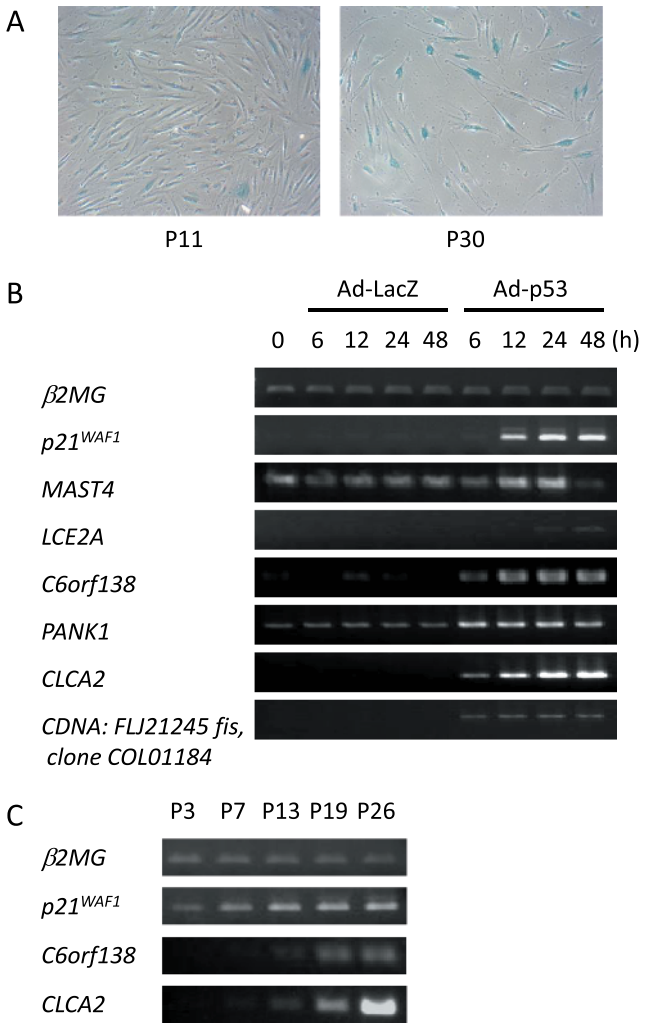

Figure W1. Replicative senescence in late-passage fibroblast. (A) After 30 times of passage, the cells were subjected to SA-β-gal staining. (B) RT-PCR analysis of six candidate mRNAs in U373MG cells at indicated times after infection with Ad-p53 or Ad-LacZ. *β2-Microglobulin* was used for the normalization of expression levels. *p21*^{WAF1} served as a positive control. (C) RT-PCR analysis of *C6orf138* and *CLCA2* mRNAs in NHDF cells at indicated passage levels. *β2-Microglobulin* was used for the normalization of expression levels. *p21*^{WAF1} served as a positive control.

Table W1. List of 85 Senescence-Associated Genes.

Gene	P26/P3	p53 Induction
MAST4	>50	++
RGS4	>50	
PPP4R4	>50	
ZNF367	>50	
SORT1	>50	
SYPL2	>50	
LCE2A	>50	++
MOSC1	>50	
TOMM40L	>50	
CDNA FLJ32320 fis	>50	
SDPR	>50	
PPFIA2	>50	
KIAA1147	>50	
C6orf138	>50	++
DSCC1	>50	
POLR3G	>50	
ETV4	>50	
PARG	>50	
SIGLEC15	>50	
Clone IMAGE:35527	>50	
LEPR	>50	
RBM24	>50	
BRI3BP	>50	
ANXA1	>50	
HSFE-1	>50	
Clone 23555	>50	
CCIN	>50	
CACNA1H	>50	
SLC7A14	>50	
KLRF1	>50	+
PANK1	>50	++
ENPP5	>50	
CPEB2	>50	
C11orf41	>50	
cDNA DKFZp686O 1044	>50	
TFDP1	>50	
RASD1	>50	
NAP1L2	>50	
DHRS12	>50	
EIF2C1	>50	
SLC7A14	>50	
CLCA2	>50	++
CYP3A5	>50	

Table W1. (*continued*).

