<i>p16^{INK4a}-2</i>	AGGGGTGTTCAATTCATGCTAT	ACACTCTGCTCCTGACCTGG	-130 ~ -50	2
<i>p16^{INK4a}-3</i>	GGAGCCACCCATTAAACTAACT	CAAAAATAAGACACTGAAAACCTCG	+341 ~ +411	2
<i>p19^{ARF}-1</i>	AGGTGCCTCAACGCCGAAG	CTGGTCCAGGATTCCGGTGCGG	-12503 ~-12222	3
<i>p19^{ARF}-2</i>	ATGGGCAACGTTACGTCAGCAGC	AGCGGTACACAAAGACCACCCA	+5135~+5417	3
<i>p21^{CIP1}</i>	CACAGTTGGTCAGGGACAGA	CAGGACCAACCCACTCCTT	-4933~-4694	4
<i>p27^{KIP1}</i>	CTGGCTCTGCTCCATTTGAC	GGCTCCCGTTAGACACTCTC	-163~+31	4
<i>Runx2</i>	GGGGGAAGCCACAGTGGTA	AAAATAGGAGTTTTAAAGCT	-137~-22	5
<i>Osteocalcin</i>	GAGGACATTACTGAACAC	CAGTGGGTCAAACCCAAA	-505~-153	6
<i>Gapdh</i>	CCACCCAGAAGACTGTGGAT	GGATGCAGGGATGATGTTCT	+3767~+3850	1

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Supplementary Table 2. Primers used for quantitative real-time PCR

Primers	Forward	Reverse
<i>p16^{INK4a}</i>	GAAAGAGTTCGGGGCGTTG	GAGAGCCATCTGGAGCAGCAT
<i>p21^{CIP1}</i>	AGAAGGTACTTACGGTGTGGT	GAGAGATTTCCCGAATTGCAGT
<i>p53</i>	ATCGCCTTCGACATCATCGC	CCCATGCGTACTCCATGAG
<i>Ki67</i>	ACCGTGGAGTAGTTTATCTGGG	TGTTTCCAGTCCGCTTACTTCT
<i>Ezh1</i>	CCAGACTGCCAGAATCGCTTT	CAGGTGCTTTTTGAGGCCA
<i>Ezh2</i>	AGTGACTTGGATTTTCCAGCAC	AATTCTGTTGTAAGGGCGACC
<i>Utx</i>	CGGGCGGACAAAAGAAGAAC	CATAGACTTGCATCAGATCCTCC