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Photoactivatable senolysis with single-cell resolution delays aging

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Strategies that can selectively eliminate senescent cells (SnCs), namely senolytics, have been shown to promote healthy lifespan. However, it is challenging to achieve precise, broad-spectrum and tractable senolysis. Here, we integrate multiple technologies that combine the enzyme substrate of senescence-associated β -galactosidase (SA- β -gal) with fluorescence-tag for the precise tracking of SnCs; construction of a bioorthogonal receptor triggered by SA- β -gal to target and anchor SnCs with single-cell resolution; and incorporation of a selenium atom to generate singlet oxygen and achieve precise senolysis through controllable photodynamic therapy (PDT). We generate KSL0608-Se, a photosensitive senolytic prodrug, which is selectively activated by SA- β -gal. In naturally-aged mice, KSL0608-Se-mediated PDT prevented upregulation of age-related SnCs markers and senescence-associated secretory phenotype factors. This treatment also countered age-induced losses in liver and renal function as well as inhibited the age-associated physical dysfunction in mice. We therefore provide a strategy to monitor and selectively eliminate SnCs to regulate aging.

Aging is the leading risk factor associated with numerous pathologies, including cancer and fibrosis¹⁻³. A fundamental aging mechanism has been proposed to be linked to the excessive, usually time-dependent, development of cellular senescence, a stable and stagnant terminal state that occurs after stress-induced cellular damage^{1,4}. This accumulation drives inflammation-mediated tissue dysfunction and aging by promoting the secretion of proinflammatory and matrix-degrading molecules (known as the senescence-associated secretory phenotype, SASP)^{5,6}. The selective removal of senescent cells (SnCs), termed “senolysis”, can alleviate these age-related features and extend the healthy lifespan, as supported by recent investigations in mice⁷, leading to an exploration of therapeutic approaches towards senolysis⁸⁻¹³.

Surgical removal of aging tissue is currently used as a therapeutic approach for the motor dysfunction caused by the accumulation of SnCs. However, because this treatment is limited to muscle aging and causes trauma and pain to patients, a better approach would be to selectively eliminate SnCs with senolytic drugs (senolytics). Senolytics were initially selected based on their ability to transiently switch off senescence-associated antiapoptotic pathways¹⁴⁻¹⁸. Typical examples include ABT-737 and ABT-263, which target SnCs to induce apoptosis by inhibiting antiapoptotic proteins in the BCL-2 family^{8,19-22}. Some natural senolytics, such as quercetin and fisetin, have also proved effective in influencing aging and age-related diseases^{16,23-25}. However, SnCs exhibit great heterogeneity and dynamics *in vivo*^{9,10,26-28}, resulting in significant limitations of these senolytics in terms of accuracy (no toxicity to non-SnCs), tractability (administration controllability) and broad-spectrum activity (effectiveness against different types of SnCs). Even though there are emerging strategies to address these issues, including glutaminolysis inhibition²⁶ and enzyme-targeted prodrugs^{9,10}, to the best of our knowledge, no report has described a smart, senotherapeutic agent able to achieve all these aims.

The integration of multiple technologies is an appealing way to achieve the development of such desirable senolytics. Photodynamic therapy (PDT) is a contemporary therapeutic technique that can destroy target cells by

55 activating a photosensitive drug with the aid of a light source²⁹⁻³², which offers the potential to improve the
56 tractability of senolytics due to the dependence on light illumination. Meanwhile, the prodrug strategy targeting
57 senescence-associated β -galactosidase (SA- β -gal) for senolysis has shown advantages in broad-spectrum activity
58 since the enhanced activity of this enzyme is a common feature of SnCs^{9,33,34}. A further challenge in the targeting
59 of small-molecule drugs in general is that they readily exit their target cells due to rapid transport across cell
60 membranes and enter the surrounding normal cells due to free diffusion. The utilization of emerging anchoring
61 technologies that can covalently bind drugs to sites of interest through bioorthogonal reactions³⁵⁻⁴⁰ could limit
62 such off-target effects.

63 Accordingly, we here report the construction of a smart senotherapeutic agent, **KSL0608-Se**, that integrates
64 PDT with β -gal-targeted prodrug and target-site anchoring technologies to achieve tractable, broad-spectrum
65 activity and accuracy. This integrated strategy enables the selective activation of **KSL0608-Se** by SA- β -gal to
66 form a bioorthogonal receptor (quinone methide). The active receptor covalently reacts with exposed nucleophilic
67 groups (e.g., sulfhydryls) on the surfaces of surrounding proteins, converting the prodrug into a near-infrared
68 (NIR)-emitting senolytic drug. The bioorthogonal reaction and fluorescence off-on behaviour occur only after
69 activation by SA- β -gal, which allows selective monitoring of and precise anchoring to SnCs. The newly formed
70 drug can be activated by light *in situ* to produce singlet oxygen in SnCs, resulting in photocontrollable senolysis
71 at single-cell resolution. **KSL0608-Se** achieved the specific recognition and selective clearance of SnCs in a
72 complex coculture system of young cells and SnCs. Of note, in naturally aged mice and mice treated with
73 doxorubicin (doxo), the **KSL0608-Se**-guided therapy effectively decreased the number of SA- β -gal-positive cells
74 and inhibited the expression of age-related genes and markers. This treatment also showed the ability to improve
75 age-associated physical dysfunctions in naturally aged mice. In brief, our unprecedented integration strategy can
76 effectively eliminate SnCs and exhibit the potential to counteract aging and ameliorate age-related diseases.

77 Results

78 **Design of a photoactivatable prodrug for senolysis.** Our main focus in designing this senolytic prodrug was
79 ensuring the accurate delivery of the photosensitive drug to SnCs for precise and controllable yet complete
80 senolysis with single-cell resolution. We then report a prodrug strategy to design a photosensitive senolytic agent
81 activated by SA- β -gal, an enzyme both specific to and widespread among SnCs. The synthesis starts with the
82 modification of a dicyanomethylene-4H-pyran (DCM) based skeleton with favorable donor- π -acceptor (D- π -A)
83 characteristics and a phenolic group for regulating electron-donating capability (Fig. 1a). We then replaced the
84 oxygen (O) atom on the skeleton with a selenium (Se) atom to enhance singlet oxygen generation upon
85 photoirradiation. It is believed that this effect is attributed to the increase in molecular dipoles and the enhanced
86 intersystem crossing ability (ISC)^{30,41,42}. To this photosensitive scaffold, a β -D-galactosyl group was attached as
87 both the SA- β -gal-responsive site and the phototoxicity blocking group, and a fluoromethyl was added as a
88 self-immobilizing moiety, creating the photosensitive prodrug **KSL0608-Se**. This prodrug is converted to a
89 bioorthogonal quinone-methide receptor *via* a 2-fluoromethylphenol intermediate immediately after its specific
90 hydrolysis by SA- β -gal. This receptor is unique in that it can covalently attach to nearby proteins and selectively
91 generate a photosensitive drug *in situ* in SnCs (Fig. 1b). Based on the same design strategy, a self-immobilizing
92 SA- β -gal fluorescent probe with O instead of Se, **KSL0608-O**, was synthesized (Fig. 1a). After hydrolysis by
93 SA- β -gal, **KSL0608-O** exhibits fluorescence similar to **KSL0608-Se** but does not produce a phototoxic substance,
94 thus it is also a desirable control. Accordingly, **KSL0608-O** and **KSL0608-Se** were synthesized as outlined in Fig.
95 1a, described in more detail in the Supplementary Information (Section “Synthesis and characterization” and
96 Supplementary Figs. 12-55).

97
98 **Spectral response towards β -galactosidase (β -gal).** The fluorescence spectra and the absorption spectra of
99 **KSL0608-O** and **KSL0608-Se** were evaluated. *E. coli* β -gal, as the SA- β -gal model protein, was used in catalytic
100 amounts for substrate recognition, and bovine serum albumin (BSA), with a strongly nucleophilic sulfhydryl
101 group⁴³, was supplied in excess in the recognition system to provide a model protein substrate for the reaction
102 with the quinone-methide receptor. As expected, prior to treatment with *E. coli* β -gal and BSA, both **KSL0608-O**
103 and **KSL0608-Se** exhibited faint fluorescence since the hydroxyl group was caged by a β -D-galactosyl group,
104 suppressing the intramolecular charge transfer (ICT) process. After treatment with *E. coli* β -gal, both compounds
105 exhibited negligible fluorescence changes. However, in the presence of BSA, the addition of *E. coli* β -gal
106 stimulated a significant fluorescence increase at 665 nm for **KSL0608-O** and at 721 nm for **KSL0608-Se** (Fig.
107 2a,b). In addition, the absorption spectra exhibited a red-shift upon the addition of both *E. coli* β -gal and BSA
108 (Supplementary Fig. 1). To our delight, the addition of BSA into the **KSL0608-O/Se** system provoked a large
109 fluorescence enhancement, while adding other nucleophilic small molecules did not (Extended Data Fig. 1a,b).
110 This should be attributed to the enhanced rigidity of the fluorophore after binding with proteins like BSA,
111 although these small-molecule species can also react with the quinone-methide receptor. Furthermore,
112

113 SDS-PAGE and in-gel fluorescence imaging were performed to confirm that both compounds could covalently
114 modify proteins after enzymatic activation. An intense fluorescent band was observed at the expected molecular
115 weight for BSA in the group containing **KSL0608-O/KSL0608-Se**, *E. coli* β -gal and BSA (Fig. 2c). In contrast,
116 no fluorescence signal was observed in the absence of BSA or *E. coli* β -gal. These results confirm that the
117 hydrolysis of the two compounds by *E. coli* β -gal leads to the formation of fluorescent adducts through
118 electrophilic intermediates capable of covalently reacting with surrounding proteins.