Gene	P26/P3	p53 Induction
RNLS	>50	
MAST4	41.74	
SFRP4	41.67	
RGS2	40.24	
DTL	39.38	
NPTX1	30.78	
INA	29.34	
NAT8L	28.66	+
MMP1	28.28	
DBNDD1	24.25	
CCND1	22.55	
ATP8B4	22.11	
GLTPD1	22.02	
MET	21.58	
CCND1	20.73	
MFAP1	20.20	
CCND2	18.71	
C9orf57	18.21	
FNDC5	16.78	
PHLDA1	15.91	
CIT	15.55	
SLC1A2	15.32	
KLHDC9	14.87	
TOR1AIP1	14.55	
IFIT2	14.42	
ENY2	14.26	
RNF219	13.97	
CDNA: FLJ21245 fis	13.40	++
PRKG2	12.32	
TNIK	12.06	
APBA1	11.76	
TMEM158	11.58	+
E2F7	11.33	+
CNTN3	10.96	
CYYR1	10.93	
SLC35A3	10.77	
GPR68	10.76	
SYNM	10.63	
ITGA6	10.56	
VWF	10.54	
PLCB1	10.38	
CENPQ	10.19	

"+" indicates more than 5-fold induction at 24 or 48 hours after infection with Ad-p53; "++," more than 10-fold induction at 24 or 48 hours after infection with Ad-p53.

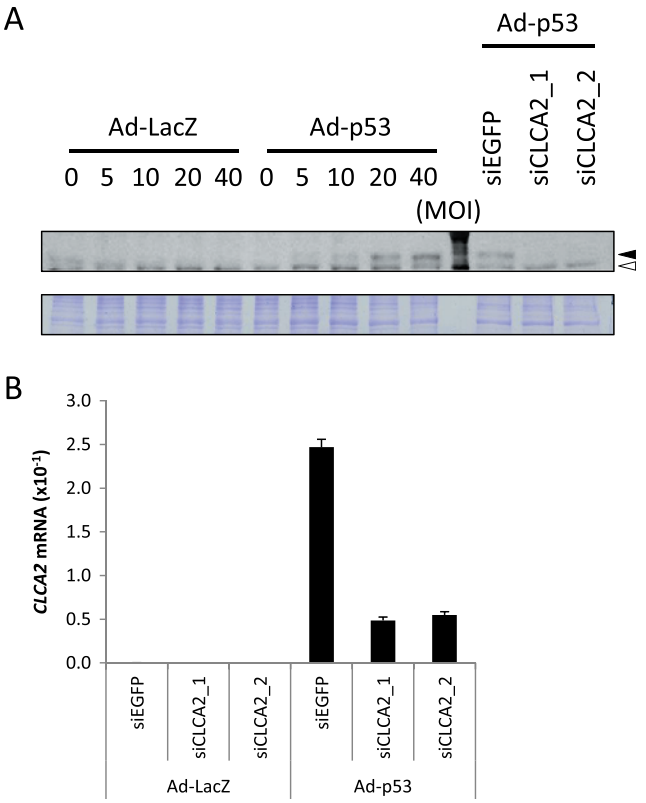

Figure W2. Ectopic expression of p53 induced CLCA2 protein. (A) Western blot analysis of CLCA2 protein in U373MG cells at 48 hours after infection with Ad-p53 or Ad-LacZ at indicated doze. CBB staining was used for the normalization protein loading. siCLCA2_1, siCLCA2_2, or siEGFP was transfected at 7 hours before adenovirus infection at 20 MOI. Black arrowhead indicates CLCA2 protein; open arrowhead, nonspecific band. (B) Quantitative RT-PCR analysis of CLCA2 in U373MG cells 48 hours after infection with Ad-LacZ or Ad-p53 at 20 MOI. siCLCA2_1, siCLCA2_2, or siEGFP was transfected 7 hours before adenovirus infection.

