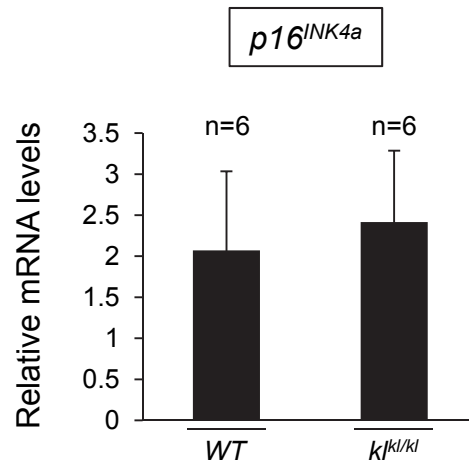


**Supplementary Figure 1. Tissue distribution of  $\alpha$ -klotho.**

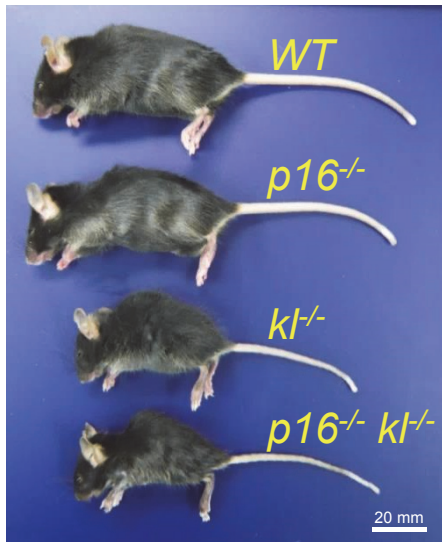
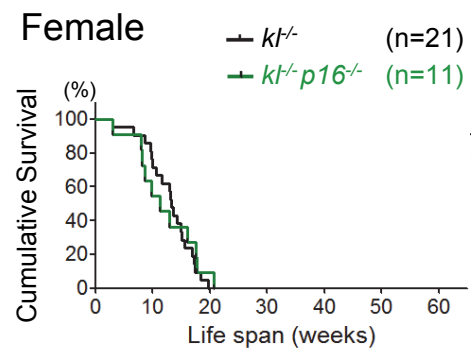
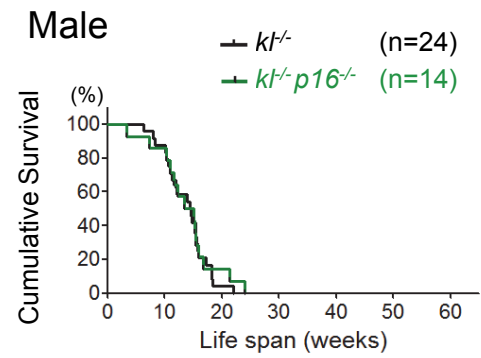
**(a)** The relative expression levels of  $\alpha$ -klotho mRNA in the indicated tissue of *WT* mice were determined by RT-qPCR. All samples except those from the ovary and uterus were collected from 16-week-old male mice. Samples from ovary and uterus were collected from 16-week-old female mice. The experiments were performed in triplicate using three individual mice, and representative results from three independent experiments are shown. Error bars indicate mean  $\pm$  s.d.

**(b)** Indicated tissue of 16-week-old female (ovary and uterus) and male (other tissues) *WT* mice were subjected to western blot analysis using antibodies shown left.  $\alpha$ -tubulin was used as a loading control. The experiments were performed in triplicate using three individual mice, and representative results from three independent experiments are shown.