119 Emission titration experiments of both compounds with *E. coli* β -gal at different concentrations were then
120 conducted. A dramatic increase in the NIR fluorescence of **KSL0608-O** at ~ 665 nm was elicited by *E. coli* β -gal,
121 and a linear relationship between fluorescence intensity and *E. coli* β -gal concentration was observed, with a
122 correlation coefficient (R^2) greater than 0.96 (Fig. 2d). Similarly, upon the addition of *E. coli* β -gal, an increase in
123 the NIR fluorescence of **KSL0608-Se** at ~ 721 nm appeared, again with a good linear relationship ($R^2 > 0.99$)
124 (Fig. 2e). Accordingly, the limits of detection (LOD) of β -gal by **KSL0608-O** and **KSL0608-Se** were calculated
125 to be 3.12×10^{-3} U/mL and 8.96×10^{-2} U/mL, respectively (Supplementary Table 1). Furthermore, a molecular
126 docking simulation of **KSL0608-O** with human β -gal (PDB: 3THC) was performed. Multiple hydrogen bonds
127 form between the galactosyl group of **KSL0608-O** and human β -gal, and the binding model significantly
128 overlaps with that adopted by galactose (Fig. 2f,g), suggesting that **KSL0608-O** could be fully hydrolyzed by
129 human β -gal to release the active precursor. The above results indicated that both compounds could be activated
130 by β -gal and immobilized on proteins of interest *in situ*, suggesting their ability to monitor fluctuations in β -gal
131 and the feasibility of our SA- β -gal-activatable prodrug and target-site anchoring design strategy.

132 The reaction kinetics of **KSL0608-O** and **KSL0608-Se** with β -gal were further investigated. After the
133 addition of *E. coli* β -gal and BSA, the fluorescence intensity for the two compounds was markedly enhanced and
134 reached a maximum within 10 min (Extended Data Fig. 1c,d). Additionally, the effect of pH on the fluorescence
135 response of **KSL0608-O/KSL0608-Se** to β -gal over a wide pH range (4-10) was then investigated. Both
136 compounds were stable and displayed a good response to β -gal over this pH range, ensuring their ability to track
137 β -gal at physiological pH (Extended Data Fig. 1e,f). Next, their photostability for the detection of β -gal was
138 evaluated and the results indicated that both compounds exhibit high photostability and the potential to track
139 β -gal over a long duration *in vivo* (Extended Data Fig. 1g,h).

140
141 **Photoinduced ROS generation *in vitro*.** We then examined the ability of **KSL0608-O** and **KSL0608-Se** to
142 convert oxygen into reactive oxygen species (ROS) after photoirradiation using a commercial ROS indicator
143 1,3-diphenylisobenzofuran (DPBF)⁴⁴⁻⁴⁷. Under light irradiation, the absorption of DPBF remained constant in the
144 presence of **KSL0608-O** or **KSL0608-Se** alone, implying that neither triggers ROS generation associated toxicity
145 even when exposed to light (Fig. 2h). In contrast, the absorption of DPBF in the group containing **KSL0608-Se**,
146 *E. coli* β -gal and BSA decreased rapidly within 1 min, confirming the efficient generation of ROS once
147 **KSL0608-Se** was activated (Fig. 2h,i). Conversely, no significant change was observed in that of the group
148 containing **KSL0608-O**, *E. coli* β -gal and BSA, indicating that replacement of the Se atom with an O atom
149 significantly decreased the ability to generate ROS. Consistent with this, the singlet oxygen quantum yields (Φ_{Δ})
150 of **KSL0608-O** and **KSL0608-Se** were calculated to be 0.07 and 0.20 and their relative fluorescence quantum
151 yields (Φ_f) were 0.33 and 0.08, after the addition of *E. coli* β -gal and BSA (Supplementary Table 1). These
152 results confirmed that **KSL0608-Se** does not produce ROS by itself, but can generate an effective photosensitive
153 drug *in situ* after being triggered by the target protein.

154
155 **Endogenous β -gal imaging in live cells.** Prior to cell imaging, the cytotoxicity of our compounds was evaluated
156 and both compounds exhibited low cytotoxicity (Supplementary Fig. 2). Human ovarian cancer cells (SKOV3)
157 with high endogenous β -gal levels, and human liver cancer cells (HepG2) containing low endogenous β -gal, were
158 incubated separately with our compounds. A marked NIR fluorescence signal was observed in SKOV3 cells,
159 whereas no significant fluorescence signal appeared in HepG2 cells (Extended Data Fig. 2a,b). D-galactose and
160 phenylethyl β -D-thiogalactopyranoside (PETG), two kinds of β -gal inhibitor^{48,49}, were used to reduce the activity
161 of β -gal in cells. As expected, after treatment with each inhibitor, SKOV3 cells incubated with
162 **KSL0608-O/KSL0608-Se** displayed reduced fluorescence in the NIR channel (Extended Data Fig. 2c,d),
163 supporting the specificity of both compounds for detecting endogenous β -gal in live cells.

164 We then evaluated their capability to monitor β -gal activity in senescent normal cells and senescent cancer
165 cells, including human lung fibroblastic cells (MRC-5) with replication-induced senescence, and rat renal tubular
166 epithelial cells (NRK-52E), human non-small lung cancer cells (A549) and normal human liver cells (HL-7702)
167 with a senescent phenotype mediated by DNA damage. Such senescent cells with DNA damage were obtained by
168 stimulations with ROS for NRK-52E, Mitomycin C (MitoC) for A549 and doxo for HL-7702^{14,15}. After
169 incubation with **KSL0608-O** or **KSL0608-Se**, all the senescent cells emitted stronger NIR fluorescence than the
170 corresponding young cells with low expression of β -gal (Fig. 3a-c). The results of X-gal staining and western blot

171 assays for p21 and p53 of young cells and SnCs were in good agreement with the above results (Fig. 3a and
172 Supplementary Fig. 3). These results confirmed that both compounds could monitor SA- β -gal in live cells.
173

174 **Lysosome-targeted function of KSL0608-O and KSL0608-Se.** Senescent MRC-5 cells (P40) were
175 co-incubated with **KSL0608-O** or **KSL0608-Se** and commercial staining dyes targeting different organelles. The
176 NIR fluorescence overlapped well with the green fluorescence of LysoTracker in senescent MRC-5 cells. In
177 contrast, there was poor overlap between the fluorescence in the NIR channel and that in the green channel for
178 MitoTracker or GolgiTracker (Fig. 3d,e and Supplementary Fig. 4). These results indicated that our molecules
179 responsible for NIR fluorescence accumulated mainly in the lysosomes of SnCs, suggesting that they can
180 function *in situ* through enzymatic reaction with the endogenous SA- β -gal located in lysosomes.
181