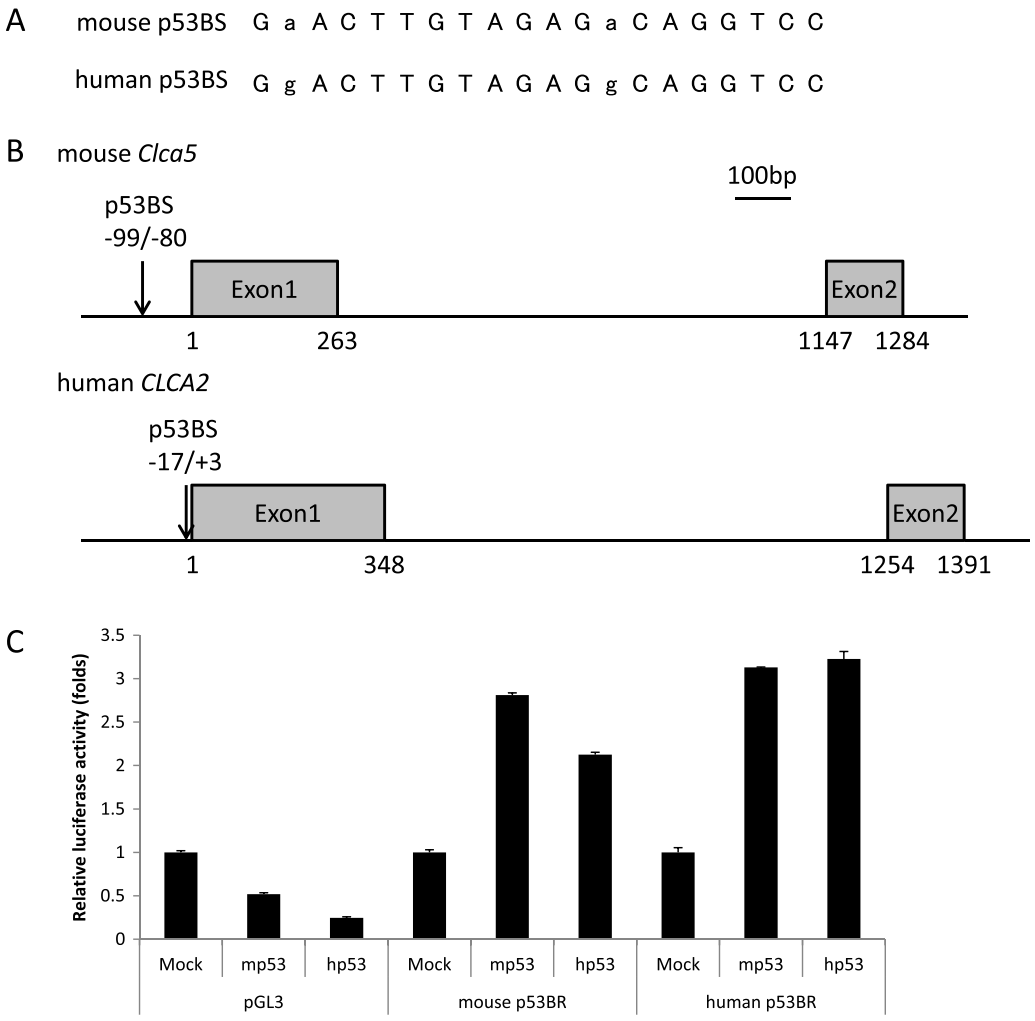

Figure W3. Comparison of mouse *Clca5* and human CLCA2. (A) Alignment of mouse p53BS and human p53BS. Nucleotides conserved between human and mouse are written in capital letters. (B) Genomic structures of the mouse *Clca5* and human *CLCA2* genes. Gray boxes indicate the locations and relative sizes of the two exons. Arrows indicate the locations of potential p53-binding sites. (C) Results of luciferase assay of p53BR are shown. Luciferase activity is indicated relative to the activity of mock vector with SDs ($n = 2$).

