Fig. S1 Luan et al.

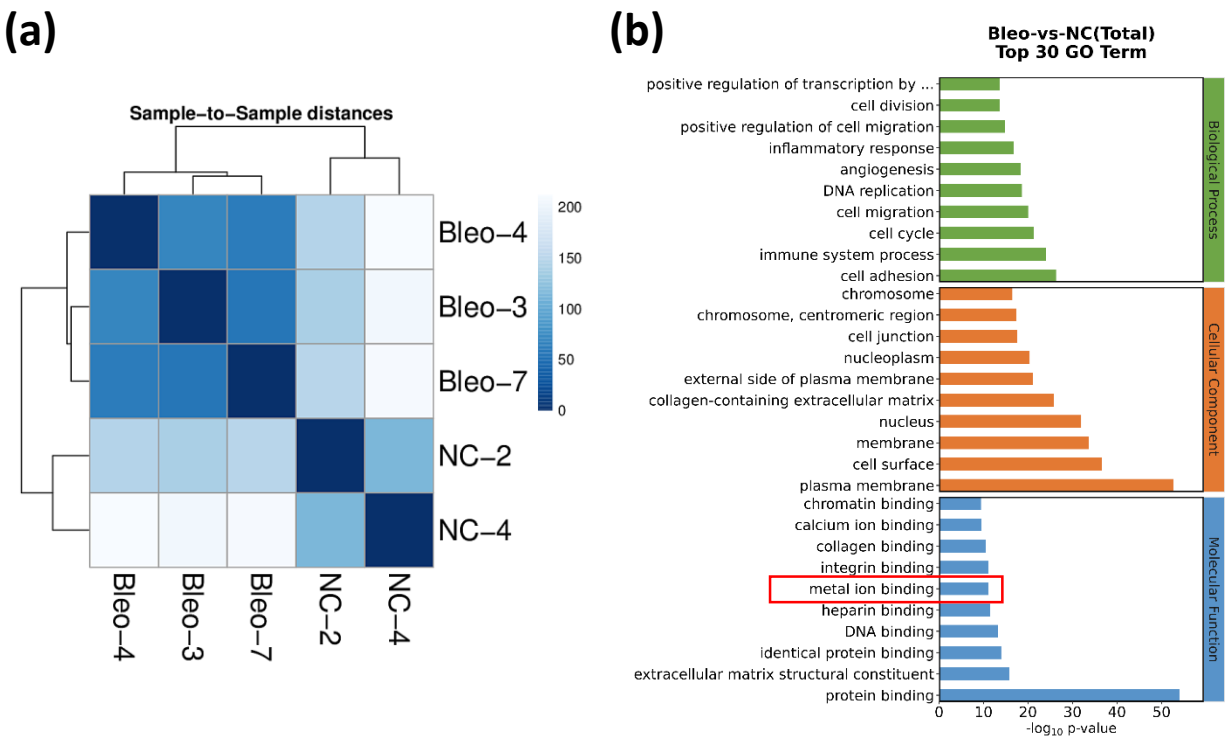


Figure S1 Sample distances and GO analysis of the Bleo *vs* NC mice. (a) Sample-sample distances were shown to evaluate the sample homogeneity. (b) GO analysis revealed the top 30 changed pathways upon Bleo *vs* NC mice.

Fig. S2 Luan et al.

(a)

Gene_id	Fold Change	Log2 Fold Change	p-value	q-value	Regulation
Clic1	0.603078	-0.72958	0.001575	0.003828	Down
Clic2	-	-	-	-	-
Clic3	15.56156	3.959914	5.14E-05	0.000174	Up
Clic4	4.084825	2.030274	1.65E-09	1.41E-08	Up
Clic5	9.603676	3.263587	2.55E-30	2.76E-28	Up
Clic6	2.975965	1.573358	0.541011	0.617205	Up