182 **Light-controllable removal of SnCs in a coculture system.** We chose a safe dose (12 J/cm²) for the following
183 PDT experiments in living cells (Extended Data Fig. 3a-d) and confirmed the ability of this dose of light to
184 induce ROS (Fig. 4a,b). From Fig. 4c, upon light irradiation, **KSL0608-Se** exhibited the specific dose-dependent
185 phototoxicity to these senescent cells at 24 h. Through our treatment, over 65% of the drug-induced senescent
186 cells were killed. The **KSL0608-Se**-mediated PDT also exhibited phototoxicity towards P40 MRC-5 cells whose
187 senescence degree was higher than that of MRC-5 cells at P28 (Fig. 4c). We next test its cytotoxicity at 48 h and
188 72 h and found that no significant toxicity to all the young cells appeared (Extended Data Fig. 3e-l). Of note,
189 upon the prolonged incubation time, this treatment is a little phototoxic to MRC-5 cells at P28, not a low passage
190 for normal cells, but the phototoxicity was far lower than that to more senescent MRC-5 cells (P40) (Extended
191 Data Fig. 3h,l). Furthermore, no dark cytotoxicity was observed in both young and senescent cells within 3 days
192 after treatment. The above cytotoxicity test results supported our claim of the photoactivatable senolysis
193 (Extended Data Fig. 4). Flow cytometry analysis using Annexin V/PI double staining was used to further confirm
194 the selective phototoxicity of our treatment to SnCs. As expected, we observed the number of apoptotic cells
195 among the MRC-5 cells (P40) higher than that among the MRC-5 cells (P28) (Fig. 4d,e). Interestingly, the
196 **KSL0608-Se**-mediated PDT was desirably only sensitive to the RAW264.7 cells (a macrophage with a certain
197 β -gal expression ability) matured by drug induction, but not to the uninduced RAW264.7 cells (Supplementary
198 Fig. 5), which can be explained by the overexpression of β -gal in macrophages occurred only at the mature
199 stage⁵⁰. Such *in-vitro* treatment on cells was conducted in PBS buffer without serum, which is a culture condition
200 of nutrient starvation with the risk of evoking cell autophagy. Thereby we evaluated effects of PBS on cells. All
201 the results indicated that PBS had negligible cytotoxicity and did not cause cell autophagy within the treatment
202 time we used (Supplementary Fig. 6), driving out this risk.
203

204 To further verify the specificity of **KSL0608-O** and **KSL0608-Se** to SnCs, a coculture system of SnCs and
205 young cells was built (Extended Data Fig. 5a,b), in which young cells were prelabelled with green fluorescence
206 and then cocultured with SnCs. As shown in Fig. 4f,g and Extended Data Fig. 5c-f, the young cells exhibited
207 negligible fluorescence in the NIR channel, whereas SnCs displayed bright NIR fluorescence, achieving the
208 specific labelling of SnCs. We then investigated the ability of the two compounds to specifically kill SnCs in this
209 coculture system. Cocultured cells were treated with **KSL0608-Se** accompanied by irradiation, and the more
210 senescent MRC-5 cells (P40) exhibited significant cell shrinkage and morphological changes that are
211 characteristic of apoptosis (Fig. 4g); however, the MRC-5 cells (P28) remained unchanged in shape. When
212 **KSL0608-O** was used instead of **KSL0608-Se**, neither the P28 MRC-5 cells nor the P40 MRC-5 cells underwent
213 a change in cell shape (Fig. 4f). These results indicated that both compounds possess the ability to recognize and
214 target SnCs, while **KSL0608-Se** is unique in its capacity to selectively kill SnCs. Furthermore, from
215 Supplementary Fig. 7, multiple fluorescent bands were observed over a wide molecular weight range for the
216 senescent HL-7702 cells incubated with **KSL0608-O** or **KSL0608-Se**, illustrating that the NIR-emitting products
217 were formed and successfully anchored to a variety of proteins in SnCs.
218

219 **Whole-body fluorescence imaging of SnCs *in vivo*.** Probe **KSL0608-O** can be activated and then emit bright
220 fluorescence under the excitation of light at almost the same wavelength as **KSL0608-Se**. This allows the use of
221 **KSL0608-O** in the *in-vivo* fluorescence imaging experiments in mice to verify the penetration ability of the
222 irradiation light used in the **KSL0608-Se**-mediated PDT to solid organs. As shown in Fig. 5a,b, the fluorescence
223 intensity in the abdomen of aged mice gradually increased over time and reached up to a maximum at 96 h,
224 whereas it was negligible in that of young mice, supporting the targeting activation of **KSL0608-O** in aged mice.
225 Additionally, the fluorescence intensity of liver, lung and kidney gained from aged mice was stronger than that
226 from young mice, whereas the heart and spleen from both aged and young mice displayed negligible fluorescence
227 signal (Fig. 5c,d), suggesting senescence mainly occurs in liver, lung and kidney of these aged mice. These
228 results provided the evidence that the irradiation light used in **KSL0608-Se**-mediated PDT has the ability to
229 penetrate tissues.

230 **Selective removal of SnCs in a mouse model with doxo-induced senescence.** We next evaluated the PDT effect
231 of **KSL0608-Se** *in vivo* against age-related pathologies (Fig. 6a). Various aging markers to evaluate the anti-aging
232 efficacy of **KSL0608-Se**-mediated PDT for senolysis were investigated. We assessed the expression levels of
233 γ -histone-2AX (γ -H2AX), a recognized age-related marker^{51,52}. The results indicated that our treatment reduced
234 the expression of γ -H2AX in the livers and kidneys of aged mice (Fig. 6b). Serum biochemical tests showed that
235 the aging induced upregulation of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as
236 age-associated indicators⁵³⁻⁵⁵ was counteracted (Fig. 6c). In addition, the PDT treatment with **KSL0608-Se**
237 markedly decreased the expression level of *p21* and *IL-1 β* , two other markers for aging^{8,53} (Fig. 6d and
238 Supplementary Fig. 8). Consistent with this observation, the expression level of SA- β -gal, as detected by both
239 X-gal and **KSL0608-O** staining, indicated that there were markedly fewer SnCs in the kidneys of aged mice after
240 **KSL0608-Se**-mediated PDT (Fig. 6e). Such results support that our treatment could effectively remove SnCs to
241 inhibit the upregulation of different age-related markers in aged mice.

242 RNA sequencing of the livers from these mice was carried out. Comparison of the gene expression in the
243 young control group and aged control group revealed 362 differentially expressed genes (Fig. 6f,g). There were
244 656 differentially expressed genes between the “**KSL0608-Se** + irradiation” group and the aged control group
245 (Fig. 6f,g). The 128 genes that were differentially expressed in both comparisons were analyzed (Supplementary
246 Fig. 9). As shown in Fig. 6h, 27 genes associated with deleterious effects of aging (SASP and fibrosis phenotypes)
247 were upregulated in doxo-treated mice and downregulated following PDT with **KSL0608-Se**. In addition to these
248 128 genes, 4 genes known to be associated with aging displayed similar but nonsignificant changes in expression.
249 These results strongly support **KSL0608-Se** as a desirable senotherapeutic agent able to delaying aging in mice
250 by reversing the expression of aging-related genes.

251
252 **Selective removal of SnCs in a naturally aged mouse model.** The related experiments were then carried out in
253 naturally aged mice (Fig. 7a). We first tested the levels of age-related indicators in the kidneys and livers of mice
254 in the four groups. The results of western blot analysis indicated the expression level of p53 from the
255 “**KSL0608-Se** + irradiation” group was significantly reduced while that from the **KSL0608-Se** group did not
256 decrease so much (Fig. 7b). We then found that p21 and SA- β -gal positive SnCs from the “**KSL0608-Se** +
257 irradiation” group was much less than those from the aged control group (Fig. 7c,d). Subsequently, we also found
258 the level of uric acid (UA), creatinine (Cr), blood urea nitrogen (BUN), ALT and AST increased in aged mice
259 (Fig. 7e,f). To our delight, the expression level of these indicators in mice from the “**KSL0608-Se** + irradiation”
260 group was downregulated, demonstrating that our treatment could counteract the age-associated loss of renal
261 function and liver function. Notably, the expression of key SASP factors in livers, kidneys and serum, including
262 CXCL1, CXCL3, IL-1 β , IL-6, MMP-1, MMP-7 and TNF- α , increased with age and was significantly reduced
263 after our treatment (Fig. 8a-g and Extended Data Fig. 6a-n). Additionally, p16, a cell cycle regulator whose
264 expression level increased with age, also decreased in level after treatment (Extended Data Fig. 6o). From these
265 results, the treatment used in the “**KSL0608-Se** + irradiation” group exhibited the more powerful ability to
266 decrease the level of all these indicators compared to that in the **KSL0608-Se** group, establishing the
267 irreplaceable role of irradiation in the treatment. Additionally, the results of hematoxylin and eosin (H&E)
268 staining assay confirmed our no-toxic treatment to the main organs of mice (Supplementary Fig. 10). The
269 **KSL0608-Se**-mediated PDT showed an effective photoactivatable senolysis in livers and kidneys of naturally
270 aged mice, and thus successfully ameliorated the age-associated losses of such organs.

271 Naturally aged mice showed significant declines in muscle strength, hanging endurance, locomotor activity
272 and walking speed, time and distance, while all of which were recovered after treatment with
273 **KSL0608-Se**-mediated PDT (Fig. 8h-k and Supplementary Fig. 11). As a control, no obvious signs of recovery in
274 mice from the **KSL0608-Se** group were observed. Additionally, there were no significantly cognitive alterations
275 in all groups of mice (Fig. 8k), further supporting the safety of our treatment. To be brief, our
276 **KSL0608-Se**-mediated PDT could markedly improve the decline symptoms of naturally aged mice in physical
277 function.