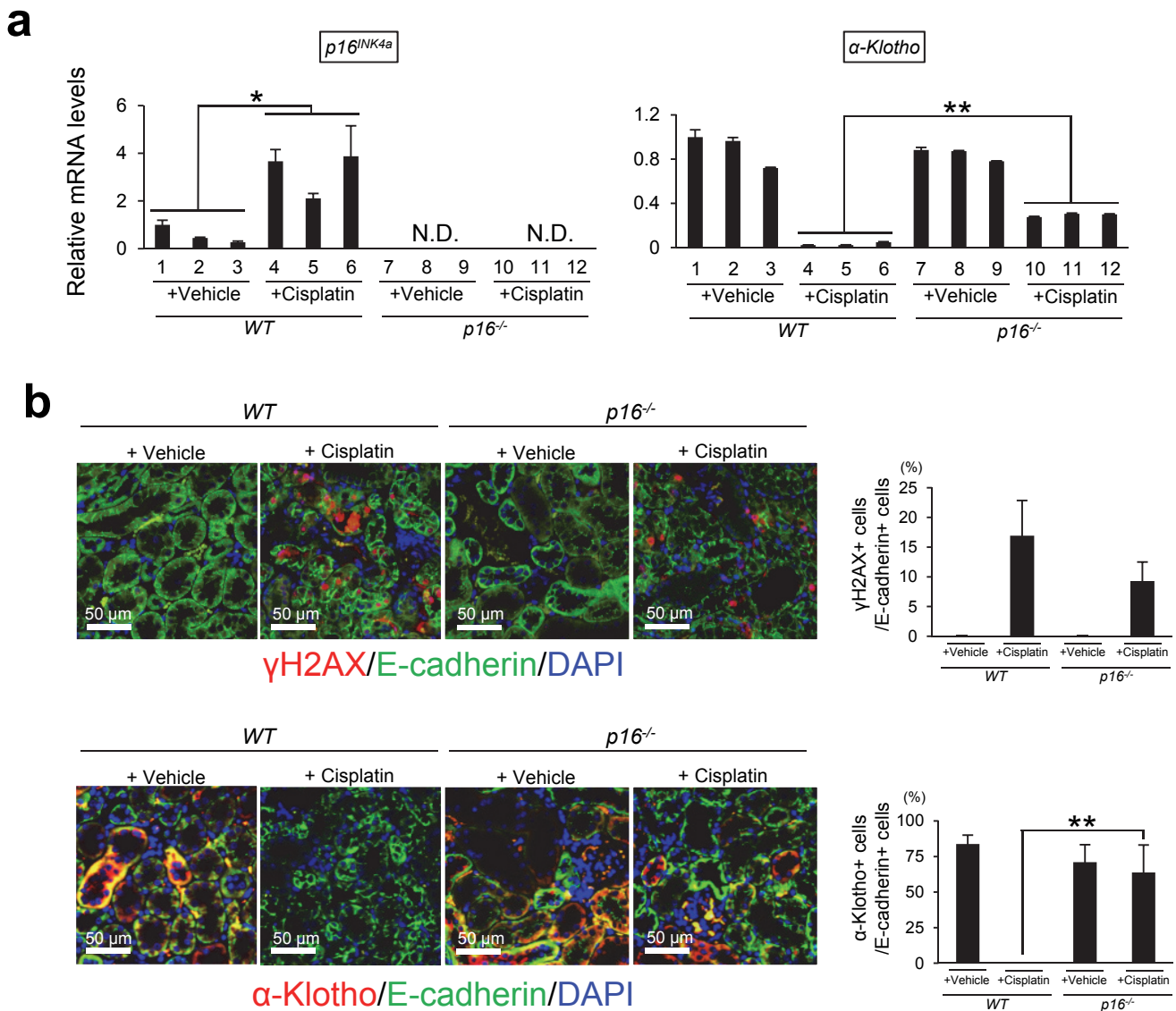


**Supplementary Figure 2. Renal expression of  $p16^{INK4a}$  in  $kl^{-/-}$  mice.**

The relative levels of  $p16^{INK4a}$  mRNA in kidneys of 2 to 6 month-old male mice of each genotype were examined by RT-qPCR.  $n$ =number of mice examined. Data were analysed by Student's  $t$ -test and are displayed as mean  $\pm$  s.d.