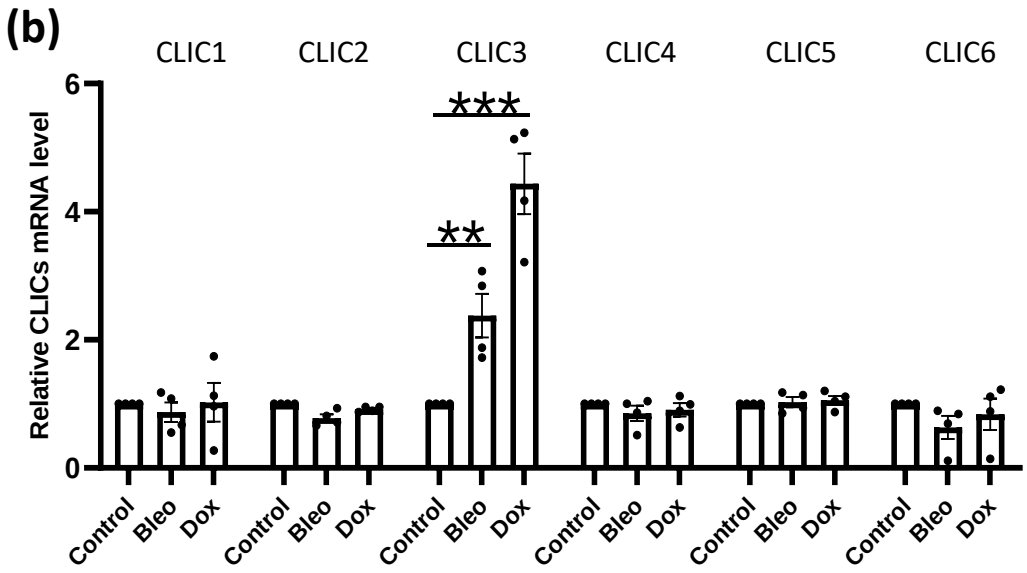


Figure S2 Expression profile of chloride intracellular channels. (a) Expression of *Clic* members from RNA-seq. (b) Detection of *CLIC1-CLIC6* mRNA levels in MRC5 after two days treatment with Bleo and Dox (n=4). Statistical analysis: paired one-way ANOVA test (**P < 0.01 and ***P < 0.001).

Fig. S3 Luan et al.

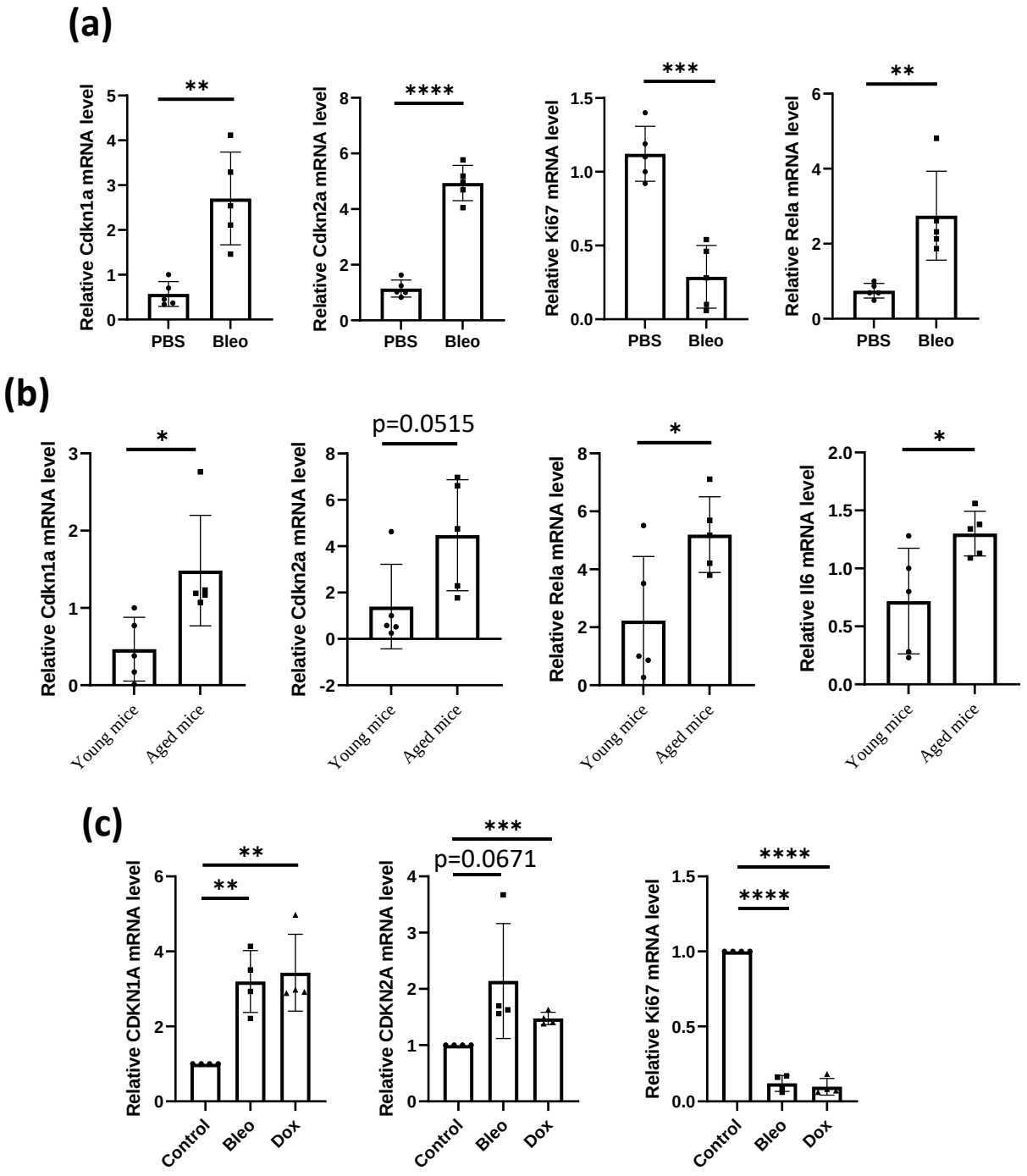


Figure S3 Validation of different aging and senescence models. (a) Detection of *Cdkn1a, Cdkn2a, Ki67* and *Rela* mRNA levels in the lung of bleomycin treated mice (n=5). (b) Detection of *Cdkn1a, Cdkn2a, Rela* and *Il6* mRNA levels in the lung of naturally aged mice (n=5). (c) Detection of *CDKN1A, CDKN2A* and *Ki67* mRNA levels in WI38 treated with Bleo or Dox (n=4). Statistical analysis: unpaired two-tailed student's t-test for (a,b), paired one-way ANOVA test for (c). (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Fig. S4 Luan et al.