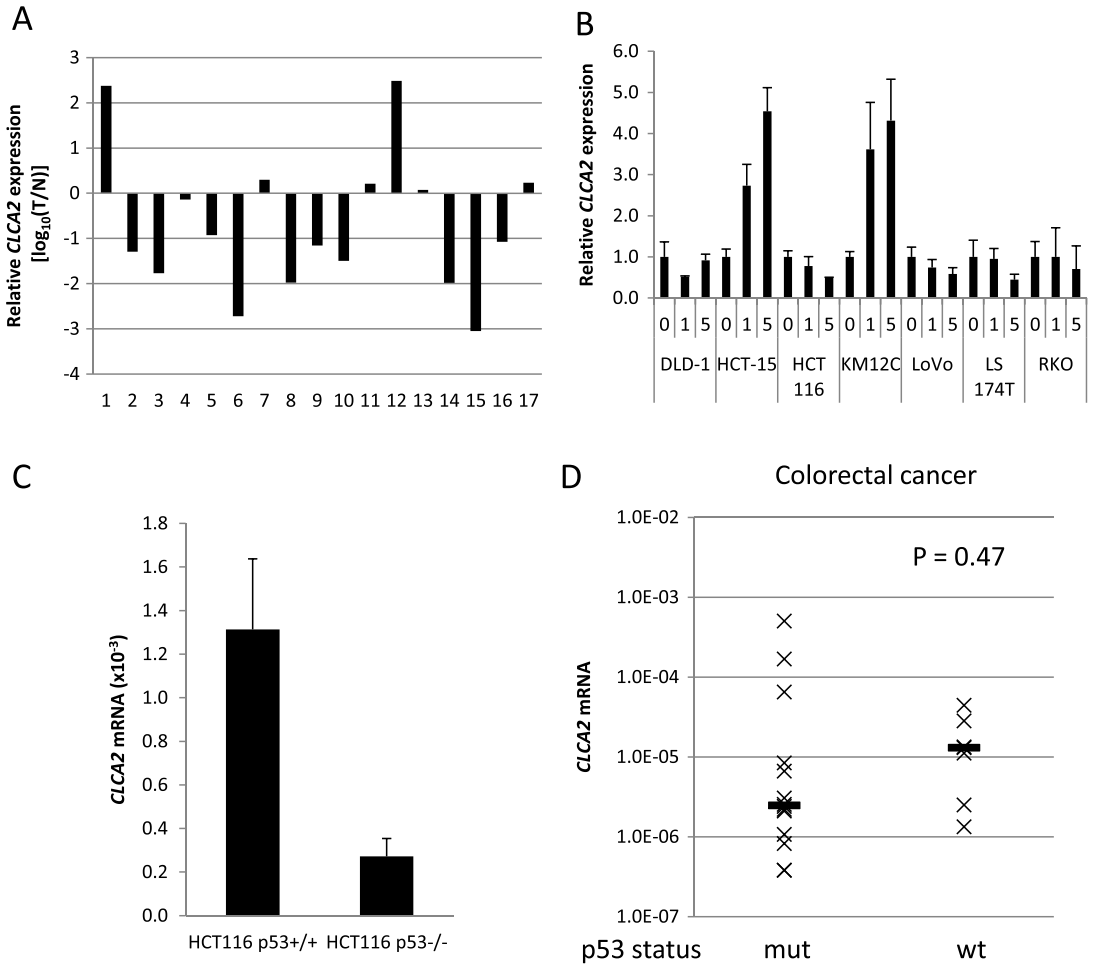

Figure W4. Suppression of *CLCA2* expression in colorectal cancer tissues. (A) Relative *CLCA2* expressions in colorectal cancer tissues compared with normal tissues were examined by quantitative real-time PCR analysis. *β-Actin* was used for the normalization of expression levels. (B) Seven colorectal cancer cell lines were treated with 1 or 5 μM of 5-Aza. *CLCA2* expressions were examined by quantitative real-time PCR analysis. *β2-Microglobulin* was used for the normalization of expression levels. (C) *CLCA2* expressions were examined by quantitative real-time PCR analysis. *β2-Microglobulin* was used for the normalization of expression levels. (D) *CLCA2* expressions were examined by quantitative real-time PCR analysis in 21 colorectal cancer cell lines. *β2-Microglobulin* was used for the normalization of expression levels. Student's *t* test was applied for comparing the *CLCA2* expressions in p53 mutant cell lines with those in p53 wild-type cell lines.

Table W2. List of Cancer Cell Lines.

No.	Colorectal Cancer Cell Lines
1	DLD-1
2	HCT-15
3	HT-29
4	KM12C
5	KM12SM
6	NCI-H508
7	NCI-H684
8	NCI-H716
9	SNU-C2A
10	SNU C5
11	SW480
12	SW620
13	SW948
14	WiDr
15	HCT 116
16	LoVo
17	LS 174T
18	NCI-H498
19	RKO
20	SNU C4
21	SW48