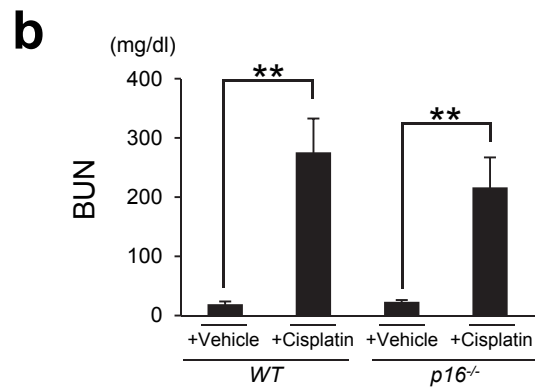
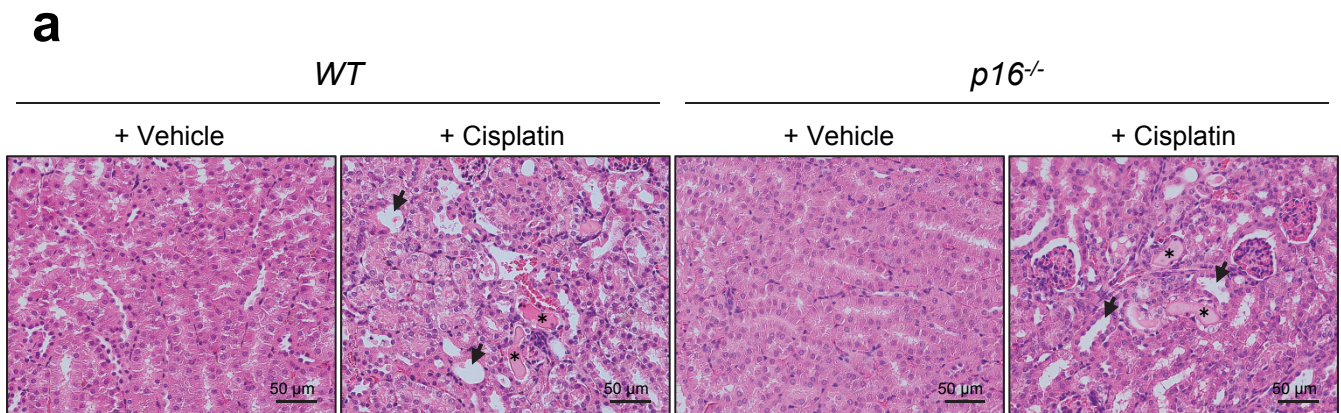
**a****b**

**Supplementary Figure 3. *p16*<sup>INK4a</sup> ablation failed to reverse the ageing phenotypes of *kl*<sup>-/-</sup> mice.** (a) Representative photographs of 11-week-old female mice of each genotype (n=3). (b) Kaplan-Meier plot showing survival of *kl*<sup>-/-</sup> mice (male, n=24; female, n=21), and *p16*<sup>-/-</sup> *kl*<sup>-/-</sup> mice (male, n=14 ; female, n= 11).



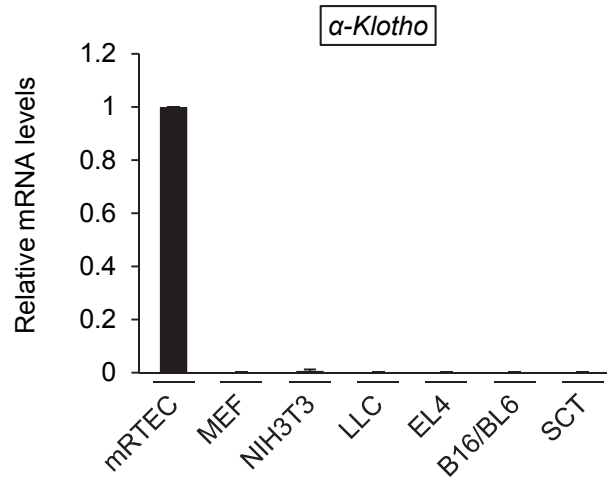
**Supplementary Figure 4. *p16<sup>INK4a</sup>* ablation mitigates Cisplatin-induced reduction of *α-klotho*.**

(a) The relative levels of indicated mRNA in kidneys of 10 to 20-week-old male mice of each genotype treated with or without Cisplatin were examined by RT-qPCR. Representative results of three individual male mice were shown (n=3 experiments). (b) Immunohistochemical analysis of  $\gamma$ -H2AX (red), E-cadherin (green), and DAPI (blue) (upper panel) and  $\alpha$ -klotho (red), E-cadherin (green), and DAPI (blue) (lower panel) in kidney sections of 10 to 20-week-old male mice of each genotype treated with or without Cisplatin. Representative results of three individual male mice were shown. The histograms indicate the percentages of E-cadherin expressing cells that were positive for  $\gamma$ -H2AX (upper panel) or  $\alpha$ -klotho expression (lower panel). At least 100 cells were scored per group (n=3 experiments). For all graphs, data were analysed by Welch's *t*-test and are displayed as mean  $\pm$  s.d. \**P*<0.05 \*\**P*<0.01.