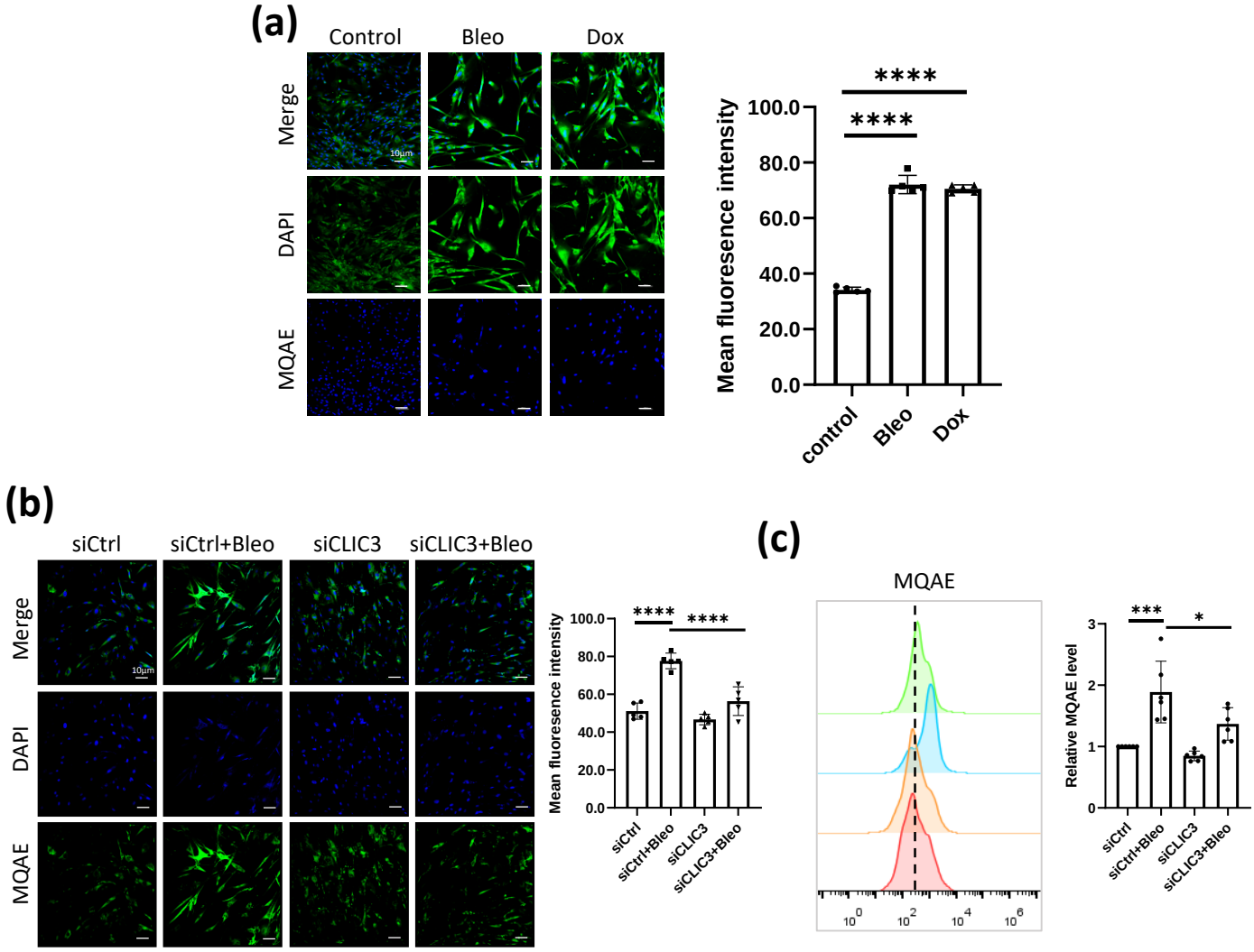


Figure S4 Chloride is accumulated during cellular senescence. (a) WI38 cells were treated with Bleo or Dox, two days later, cells were stained with MQAE and DAPI. Representative photos and calculation of the mean fluorescence intensity were shown (example of more than 3 biological repeats). (b,c) WI38 was transfected with siCtrl or siCLIC3 and was treated with Bleo in the next day. (b) Three days after transfection, cells were stained with MQAE and DAPI. Representative photos and calculation of the mean fluorescence intensity were shown (example of more than 3 biological repeats). (c) Three days after transfection, cells were analyzed with flow cytometry after being incubated with MQAE, cell frequency deviations and the average of biological repeats ± SD were shown (n=6). Statistical analysis: unpaired one-way ANOVA test for (a,b), paired one-way ANOVA test for (c). (*P < 0.05, ***P < 0.001 and ****P < 0.0001).