278 We then carried out the RNA sequencing analysis of differentially expressed genes in the mouse livers. By
279 comparing the gene expression in the young control group with that in the aged control group, 1399 differentially
280 expressed genes were found (Fig. 8l,m). While 578 differentially expressed genes were observed between the
281 aged control group and the “**KSL0608-Se** + irradiation” group (Fig. 8l,n). As shown in Fig. 8l and Extended Data
282 Fig. 7, there were 146 common genes in these two groups of differentially expressed genes. Among them, after
283 **KSL0608-Se**-mediated PDT, 18 age-related genes upregulated with age were downregulated and 6 genes
284 downregulated with age were upregulated (Fig. 8o). Another 10 genes upregulated with age, which are associated
285 with SASP and fibrosis phenotypes, also were downregulated after our treatment (Fig. 8o). The successful
286 modulation of these age-related genes further confirmed the reliability of our treatment strategy in delaying
287 natural aging.

288

289 Discussion

290 We present a general strategy to construct senolytics, which combines the advantages of tractability,
291 broad-spectrum activity, and accuracy through the integrated use of PDT, β -gal-targeted prodrug and target-site
292 anchoring technologies. Using the strategy, we generated a smart senolytic prodrug, **KSL0608-Se**, that is capable
293 of being selectively activated by SA- β -gal and then bioorthogonally anchored to nearby proteins in SnCs. This
294 results in the *in situ* formation of a photosensitive drug with the capability of generating highly cytotoxic $^1\text{O}_2$ and
295 emitting fluorescence in the NIR region, facilitating the fluorescence-guided photoactivatable senolysis with
296 single-cell resolution. We also developed **KSL0608-O** with O rather than Se as both a control molecule and an
297 excellent self-immobilizing NIR-emitting probe for the precise imaging of senescence *in vivo*. Compared with
298 **KSL0608-O**, **KSL0608-Se** exhibited a weaker fluorescence response but stronger phototoxicity towards SnCs,
299 confirming that the introduction of the Se atom was key to improving photosensitivity.

300 Cell imaging studies confirmed that **KSL0608-Se** can be activated specifically to emit NIR fluorescence in
301 different types of SnCs and exhibits the ability to distinguish SnCs from young cells. Importantly, cytotoxicity
302 and flow cytometry assays confirmed low dark toxicity of **KSL0608-Se** to both young cells and SnCs but high
303 phototoxicity to SnCs. From these results, **KSL0608-Se** exhibits high efficiency against different types of SnCs
304 (broad-spectrum activity) and minimal side effects to nonirradiated areas (tractability). In an *in vitro* coculture
305 system of SnCs and young cells to mimic the aging environment, we observed that only SnCs exhibited
306 non-negligible NIR emission and were destroyed after **KSL0608-Se**-mediated PDT for 1 h, confirming the
307 accurate fluorescence-guided senolysis by **KSL0608-Se** (precision). In both the X-gal and fluorescence imaging
308 assays of the kidneys of doxo-treated mice, **KSL0608-Se** proved efficient at reducing the percentage of
309 SA- β -gal-positive cells. Western blot assays confirmed that the expression levels of the aging marker γ -H2AX
310 were significantly decreased in the kidney and liver tissues from the “**KSL0608-Se** + irradiation” group. In
311 addition, the aging markers *p21* and *IL-1 β* , and the liver damage indicators AST and ALT, were inhibited after
312 **KSL0608-Se**-mediated PDT according to the results of RT-qPCR and serum tests. Consistent with these results,
313 we found through RNA sequencing that our treatment attenuated the expression of 31 senescence-associated
314 genes in livers of the doxo group. We further tried treating naturally aged mice whose aging symptoms are
315 usually more difficult to be improved than those of the mice with drug-induced senescence. As evident from the
316 results of X-gal and IF assays, the **KSL0608-Se**-mediated PDT successfully eliminated the SnCs in livers and
317 kidneys of the naturally aged mice. Our treatment also desirably inhibited the upregulation of various age-related
318 markers, which was supported by the results of western blot and ELISA assays. In addition, it played the
319 counteraction against the age-induced losses in liver function and renal function and achieved the recovery of the
320 age-associated physical dysfunctions. Most notably, 34 key age-related genes in livers of naturally aged mice
321 were inhibited, suggesting the powerful ability of our strategy to treat signs of natural aging.

322 These successes in mice confirm our strategy to monitor and selectively eliminate SnCs to regulate aging.
323 Since our treatment regime is photoactivated and thus non-invasive, the biological effects depend on the
324 wavelength of the light irradiation absorbed by the photosensitive drug. One major concern is that for translation
325 of this treatment for deep tissues and organs of larger species the 535 nm light irradiation used could result in
326 limited tissue penetration, nevertheless we believe that **KSL0608-Se** can be successfully applied for the PDT of
327 skin, muscle and superficial organs of large species. With our future research we intend to extend the absorption
328 wavelength of the photosensitive drug to the NIR region enabling improved tissue penetration to facilitate full
329 body treatment of larger species.

330 Overall, our results indicate that **KSL0608-Se** is a potent senolytic drug with the advantages of tractability,
331 broad-spectrum activity, and precision. The unprecedented integrated strategy provides a paradigm for the
332 development of senolytics that can overcome the limitations of regular senolytics. Another crucial insight that has
333 emerged from our research is that PDT as an approach for senolysis and scavenging SnCs has numerous
334 advantages over traditional senolytics, particularly if it is based on the integrated use of emerging technologies
335 for the design of senolytic agents. As such, our strategy not only provides a route for eliminating SnCs but also
336 provides the basis for PDT to precisely regulate aging.

337 Methods

338 **Statement of ethical regulations.** All animal experiments were performed in accordance with institutional guidelines and were
339 approved by the Institutional Animal Care and Use Committees of Tongji University in compliance with Chinese law for
340 experimental animals with an approval number of SYXK (Shanghai) 2020-0002.

341 **Preparation of the spectral measurements.** **KSL0608-O** and **KSL0608-Se** were respectively dissolved in DMSO to obtain stock
342 solutions (1 mM) and diluted to 10 μM for all spectral studies. *E. coli* β -gal was dissolved in phosphate buffer saline (PBS) buffer
343 (pH = 7.4, 10 μM) to obtain 10 U/mL stock solutions. Similar, stock solutions of BSA (10 mg/mL) were prepared in PBS buffer (pH
344 = 7.4, 10 mM). PBS buffers with different pH values including 4.74, 5.66, 6.86, 7.40, 8.05, 8.68 and 10.07 were prepared using

348 standard procedures. Absorption and fluorescence spectra of those compounds with *E. coli* β -gal were performed at 37 °C in a 2 mL
349 total volume of PBS buffer (10 μ M, pH 7.4) containing 1 mg/mL BSA in a 1 cm cuvette.

350

351 **Calculation of the LOD.** The LOD was determined from the fluorescence titration data based on an acknowledged method. The
352 plots of fluorescence intensity of **KSL0608-O** and **KSL0608-Se** toward different concentrations of *E. coli* β -gal, all showed a good
353 linear relationship (with R^2 greater than 0.96), and the LOD was calculated using the following equation:

354

$$\text{LOD} = 3\sigma/k$$

355

where σ is the standard deviation of eleven blank measurements and k is the slope of the linear equation.

356

357 **Calculation of the Φ_{fl} .** The Φ_{fl} of **KSL0608-O/KSL0608-Se** before and after the addition of *E. coli* β -gal and BSA was determined
358 by comparison with the fluorescence of fluorescein ($\Phi_{fl,st} = 0.95$ in 0.1 M NaOH). The Φ_{fl} was calculated according to following
359 equation⁵⁶:

360

$$\Phi_{fl,x} = \Phi_{fl,st} \left(\frac{F_x A_{st}}{F_{st} A_x} \right)$$

361

where subscripts x and st represent the sample to be tested, respectively. F is the integrated fluorescence emission. A represents the
362 absorbance of compound at its respective excitation wavelengths.

363

364 **Calculation of the Φ_{Δ} .** The Φ_{Δ} was determined using chlorin e6 (Ce6) as a reference and 1,3-diphenylisobenzofuran (DPBF) as a
365 ROS trapping agent. DPBF (abs < 1.00), and **KSL0608-O** or **KSL0608-Se** (10 μ M) were added in a cuvette containing air-saturated
366 solvents and the solutions were kept in the dark until the absorbance reading was stable, followed by continuous light irradiation
367 (535 nm, 10 mW/cm²) at 5 s intervals. The Φ_{Δ} was calculated by following equation^{57,58}:

368

$$\Phi_{\Delta,x} = \Phi_{\Delta,st} \left(\frac{S_x}{S_{st}} \right) \left(\frac{F_{st}}{F_x} \right)$$

369

where subscripts x and st represent the sample to be tested and the standard reference Ce6 ($\Phi_{\Delta,st} = 0.65$), respectively. S is the slope
370 of the absorption curve of DPBF at the wavelength of 410 nm over time. F is the absorption correction factor, which is given by $F =$
371 $1 - 10^{-OD}$ (OD represents the optical density of sample and Ce6 at 535 nm).