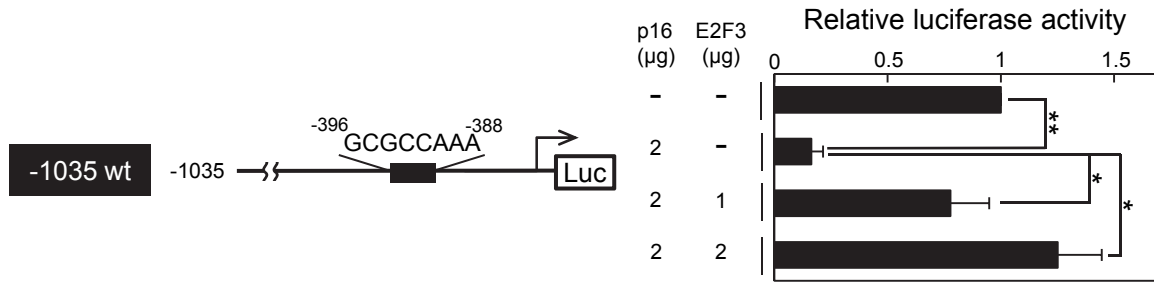


**Supplementary Figure 5. Cisplatin-induced acute kidney injury.**

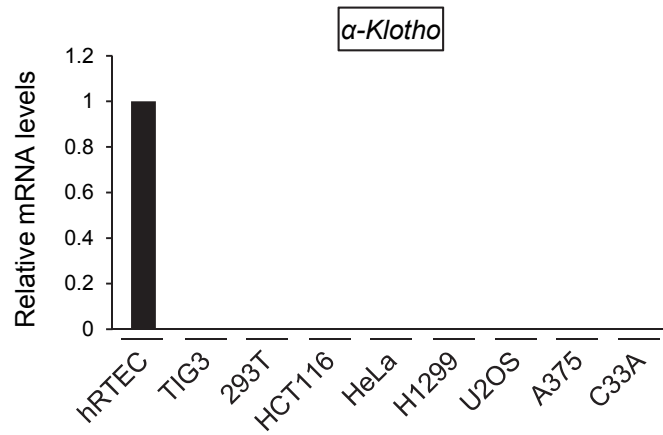
(a) Representative HE staining of kidney sections from 10 to 20-week-old *WT* and *p16<sup>-/-</sup>* male mice treated with or without cisplatin (see supplementary Figure 4) (n=3). An asterisk represents renal tubular casts and an arrow represents dilated renal tubules. (b) Serum levels of blood urea nitrogen (BUN) were measured after treatment with cisplatin three times (n=3). Data were analysed by Student's *t*-test and are displayed as mean  $\pm$  s.d. \*\**P*<0.01.



**Supplementary Figure 6. Comparison of  $\alpha$ -klotho expression among murine cells.** Relative expression levels of  $\alpha$ -klotho mRNA in the indicated murine cells were determined by RT-qPCR. SCT: Spindle cell tumour cell line prepared from mice lacking  $p16^{INK4a}$  genes<sup>54</sup>. The experiments were performed in triplicate, and representative results from three independent experiments are shown. Error bars indicate mean  $\pm$  s.d.