Fig. S5 Luan et al.

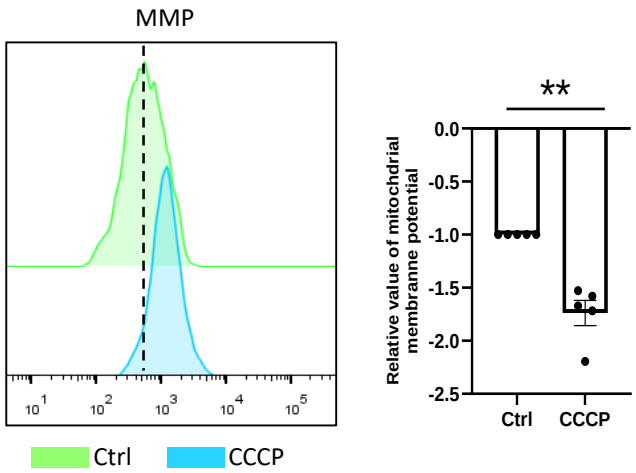


Figure S5 CCCP promotes a drop of MMP. WI38 was treated with CCCP for eight hours. Cells were analyzed with flow cytometry after being incubated with MMP drop indicator Rhodamine123 (n=5). Statistical analysis: paired two-tailed student's t-test (**P < 0.01).

Fig. S6 Luan et al.

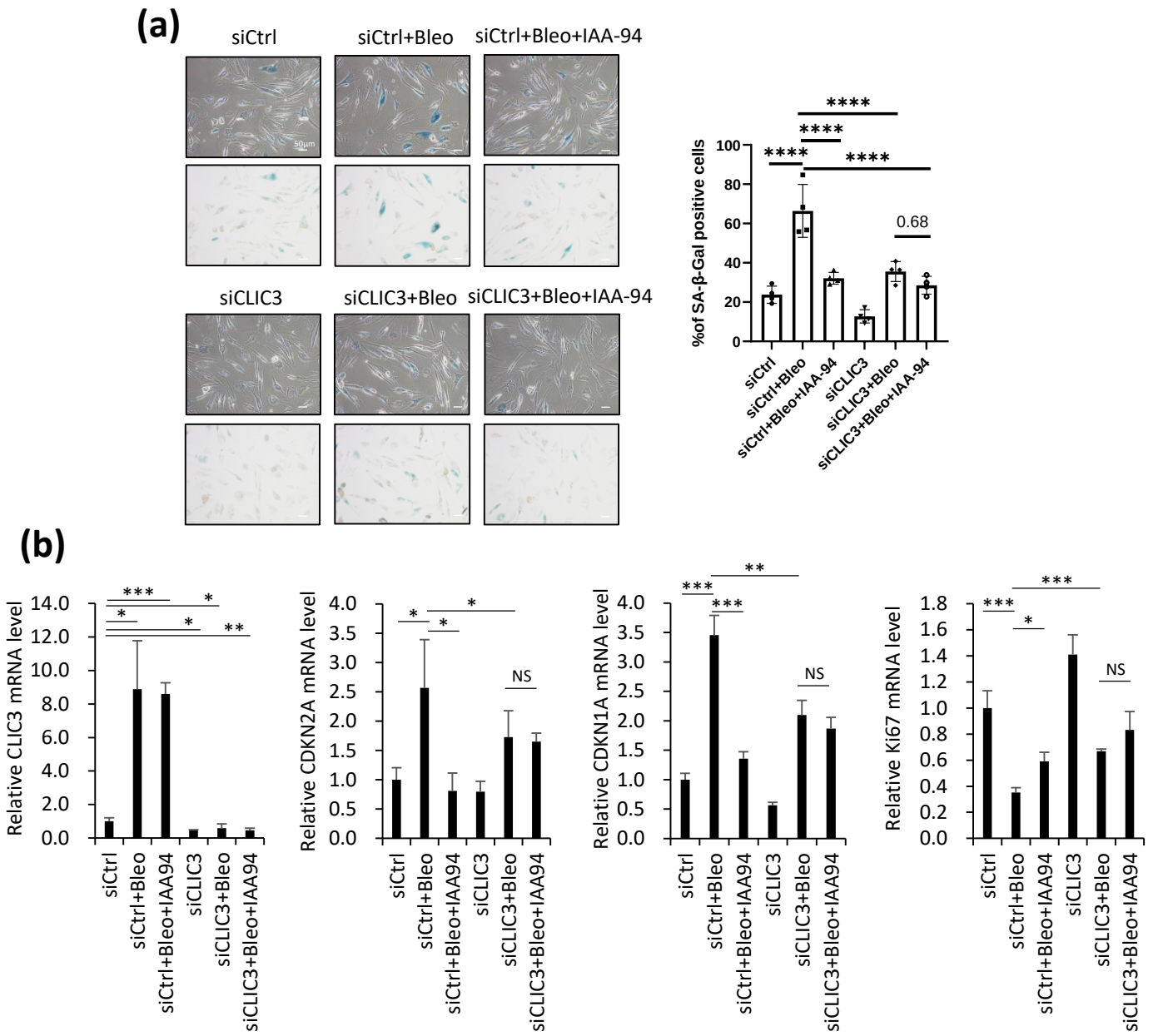
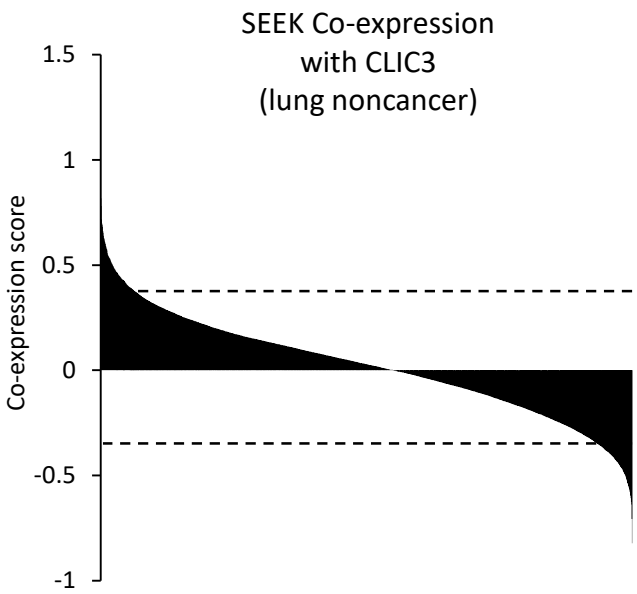