372

373 **Cell culture.** MRC-5 cells, HepG2 cells, RAW264.7 cells, A549 cells and SKOV3 cells were purchased from Cell Bank of Chinese
374 Academy. HL-7702 cells were purchased from Shanghai FuHeng BioLogy Co., Ltd. NRK-52E cells were purchased from Procell
375 Life Science&Technology Co., Ltd. MRC-5 cells were cultured in MEM (Gibco) supplemented with 10% FBS (Gibco), 1%
376 penicillin/streptomycin (Yeasten), 1 mM sodium pyruvate solution (BI), and 1% non-essential amino acids solution (BI). SKOV3
377 cells, A549 cells and HL-7702 cells were cultured in RPMI-1640 (HyClone) medium supplemented with 10% FBS and 1%
378 penicillin/streptomycin. NRK-52E cells, RAW264.7 cells and HepG2 cells were cultured in DMEM (Gibco) medium supplemented
379 with 10% FBS and 1% penicillin/streptomycin. All cells were cultured and maintained at 37 °C with 5% CO₂. To obtain
380 drug-induced senescent cells, A549 cells were stimulated with MitoC (0.5 μ M) twice for two days and HL-7702 cells were treated
381 with doxo (1 μ M) for 1 day. NRK-52E cells were stimulated with 150 nM H₂O₂ for 1 day and cultured for 2 days to induce
382 senescence. To obtain mature RAW 264.7 cells, RAW 264.7 cells were stimulated by Lipopolysaccharide (Lps, 50 ng/mL) and
383 interferon- γ (INF- γ , 100 ng/mL) for 3 days⁵⁹.

384

385 **Cytotoxicity assay.** The percentage cell survival of these cells after treatment with **KSL0608-O** or **KSL0608-Se** was assessed by
386 using a Cell Counting Kit-8 (CCK-8) assay. Cells were seeded into a 96-well plate at 6000 cells per well and treated with
387 **KSL0608-O** and **KSL0608-Se** at different concentrations for 24 h. For irradiation experiment, the cells were separately incubated
388 with **KSL0608-Se** in PBS buffer for 30 min, followed by irradiation with an LED lamp (535 nm, 10 mW/cm²) for 20 min. Then, the
389 PBS buffer was removed from the petri dish and the culture medium was added into the dish, the cells were further incubated in the
390 incubator for 24 h, 48 h and 72 h, respectively. As a control experiment, all these cells were treated with PBS buffer for different
391 times. Then the PBS buffer was replaced by the culture medium and further incubated in the incubator for 24 h. For cytotoxicity
392 tests, the culture medium was removed, 10 μ L of CCK-8 solution was added to each well, and the 96-plates were incubated at 37 °C
393 with 5% CO₂ for 2 h. A Microplate Reader (Bio-Tek Instruments, Synergy H1) was employed to record the absorbance of each well
394 at 450 nm. The percentage cell survival was evaluated by measuring the absorbance at 450 nm and calculated by the formula
395 (percentage cell survival = (OD_{positive} - OD_{control})/(OD_{negative} - OD_{control})). All experiments were carried out at least in triplicate.

396

397 **Influences of autophagy process.** To ensure the reliability of our treatment, we have further evaluated the cytotoxicity of PBS on
398 senescent/young cells at different treatment times. The cells were treated with PBS buffer for different times and the cytotoxicity of
399 PBS to these cells was evaluated by CCK8 assays. **AF-C**, an autophagy-pH-sensitive ratiometric fluorescence probe^{60,61}, was used
400 to monitor the autophagy process of cells in PBS buffer within 2 h. The PBS-treated cells were incubated with **AF-C** (20 μ M) for 15
401 min and then imaged by a Leica TCS SP8 SMD confocal microscope.

402

403 **Confocal imaging.** Cells were plated on a glass-bottomed cell culture dish (NEST Biotechnology, 801001) and incubated with
404 normal medium for 1-2 days. Then, the medium was replaced with PBS buffer containing 10 μ M (or 5 μ M) probe (**KSL0608-O** or
405 **KSL0608-Se**) and incubated for 30 min. Then the above cells were washed with PBS for three times. The tissue sections were
406 immersed in a PBS buffer containing 10 μ M probe (**KSL0608-O**) for 1 h at 37 °C, and then washed with PBS buffer and mounted
407 with 75% glycerol. The fluorescence imaging of cells and tissue sections were performed by a Leica TCS SP8 SMD confocal
408 microscope and a Nikon csu-w1 sora confocal microscope, respectively.

409 MRC-5 cells (P28) and young HL-7702 cells were stained with 5 μ M CellTracker Green CMFDA (Yeasen, 40721ES50) for 20
410 min, respectively. The above CMFDA-stained young cells and senescent cells were co-cultured for 1 day. Then, the co-cultured cells
411 were incubated with **KSL0608-O** (10 μ M) or **KSL0608-Se** (10 μ M) for 30 min and fluorescence imaging was carried out.
412

413 **X-gal staining assay.** Cultured cells were washed with PBS once and fixed for 10 min at room temperature in 4% formaldehyde and
414 2% glutaraldehyde. Then, after washing three times with PBS, MRC-5 cells, HL-7702 cells, A549 cells, NRK-52E cells were
415 respectively incubated with SA- β -gal staining solution (1 mg/mL X-gal, pH = 6.0) overnight at 37 °C and RAW 264.7 cells were
416 incubated with β -gal staining solution (1 mg/mL X-gal, pH = 4) for 7 h at 37 °C. Additionally, the frozen sections were dried at
417 37 °C for 15 min and then fixed with 2% formaldehyde and 0.2% glutaraldehyde in PBS for 10 min. Subsequently, the sections were
418 washed with PBS for three times and then incubated with 1 mg/mL X-gal staining solution (pH = 6.0 for kidney sections, pH = 4.0
419 for liver sections⁶²) overnight at 37 °C. After completion of β -gal staining, the sections were washed using PBS for three times. The
420 images of X-gal-staining were collected using a Ti-S microscope (Nikon).
421

422 **Flow cytometric assay.** MRC-5 cells were seeded in culture plates and were cultured for 24 h. Then the cells were incubated with
423 **KSL0608-Se** (10 μ M) for 30 min and then were exposed under an LED lamp (535 nm, 10 mW/cm²) for 20 min. Cells were further
424 cultured for 5 h and were digested by trypsin-EDTA solution. Finally, the above cells were washed with PBS, and stained with PI
425 and Annexin V-FITC following the protocol (Byotime Biotechnology, C2015M). Then the samples were analyzed by flow
426 cytometry (CytoFLEX LX, Beckman Coulter, CA, USA).
427

428 **Drug treatment *in vivo*.** C57BL/6J mice (male and female, 2-month-old and 21-month-old) were purchased from Charles River
429 Laboratory Animal Co., Ltd, maintained under specific pathogen-free facility (SPF) at 25 °C and 40-60% humidity conditions with a
430 12 h light/12 h dark cycle and free access to food and water.

431 **KSL0608-O** or **KSL0608-Se** was mixed in mixture solution containing 70% normal saline, 25% castor oil and 5% DMSO.
432 Doxo was dissolved in normal saline. All drugs were administered to the mice by intraperitoneal (i.p.) injection.