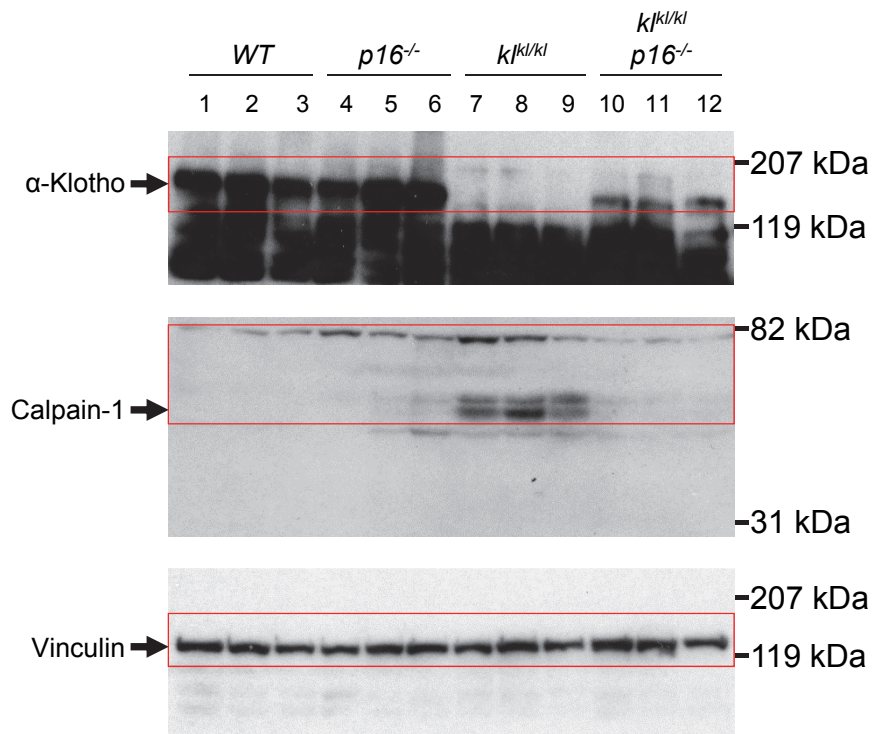


**Supplementary Figure 7. Ectopic expression of E2F3 blunts negative effect of p16<sup>INK4a</sup>.** Schematic representation of the reporter construct of mouse *α-klotho* gene promoter used in the analysis (left panel). The E2F binding element is shown as a black rectangle with the sequence and firefly luciferase is shown as Luc. The reporter construct was co-transfected into mRTECs along with LacZ plasmid. Where indicated, cells were also co-transfected with a p16<sup>INK4a</sup> expression plasmid and increasing amounts of E2F3 expression plasmid. The experiments were performed in triplicate, and representative results from three independent experiments are shown. Data were analysed by Welch's *t*-test and are displayed as mean ± s.d. \**P*<0.05 \*\**P*<0.01.

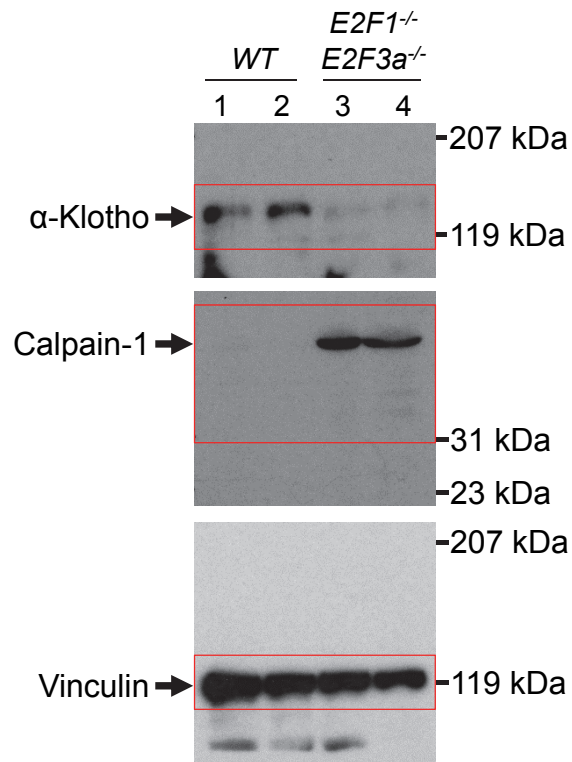


**Supplementary Figure 8. Comparison of  $\alpha$ -klotho expression among human cells.** Relative expression levels of  $\alpha$ -klotho mRNA in the indicated human cells were determined by RT-qPCR. The experiments were performed in triplicate, and representative results from three independent experiments are shown. Error bars indicate mean  $\pm$  s.d.