Figure S6 CLIC3 is key among chloride channels during senescence regulation. WI38 was transfected with siCtrl or siCLIC3 and was treated with Bleo or IAA-94 where indicated. (a) Six days post transfection, cells were fixed and stained with SA-β-Gal staining solution. Representative images and the percentage of SA-β-Gal positive cells were shown. (b) Three days after transfection, mRNA levels of *CLIC3*, *CDKN2A*, *CDKN1A* and *Ki67* were detected by RT-qPCR (example of more than 3 biological replications). Statistical analysis: unpaired one-way ANOVA test. (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Fig. S7 Luan et al.



Symbol	Co-expression score	P value
IGFBP6	0.6469	0.0037
IL32	0.6128	0.0016
IL2RB	0.562	0.0115
CX3CR1	0.5444	0.0029
IL11RA	0.5389	0.0149
CCL5	0.5134	0.0215
IGFBP3	0.4266	0.0179
CXCR3	0.4233	0.0695
CXCR6	0.4141	0.0449

Figure S7 SEEK co-expression of CLIC3 with SASP genes. The SEEK co-expression database was interrogated for SASP associated genes co-expressed with CLIC3 in lung noncancer samples (threshold 0.4).

Fig. S8 Luan et al.

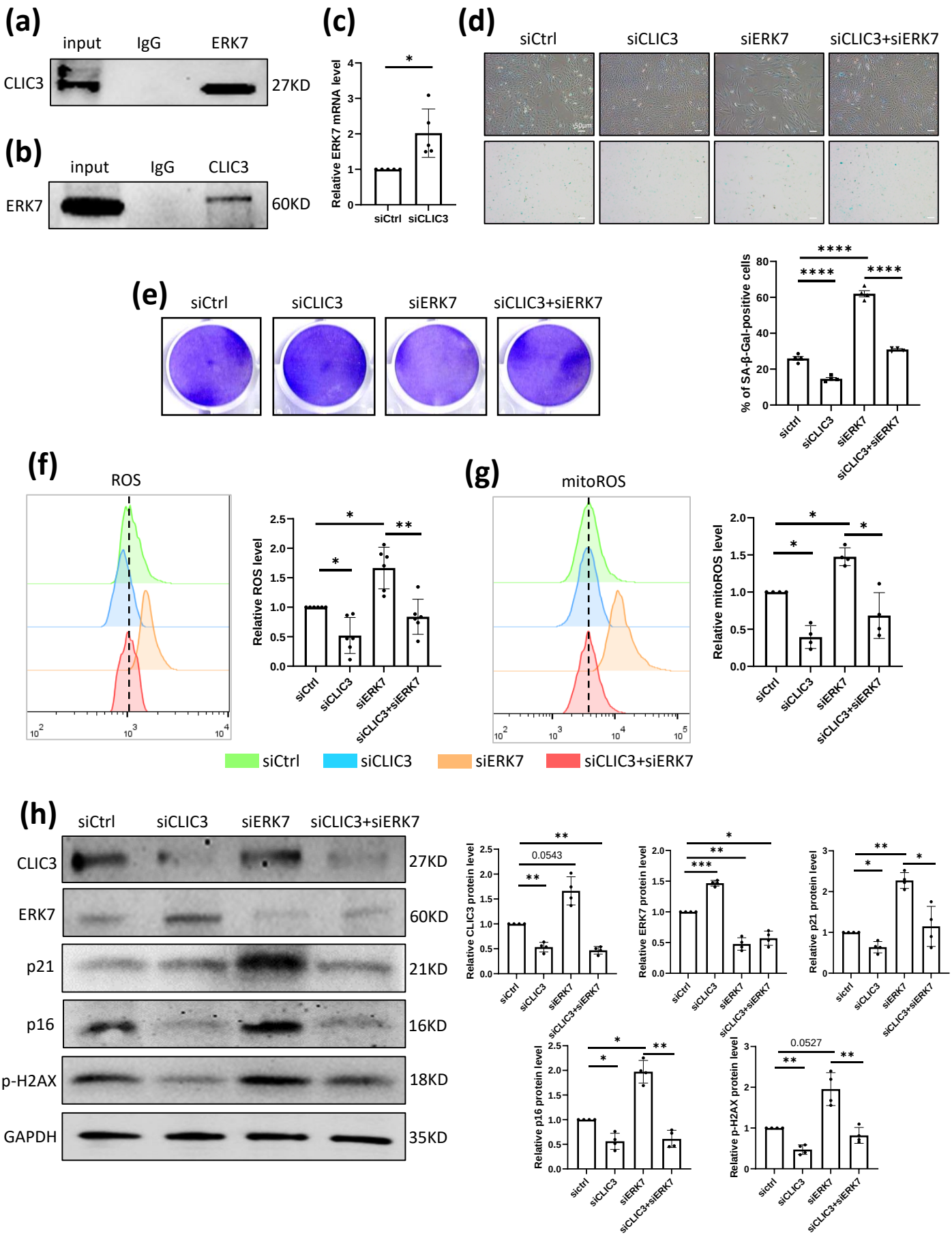


Figure S8 CLIC3 triggers cellular senescence through repressing ERK7 in lung epithelial cells. The interaction between CLIC3 and ERK7 was detected by co-immunoprecipitation with anti-IgG and anti-ERK7 (a) or anti-CLIC3 (b) antibodies in 293T cells (example of more than 3 repeats). (c) BEAS-2B was transfected with siCtrl or siCLIC3. Three days after transfection, mRNA level of *ERK7* (n=5) was