433 To construct mouse model with doxo-induced senescence, doxo (3 mg/kg) was administrated to mice (male) once on the day 1
434 and day 10. Then, **KSL0608-Se** (20 mg/kg) was administrated to aged mice on the day 24. And the mice were sacrificed on
435 thirty-eighth day. For young mice and naturally aged mice (female), mice were divided into the young (2-month-old) control group,
436 the aged (21-month-old) control group to be treated with vehicle, the **KSL0608-Se** group (21-month-old) to be treated with
437 **KSL0608-Se** (10 mg/kg), and the “**KSL0608-Se** + irradiation” group (21-month-old) to be treated with both **KSL0608-Se** (10
438 mg/kg) and irradiation. Both the vehicle and **KSL0608-Se** were administrated by intraperitoneal injection once every two weeks and
439 for eight weeks. According to the *in-vivo* imaging results using **KSL0608-O**, the mice of “**KSL0608-Se** + irradiation” group were
440 irradiated four times every two weeks after the administration of **KSL0608-Se**. All mice were sacrificed on 68 days. For imaging
441 experiments *in vivo*, mice aged 2 months and 21 months (male, n = 3) were intraperitoneally injected with **KSL0608-O** (20 mg/kg),
442 respectively.
443

444 **Tissue sections and blood samples.** Main organs of mice (heart, liver, spleen, lung and kidney) were isolated, washed with PBS,
445 and transferred to liquid nitrogen. All these organs were paraffin-embedded with paraffin and sectioned into 5 μ m slices for H&E
446 staining. The frozen kidneys and livers were sliced at -20 °C and sectioned into 20- μ m-thick sections using a cryocut microtome
447 (CRYOSTAR NX50, Thermo) and mounted onto glass slides (Servicebio). The sections were stored at -20 °C and used in one day.
448 Additionally, the blood samples of all mice were collected and stewed for 1 h at room temperature. Then, the blood samples were
449 centrifuged (3000 g, 20 min) at 4 °C to obtain serum.
450

451 **PDT and imaging *in vivo*.** The PDT experiments were performed on *in vivo* fluorescence imaging system (Perkin Elmer IVIS
452 Spectrum CT imaging system) with an excitation wavelength at 535 nm for 20 min. The hair on the back and abdomen of mice was
453 shaved. Then, the mice were anaesthetized with isoflurane and were placed on the loading platform of instrument (3-4 mice at a
454 time), followed by intermittent irradiation using sequence imaging mode (Excitation filter: 535; Subject size: 1.5; Field of view:
455 22.4). The mice with doxo-induced senescence were irradiated once a day for three consecutive days after the administration of
456 **KSL0608-Se**. The naturally aged mice were irradiated once a day for four consecutive days after the administration of **KSL0608-Se**.
457 The PDT treatment was performed on day 24, 25 and 26 for mice with doxo-induced senescence and was carried out on day 1, 2, 3,
458 4, 15, 16, 17, 18, 29, 30, 31, 32, 43, 44, 45 and 46 for naturally aged mice.

459 The imaging experiments in those mice were performed on *in vivo* fluorescence imaging system (Perkin Elmer IVIS Spectrum
460 CT imaging system) (Ex: 535 nm, Em: 640-660 nm). The hair on the back and abdomen of mice was shaved. The images were taken
461 on at 1 h, 12 h, 24 h, 72 h and 96 h after injection. Then, these mice were sacrificed and their major organs (heart, liver, spleen, lung

462 and kidney) were harvested and subjected to *ex vivo* imaging.

463

464 **Western blot analysis.** The prepared liver and kidney samples of mice were placed in grinders. Total protein was extracted using
465 RIPA lysis buffer containing protease inhibitor. The concentration of protein was quantified by BCA protein assay kit (Yeesen). After
466 mixing the SDS-PAGE protein loading buffer, the solution was heated at 100 °C for 10 min. Then, samples with equal protein
467 amount were added into the wells with 10% (or 12%) SDS-PAGE gel and were separated and transferred to a PVDF membrane. The
468 membranes were then blocked and incubated with primary antibody (γ -H2AX: Abcam, ab81299, 1/10000 dilution; p21: Cell
469 Signaling Technology, 2947S, 1/1000 dilution; p53: Santa Cruz, sc-126, 1/1000 dilution; GAPDH: Abclonal, ac033, 1/2000 dilution)
470 at 4 °C overnight and then was washed with TBST for five times. After incubating with secondary antibodies (Anti-rabbit IgG
471 antibody, CST, 7074P2; Goat anti-Mouse IgG antibody, Arigo, ARG65350) for 1 h, the membranes were imaged using an imaging
472 system (Tanon-4600SF).

473

474 **Reverse transcription (RT)-quantitative PCR (RT-qPCR).** The prepared liver and kidney samples of mice were placed in
475 grinders. Total RNA was extracted using RNA Isolation Kit (Omega, Biotek) according to the manufacturer's instructions. cDNA
476 was generated by using Hifair II 1st strand cDNA synthesis supermax for qPCR (gDNA digester plus) (Yesen). RT-qPCR was
477 performed using Hieff UNICON[®] Universal Blue qPCR SYBR Green Master Mix (Yesen) on a qPCR detecting system (BIO-RAD,
478 CFX96) by following the manufacturer's instructions.

479 Four biological repeats were conducted for each group. The test procedure was in accordance with the instruction of kit. GAPDH
480 was used as a control to normalize the expression of target genes. The primers (Sangon Biotech) used are listed as follows:

481 *GADPH*, forward: GTGGCAAAGTGGAGATTGTTG;

482 reverse: AGTCTTCTGGGTGGCAGTGAT.

483 *p21*, forward: CAGATCCACAGCGATATCCA;

484 reverse: ACGGGACCGAAGAGACAAC.

485 *IL-1 β* , forward: TGCCACCTTTTGACAGTGATG;

486 reverse: TGATGTGCTGCTGCGAGATT.

487

488 **Immunofluorescence staining.** Kidney and liver samples for immunofluorescence staining assays were incubated with primary
489 antibody (p21: Cell Signaling Technology, 2947S, 1/200) at 4 °C overnight, washed three times with PBS and then incubated with
490 appropriate secondary antibodies (Anti-rabbit IgG antibody, CST, 7074P2, 1/500) for 1 h at 37 °C. And then the samples were
491 stained by Hoechst.

492

493 **ELISA analysis.** SASP factors (IL-6, IL-1 β , CXCL1, CXCL3, MMP-1, MMP-7 and TNF- α), and cell cycle regulators p16
494 (CDKN2A) in serum, liver or kidney were monitored using ELISA Kit (RF6857, RF7630, RF8477, RF7606, RF7480, RF7678,
495 RF7722, RF6978). AST, ALT, UA, Cr and BUN in serum were teste using ELISA Kit (RF8502, RF8547, RF8520, RF8275,
496 RF8274). All ELISA Kits were purchased in Shanghai ruifan Biological Technology Co, Ltd. And the data of ELISA analysis were
497 collected by this company.

498

499 **Physical function measurements.** All functional assays were conducted one week before the sacrifice of mice.

500 The rotarod test was performed using an accelerating RotaRod system (Shanghai XinRuan Information Technology Co., Ltd,
501 XR-6C). Mice were placed in separate lanes on the rod rotating at an initial speed of 4 rpm/min and then the speed accelerated from
502 4 to 40 rpm/min in 300 s. The time, speed and distance were recorded when the mouse fell to the bottom of machine. All mice were
503 trained once a day for three consecutive days (day 1, 2 and 3) and the test were recorded on day 4, 5 and 6. The results were
504 averaged from 3 trials.

505 The grip strength of mice was measured by a grid strength meter (Shanghai XinRuan Information Technology Co., Ltd, XR501).
506 Mice were placed in the grid of the machine and grasped the grid with all four paws. The meter recorded the peak tension over six
507 trials. The test was performed once a day for three consecutive days and the results were averaged from 3 trials.

508 For the Y-maze spontaneous-alternation test, the mice were placed in the centre of a Y-shaped maze (XinRuan Information
509 Technology Co., Ltd, XR-XY1032). The mice are allowed to explore all three arms freely. The number of arm entries and sequences
510 of arm visits of mice within 8 min were recorded and analyzed.

511 To perform the hanging test, mice were kept to grab a 2-mm-thick metal wire (35 cm above a padded surface) with their
512 forelimbs only and recorded the hanging duration. Hanging time was normalized to body weight as hanging duration (sec) \times body
513 weight (g), and the results were averaged from 3 trials for each mouse.

514

515 **RNA sequencing.** For the PDT experiments on doxo-treated mice and naturally aged mice, the liver samples of mice in different
516 groups were collected for RNA sequencing in Shanghai Majorbio Bio-pharm Technology Co., Ltd. Data was analyzed using the free
517 online platform of Majorbio Cloud Platform. The accession number for RNA-seq reported in this paper is GEO: GSE186522 and
518 GSE213846.