Table W3. Sequences of Primers and RNA Nucleotides.

	Sense	Antisense
siRNA oligonucleotides		
siCLCA2_1	GGAAUUUACUCGAGGUAUUTT	AAUACCUCGAGUAAAUUCCTT
siCLCA2_2	GAUGAAUGCUCCAAGGAAATT	UUUCCUUGGAGCAUUCAUCTT
sip53	GACUCCAGUGGUAAUCUACTT	GUAGAUUACCACUGGAGUCTT
siEGFP	GCAGCACGACUUCUUCAAGTT	CUUGAAGAAGUCGUGCUGCTT
	Forward	**Reverse**
Quantitative real-time PCR (SYBR Green I Master)		
CLCA2	ATACCTGCCACATGGAAAGC	CCTCTTTTCCACACCCTCTG
$p21^{WAF1}$	AAGATCAGCCGGCGTTTG	GACCTGTCACTGTCTTGTACCC
β2-Microglobulin	TCTCTCTTTCTGGCCTGGAG	AATGTCGGATGGATGAAACC
Clca5 (mouse)	CCGAGTGGTCTGCTTAGTGA	TACAGTTCGGCTGCTTGTTG
β-Actin (mouse)	CTAAGGCCAACCGTGAAAAG	ACCAGAGGCATACAGGGACA
p16	AGCATGGAGCCTTCGGCTGA	CCATCATCATGACCTGGATCG
Quantitative real-time PCR (Probe Master)		
CLCA2 no. 21	AGAAGAGGTCAGCAGGGAGA	CTCTTGATGGAGAAAGGATTAAAGA
β-Actin no. 55	TAGGAGGGCTGGCAACTTAG	CCAAGATGTTGATGTTGGATAAGA
Site-directed mutagenesis		
mClca5_mtp53BS1	AGATAGTTCCCAGATAGATCCACCCCA	CTAAAAATTCCTCTCCAAGGCAACGC
mClca5_mtp53BS2	GAATGTTCTCAAGAGGTGTGACTTAA	AGGACTATGCCTTTTAAAAACATTATT
mClca5_mtp53BS3	TAATATTTAATCAGTGTGCTAAAATCT	GGGATTACGCTCATGATCTCCCCAAGT
Semiquantitative RT-PCR		
CLCA2	CAGATGTGCAGCCTCAGAAG	TGCTGAGCACAGTGGGTAAG
$p21^{WAF1}$	GTTCCTTGTGGAGCCGGAGC	GGTACAAGACAGTGACAGGTC
β2-Microglobulin	CACCCCCACTGAAAAAGATGA	TACCTGTGGAGCAACCTGC