detected by RT-qPCR. (d-h) BEAS-2B was transfected with siCtrl or siRNA against CLIC3 and ERK7 as indicated. Six days after transfection, cells were fixed and stained with either crystal violet (e), or SA-β-Gal staining solution (d). Representative images and the percentage of SA-β-Gal positive cells were shown (example of more than 3 biological replications). Three days after transfection, cells were analyzed with flow cytometry after being incubated with dihydroethidium (f) or mitoSOX RED mitochondrial (g), cell frequency deviations and the average of biological repeats $\pm$ SD were shown (n=6 for (f), n=4 for (g)). (h) Three days post transfection, the protein levels of CLIC3, ERK7, p21, p16, p-H2AX and GAPDH were quantified by western blot. Representative photos and biological repeats were shown (mean $\pm$ SD, n=4). Statistical analysis: paired two-tailed student's t-test for (c), unpaired one-way ANOVA test for (d), and paired one-way ANOVA test for (f-h). (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Fig. S9 Luan et al.

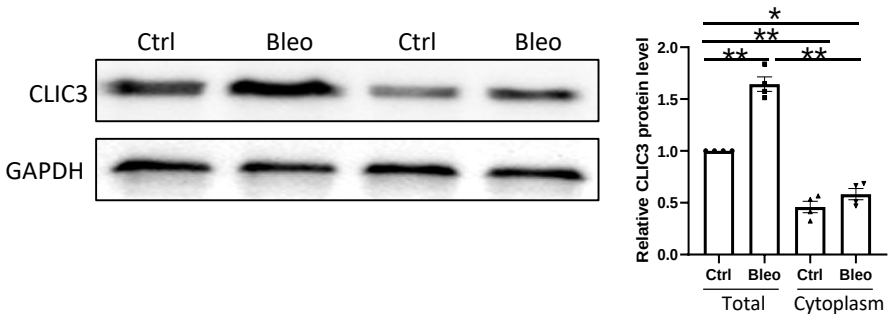


Figure S9 Detection of CLIC3 in whole cell lysate and cytoplasm. Two days after Bleo treatment in WI38, the whole cell lysate and cytoplasm fraction were extracted and the protein levels of CLIC3 and GAPDH were quantified by western blot. Representative photos and biological repeats were shown (mean ± SD, n=4). Statistical analysis: paired one-way ANOVA test. (*P < 0.05 and **P < 0.01).

Fig. S10 Luan et al.