519

520 **Statistics and reproducibility.** Leica Application Suite X and ImageJ 1.49k were used to process imaging data. Graphpad Prism

521 6.01, OriginPro 8.0 and CytExpert 2.4 were used for data analysis. Statistical analyses were performed in GraphPad Prism 6.01 with
522 the Student's t-test and one-way ANOVA. Statistical significance is represented in the figures. For *in vivo* studies, mice were
523 randomly assigned to treatment groups. All replicates in this work represent different mice. The experiments were repeated
524 independently at least twice with similar results. No sample sizes were pre-determined, but our sample sizes are similar to those
525 reported in previous publications^{9,11,26}. Blinding was not conducted and all data collection and analysis were objective in nature. No
526 data were excluded from the analysis. Data distribution was assumed to be normal but this was not formally tested.
527

528 **Reporting summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this
529 article.
530

531 **Data availability**

532 All data during the current study are available within the paper and its Supplementary Information, or from the corresponding
533 author upon reasonable request.
534

535 **Code availability**

536 Source code used for RNA-seq analysis can be found at <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE186522>.
537 <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE213846>.
538

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654

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665

666 **Author contributions**

667 Yuan Guo and J.L. conceived and designed the project. Yuan Guo, J.L., D.S., Ying Gao and T.D.J. wrote and revised the manuscript.
668 D.S. and Xinming Li performed the synthetic work. D.S. and W.L. performed and analyzed the experiments. Y.H. performed
669 modelling assay. Xiaokang Li assisted with data analysis.
670

671 **Competing interests**

672 The authors declare no competing financial interests.
673
674

675 Figure Legends

676 Figs. 1-8

677 **Fig. 1 | Integrated design strategy.** **a**, Synthetic routes of **KSL0608-O** and **KSL0608-Se**. **b**, Integrated strategy used to
678 design the senotherapeutic agent **KSL0608-Se**.

679

680 **Fig. 2 | Photochemical properties and β -gal-triggered protein labelling.** **a,b**, The normalized fluorescence intensity of
681 **KSL0608-O** (10 μ M, **a**) and **KSL0608-Se** (10 μ M, **b**) in the presence and absence of *E. coli* β -gal (10 U/mL) and BSA (1
682 mg/mL). **c**, SDS-PAGE analysis of **KSL0608-O** (10 μ M) and **KSL0608-Se** (10 μ M) in the presence and absence of *E. coli*
683 β -gal (10 U/mL) and BSA (1 mg/mL). **d,e**, Fluorescence spectra of **KSL0608-O** (10 μ M, **d**) and **KSL0608-Se** (10 μ M, **e**)
684 upon the addition of different concentrations of *E. coli* β -gal at 37 °C in PBS buffer containing BSA (1 mg/mL). **f**,
685 Schematic diagram of human- β -gal-**KSL0608-O** interactions (PDB: 3THC). **g**, Electrostatic surface potential of the
686 substrate-binding pocket in human β -gal with galactose and **KSL0608-O**. **h**, Normalized absorption spectra of DPBF in
687 different groups. **i**, Normalized absorption spectra of DPBF in a solution with **KSL0608-Se**, *E. coli* β -gal (4 U/mL) and
688 BSA (1 mg/mL) under light irradiation (535 nm, 10 mW/cm²). Error bars (**a**, **b**, and **h**) represent the standard deviation (\pm
689 S.D.). n = 3 independent samples.

690

691 **Fig. 3 | Cell imaging and intracellular localization of KSL0608-O and KSL0608-Se.** **a**, X-gal staining of young cells
692 and SnCs for β -gal expression (Scale bar: 200 μ m) and confocal fluorescence images of **KSL0608-O** and **KSL0608-Se**
693 in different cells (Scale bar: 50 μ m), including young A549 cells, MitoC-induced senescent A549 cells, young HL-7702
694 cells, doxo-induced senescent HL-7702 cells, young NRK-52E cells, ROS-induced senescent NRK-52E cells, MRC-5
695 cells (P28) and MRC-5 cells (P40). A549 cells, HL-7702 cells and NRK-52E cells were incubated **KSL0608-Se** (10 μ M)
696 for 30 min, respectively. MRC-5 cells were incubated with **KSL0608-Se** (5 μ M) for 30 min; All these cells were incubated
697 with **KSL0608-O** (10 μ M) for 30 min. **b,c**, Normalized fluorescence intensity of the cells incubated with **KSL0608-O** and
698 **KSL0608-Se** from parallel images including (**a**). (**b**: n = 10 for A549 cells, n = 11 for NRK-52E cells, n = 9 for HL-7702
699 cells and n = 6 for MRC-5 cells; **c**: n = 10 for A549 cells, n = 10 for NRK-52E cells, n = 9 for HL-7702 cells and n = 7 for
700 MRC-5 cells). Error bars represent the standard deviation (\pm S.D.). The significance of differences was analyzed with
701 two-sided Student's t-test. **c**, Confocal fluorescence images showing the subcellular localization of **KSL0608-O** (10 μ M)
702 and **KSL0608-Se** (10 μ M) in replication-induced senescent MRC-5 cells (P40). All cells were stained with Hoechst33258
703 (Hoechst, 1 μ M). Column 3 and column 6 represent cross-sectional analyses along the white lines in the insets. Scale bar:
704 25 μ m. Abbreviations: yng, young; sct, senescent. Blue channel: $\lambda_{ex}/\lambda_{em}$ = 405/420–440 nm. Green channel: $\lambda_{ex}/\lambda_{em}$ =
705 488/500–530 nm. NIR channel (**KSL0608-O**): $\lambda_{ex}/\lambda_{em}$ = 561/600–700 nm. NIR channel (**KSL0608-Se**): $\lambda_{ex}/\lambda_{em}$ =
706 561/650–750 nm. 'n' stands for the number of image and the images in each group from three biological replicates.
707

708 **Fig. 4 | Photo-induced ROS generation and PDT effect *in vitro*.** **a**, Fluorescence image of intracellular ROS stained by
709 2,7-dichlorofluorescein diacetate (DCFH-DA, 10 μ M) in SKOV3 cells incubated with **KSL0608-Se** (10 μ M) before and
710 after irradiation (n = 10). Scale bar: 50 μ m. **b**, Live/dead cell assays of SKOV3 cells in different groups using calcein-AM
711 and propidium iodide (PI). Scale bar: 100 μ m. **c**, Cytotoxicity assay of **KSL0608-Se**-mediated PDT to different cells,
712 including young A549 cells, young HL-7702 cells, young NRK-52E cells and MRC-5 cells (P28), MitoC-induced
713 senescent A549 cells, doxo-induced senescent HL-7702 cells, ROS-induced senescent NRK-52E cells and MRC-5 cells
714 (P40) (n = 3 for each group). **d,e**, Flow cytometric plots to measure apoptosis (**d**) and quantitate the percentage of
715 apoptotic cells, surviving cells and necrotic cells (**e**) in **KSL0608-Se** (10 μ M)-loaded cells (MRC-5 cells (P28) and MRC-5
716 cells (P40)) treated with or without irradiation. **f,g**, Real-time confocal imaging of cells in co-cultured systems incubated
717 with 10 μ M **KSL0608-O** (**f**) and 10 μ M **KSL0608-Se** (**g**) after irradiation. Young cells were pre-labelled by CellTracker
718 Green (5 μ M), and all cells were pre-stained by Hoechst (1 μ M). The co-cultured cells were incubated with our compound
719 for 30 min at 37 °C and then treated with laser irradiation at 561 nm. Scale bar: 50 μ m. Error bars (**a** and **c**) represent the

720 standard deviation (\pm S.D.). Significant differences were analyzed with the two-sided Student's t-test. 'n' stands for the
721 number of image and the images in each group from three biological replicates.

722

723 **Fig. 5 | Fluorescence imaging using KSL0608-O *in vivo*.** **a**, Representative whole-body imaging in young mice
724 (2-month-old) and naturally aged mice (21-month-old) at different times after injection of **KSL0608-O**. **b**, Quantitative
725 analysis of the fluorescence imaging of mice from parallel images including (**a**). **c**, Representative images of *ex vivo*
726 imaging of five major organs (heart, liver, spleen, lung and kidney) harvested from young mice (2-month-old) and
727 naturally aged mice (21-month-old) at 96 h after injection of the **KSL0608-O**. **d**, Quantitative analysis of the fluorescence
728 imaging of major organs from parallel images including (**c**). Error bars (**b** and **d**) represent the standard deviation (\pm S.D.).
729 n = 3 for each group and 'n' stands for the number of mice

730

731 **Fig. 6 | Selective removal of SnCs in doxo-treated mice.** **a**, Experimental design of **KSL0608-Se**-mediated PDT in
732 doxo-treated mice. **b**, Western blot analysis of γ -H2AX expression levels in kidneys and livers of mice in different groups
733 (n = 3 for each group). The samples derive from the same experiment and that gels/blots were processed in parallel. **c**,
734 The serum biochemical test of AST (left) and ALT (right) in different groups (n = 4 for each group). **d**, The relative mRNA
735 levels of *p21* in livers (left) and kidneys (right) of mice in different groups (n = 4 for each group) analyzed by RT-qPCR. **e**,
736 Representative images of SA- β -gal staining (X-gal and **KSL0608-O**) of kidney sections of mice in different groups. NIR
737 channel (**KSL0608-O**): $\lambda_{ex}/\lambda_{em}$ = 561/600–700 nm. Scale bar: 100 μ m. **f**, A volcano plot of gene expression in doxo
738 control group compared to young control group (left), and a volcano plot of gene expression in **KSL0608-Se** + irradiation
739 group compared to doxo control group (right) (more than twofold, $P < 0.05$, calculated by raw count value). **g**, Venn
740 diagram showing the number of differential genes and overlapped genes between A and B (A, doxo control group vs.
741 young control group; B, **KSL0608-Se** + irradiation group vs. doxo control group). **h**, Heatmap of senescence-related
742 genes of livers in different groups, where '#' represents no significance genes in livers of all mice. n = 4 for each group in
743 RNA sequencing analysis. Error bars (**b-d**) represent the standard deviation (\pm S.D.). 'n' stands for the number of mice.
744 Significant differences (**b-d**) were obtained by analysis with two-sided Student's t-test.