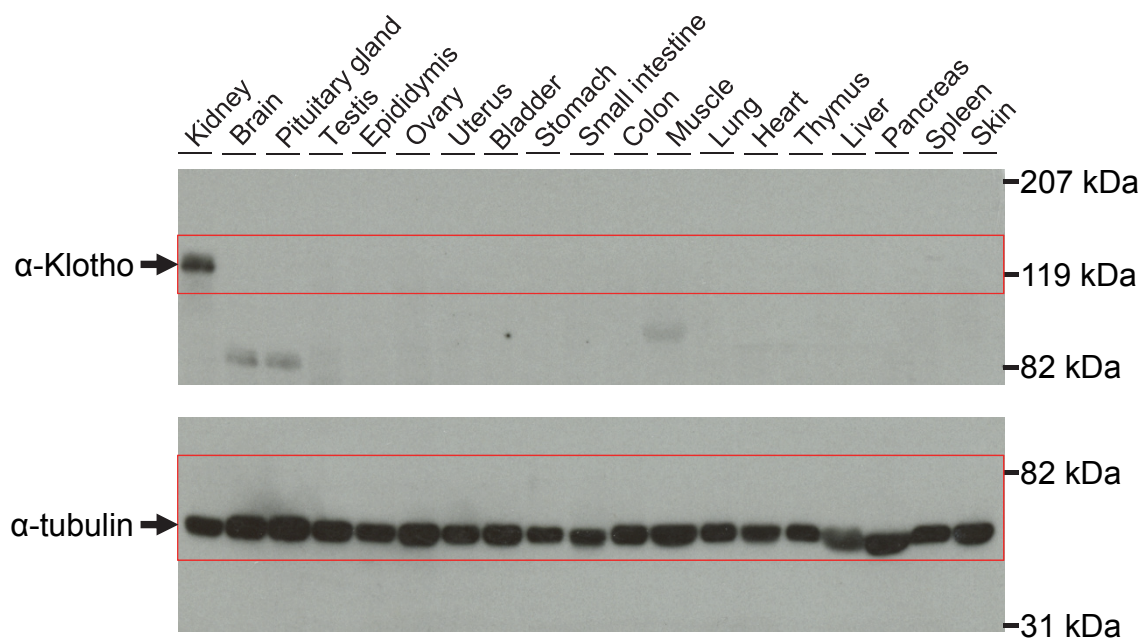




**Supplementary Figure 9. Expanded blots from Figure 4c.**



**Supplementary Figure 10. Expanded blots from Figure 6d.**



**Supplementary Figure 11. Expanded blots from Supplementary Figure 1b.**

**Supplementary Table 1. Primers used for RT-qPCR.**

Target gene	Sequence
<i>mouse GAPDH</i>	5'-CAACTACATGGTCTACATGTTC-3' (forward)
	5'-CGCCAGTAGACTCCACGAC-3' (reverse)
<i>mouse α-klotho</i>	5'-GATGGCAGAGAAATCAACACAGT-3' (forward-1)
	5'-ACTACGTTCAAGTGGACACTACT-3' (reverse-1)
<i>mouse α-klotho</i>	5'-CTGAAAACCAGCCCCTTGAA-3' (forward-2)
	5'-AATAAGCCTCTTACTGTGAT-3' (reverse-2)
<i>mouse p16</i>	5'-AGGGCCGTGTGCATGACGTG-3' (forward-1)
	5'-GCACCGGGCGGGAGAAGGTA-3' (reverse-1)
<i>mouse p16</i>	5'-GAACTCTTTCGGTCGTACCC-3' (forward-2)
	5'-CGAATCTGCACCGTAGTTGA-3' (reverse-2)
<i>mouse Cyp27b1</i>	5'-TGCTTGCGGATTGCTAACG-3' (forward)
	5'-CCTTAGTCGTCGCACAAGGTC-3 (reverse)
<i>human GAPDH</i>	5'-CAACTACATGGTTTACATGTTC-3' (forward)
	5'-GCCAGTGGACTCCACGAC-3' (reverse)
<i>human α-klotho</i>	5'-TACCTGGTGGCGCACAACCT-3' (forward)
	5'-TGTGGTTCGGTCATTCTTCGA-3' (reverse)
<i>human p16</i>	5'-CGAATAGTTACGGTCGGAGG-3' (forward)
	5'-TGAGAGTGGCGGGGTCG-3' (reverse)
<i>human Cdc6</i>	5'-GGCACAGGCTACAATCAGTT-3' (forward)
	5'-AATCTCCTGAGCAATAGCTG-3' (reverse)