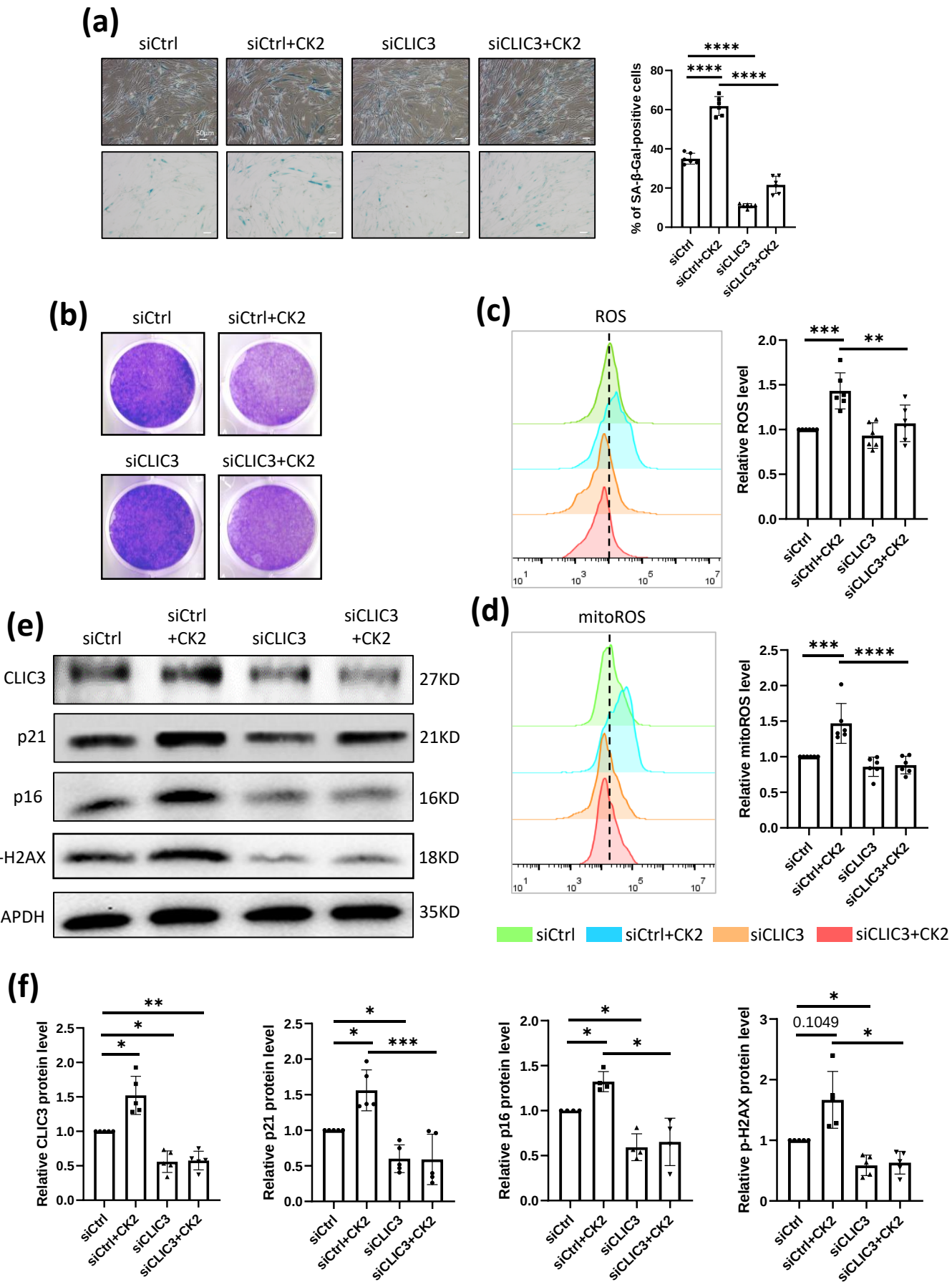


Figure S10 Knockdown of CLIC3 delays cellular senescence induced by CK2 in fibroblasts. WI38 was transfected with siCtrl or siCLIC3 and was treated with CK2 in the next day. Six days after transfection, cells were fixed and stained with either crystal violet (b), or SA-β-Gal staining solution (a). Representative images and the percentage of SA-β-Gal positive cells were shown (example of more than 3 biological replications). Three days after transfection, cells were analyzed with flow cytometry after being incubated with dihydroethidium (c) or mitoSOX RED mitochondrial (d), cell frequency deviations and the average of biological repeats ± SD were shown (n=6). (e,f) Three days post transfection, the protein levels of CLIC3, p21, p16, p-H2AX and GAPDH were quantified by western blot. Representative photos and biological repeats were shown (mean ± SD, n=5 for CLIC3, p21 and p-H2AX, n=4 for p16). Statistical analysis: unpaired one-way ANOVA test for (a), paired one-way ANOVA test for (c,d,f). (*P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001).

Table S1 Human primers used for RT-qPCR in this study.

Gene symbol	Forward primers	Reverse primers
PGK1	GCCAAGTCGGTAGTCCTTATG	CCCAGCAGAGATTTGAGTTCTA
HPRT1	CGAGATGTGATGAAGGAGATGG	TTGATGTAATCCAGCAGGTCAG
CDKN1A	CGGAACAAGGAGTCAGACATT	AGTGCCAGGAAAGACAACTAC
Ki67	GACCTCAAACTGGCTCCTAATC	GCTGCCAGATAGAGTCAGAAAG
CLIC3	ATCGAGGACTTTCTGGAGGA	TCTTGATGAACGCGGAGAAC
CDKN2A	GCACATTCATGTGGGCATTT	GACTCAAGAGAAGCCAGTAACC
IL6	GGAGACTTGCCTGGTGAAA	CTGGCTTGTTCCTCACTACTC
PFKM	GGCTGACACAGCACTCAATA	GTAGCCACCCATAGTCTCAATG
PKM	CCACCCAATCAAGGGAAGAA	GGACAGAGTACACACAGGAAAG
CS	TCCACAGAATACAAGCCACTAC	CTGGTCATTCCGGAGTTCTATG
IDH1	CTAAGGGTTGGCCTTTGTATCT	GGGACTTGTACTGCTTGTCATA
DLD	AGCTGCTTCTGGTGGTAAAG	CAATTCCCAGCTCTTCTAGTCC
SDHA	TGGGAAGGTCACTCTGGAATA	GTCTCATCAGTAGGAGCGAATG
FH	CTGGATCAGGAACTCAGACAAA	TGCCAAGTTCACCTCCTAAC
ME1	TTGTGGCGTGTGGATTGA	CGACCCTCTTCCAAGTGTTTAT
IGFBP6	TGGAGCTGTCATCACTCAAC	GCCAACACCAACACTCTTTC
IL32	GAGACAGTGGCGGCTTATTA	GGCACCGTAATCCATCTCTT
IL2RB	CACCAACCAGGGTTACTTCTT	ATCAGGGTCTTCCTCTGAGTAG
CX3CR1	CCAAGCTAGAATTCCCTCTCTC	CAGTCCCTCTCTGGTGATAAAG
IL11RA	GTCTTGCTGGTGTGGATAGAA	ATACAGGGTCTCACAGAAATGG
CCL5	CTTGAAGGGCCCAGATTCTAC	TCCCAAAGTGCTGGGATTAC
IGFBP3	GCGCTACAAAGTTGACTACGA	TCTACGGCAGGGACCATATT
CXCR3	GGTGCCCTCTTCAACATCAA	CTGGGTGGCATGAACTATGT
CXCR6	GACTACTGGGCAAAGAGTGTAG	GGCAGAGCCTGATCTCATTT
CLIC1	GCCTCCTAAAGTGTTGGGATTA	TGGGATTGGGCATAGGAAATAG
CLIC2	GAACTTAACTCTTGCTCCCTCAA	CCCTTCCACCAAAGCCTAAA
CLIC4	AGTACACAGGCAGGCATTATC	TAAGCTATGAGGCAAGGTTCAG
CLIC5	CAGGAGAATGGGAGTGTGATG	GCAGGAATACTTGGTGGTAGAG
CLIC6	CCAGTTAGGGAGGGAGTAGAA	CTGTGATGAGAGGTTGGTTAGG
ERK7	GATAAGACAGATGCCCAGAGAAC	GTCAAGGAGGCTGATGATGTT

Table S2 Murine primers used for RT-qPCR in this study.

Gene symbol	Forward primers	Reverse primers
Pgk1	CACAGAAGGCTGGTGGATTT	CTTTAGCGCCTCCCAAGATAG
Gapdh	AACAGCAACTCCCACTCTTC	CCTGTTGCTGTAGCCGTATT
Cdkn1a	GTTCCTTGCCACTTCTTACCT	TCATCCTAGCTGGCCTTAGA
Cdkn2a	GCTGGGTGGTCTTTGTGTA	TTAGCTCTGCTCTTGGGATTG
Rela	CCGACTTGTTTGGGTGATCT	TCCGTCTCCAGGAGGTTAAT
Clic3	CTCTTTGTGAAGGCCAGTGA	TGAGGAGTAGGACCATGAAGAG
Ki67	CCACACAGATGCCCTGTAAT	CTCTACTTTCCCACGTCTTGTC
Il6	GTCTGTAGCTCATTCTGCTCTG	GAAGGCAACTGGATGGAAGT

Table S3 Antibodies and probes used in this study.

Application	Name	Reference	Company	Dilution
IF/CO-IP/WB	CLIC3	sc-390006	santa cruz	1:200/1:200/ 1:300
CO-IP/WB	ERK7	13452	Proteintech	1:200
IF	53BP1	4937	Cell Signaling Technology	1:200
WB	p-H2AX	sc-517348	santa cruz	1:300
WB	p16	sc-56330	santa cruz	1:100
WB	p21	sc-6246	santa cruz	1:300
WB	GAPDH	sc-365062	santa cruz	1:500
WB	ATP1A1	14418-1-AP	Proteintech	1:500
WB	α-tubulin	T6199	Sigma	1:1000
FACS	Rhodamine123 (2mM)	R8004	Sigma	1:3000
FACS	mitoSOX RED mitochondrial (1μg/μl)	M36008	Invitrogen	1:166.67
FACS	Dihydroethidium (5μM)	D7008	Sigma	1:100

Table S4 Sequences of siRNAs used in this study.

siRNA name	Sequences	Company
siRNA control	GGCTCTAGAAAAGCCTATGC	GeneAdv
siCLIC3-1	TCTCCGCGTTCATCAAGAA	GeneAdv
siCLIC3-2	AGGAGAAAGAGTTCAAATA	GeneAdv
siCLIC3-3	CTCGTTACAGGGAGTCCAA	GeneAdv
siERK7-1	CCUAUGGCAUUGUGUGGAATT	GeneAdv
siERK7-2	GCUCCAAACUGCUCUCCUATT	GeneAdv
siERK7-3	CAGAGAACAUUCCGGGAAATT	GeneAdv