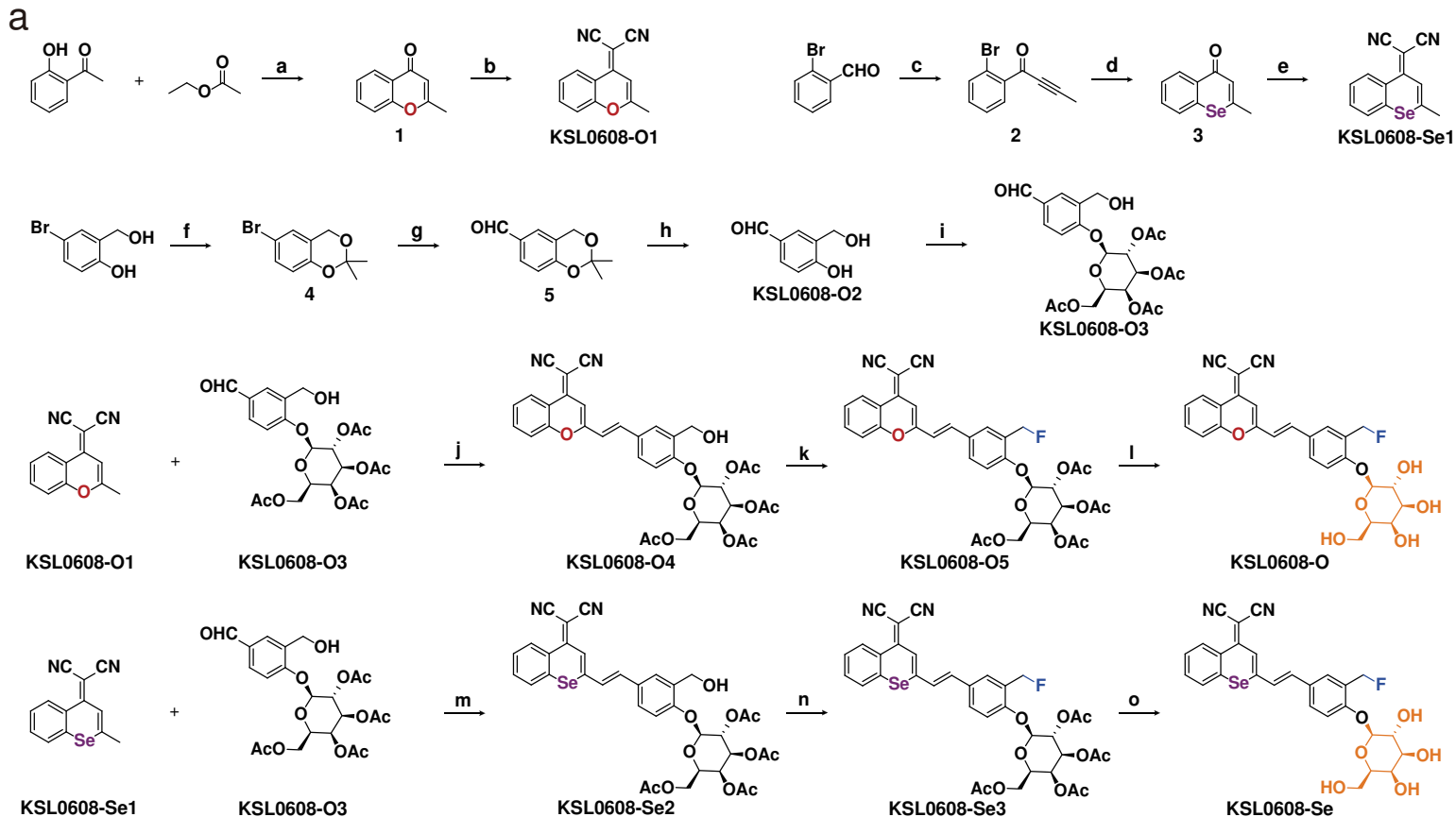
745

746 **Fig. 7 | Selective removal of SnCs in naturally aged mice.** **a**, Experimental design of **KSL0608-Se**-mediated PDT in
747 naturally aged mice. **b**, Western blot analysis of p53 expression levels in kidneys and livers of mice in different groups (n
748 = 3 for each group). The samples derive from the same experiment and that gels/blots were processed in parallel. **c**,
749 Representative images of SA- β -gal staining of livers sections and kidney sections, and the percentage of
750 SA- β -gal-positive area in these sections from mice in different groups (young control, n = 10; aged control, n = 8;
751 **KSL0608-Se**, n = 11; **KSL0608-Se** + irradiation, n = 11). Scale bar: 100 μ m. **d**, Representative images of p21 IF staining
752 of kidney sections and liver sections, and the p21 expression levels in these sections from mice in different groups (n = 6
753 for each group). Scale bar: 100 μ m. **e**, The serum biochemical test of UA (left), Cr (middle) and BUN (right) from mice in
754 different groups. **f**, The serum biochemical test of ALT (left) and AST (right) from mice in different groups. For **e,f**, young
755 control, n = 12; aged control, n = 9; **KSL0608-Se**, n = 11; **KSL0608-Se** + irradiation, n = 11. Error bars (**b-f**) represent as
756 the mean values (\pm S.D.). 'n' stands for the number of mice. Significant differences (**b-f**) were obtained by analysis with
757 two-sided Student's t-test (**b-d**) and one-way ANOVA followed by Tukey's multiple comparisons test (**e,f**).

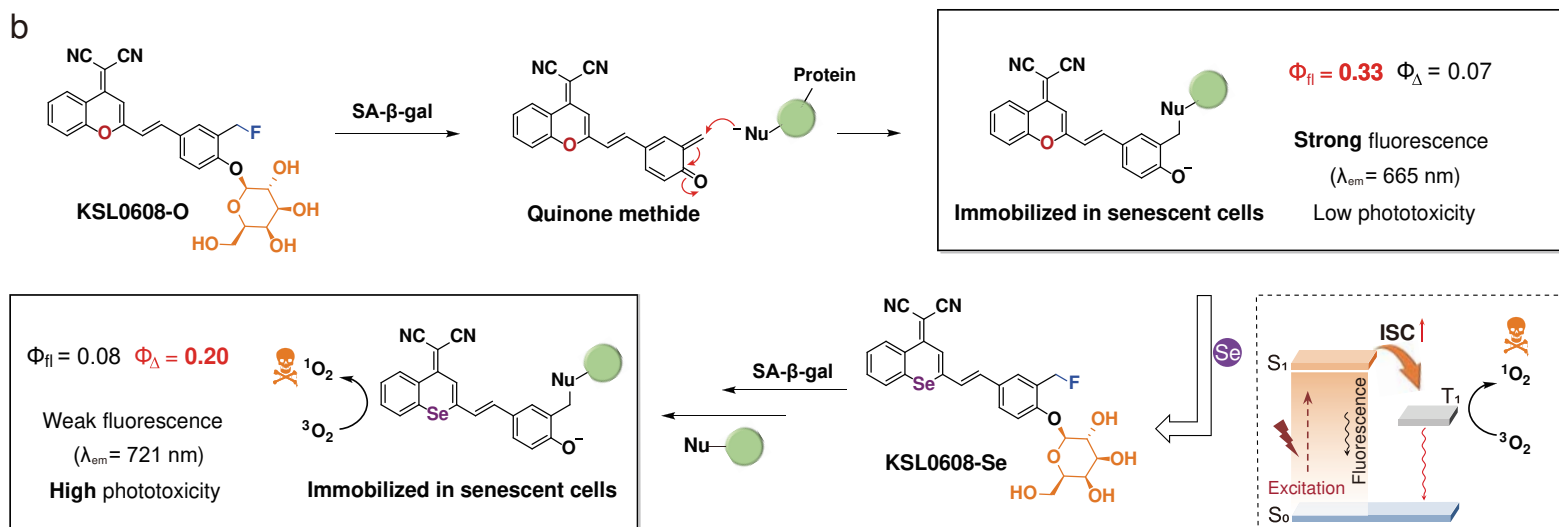
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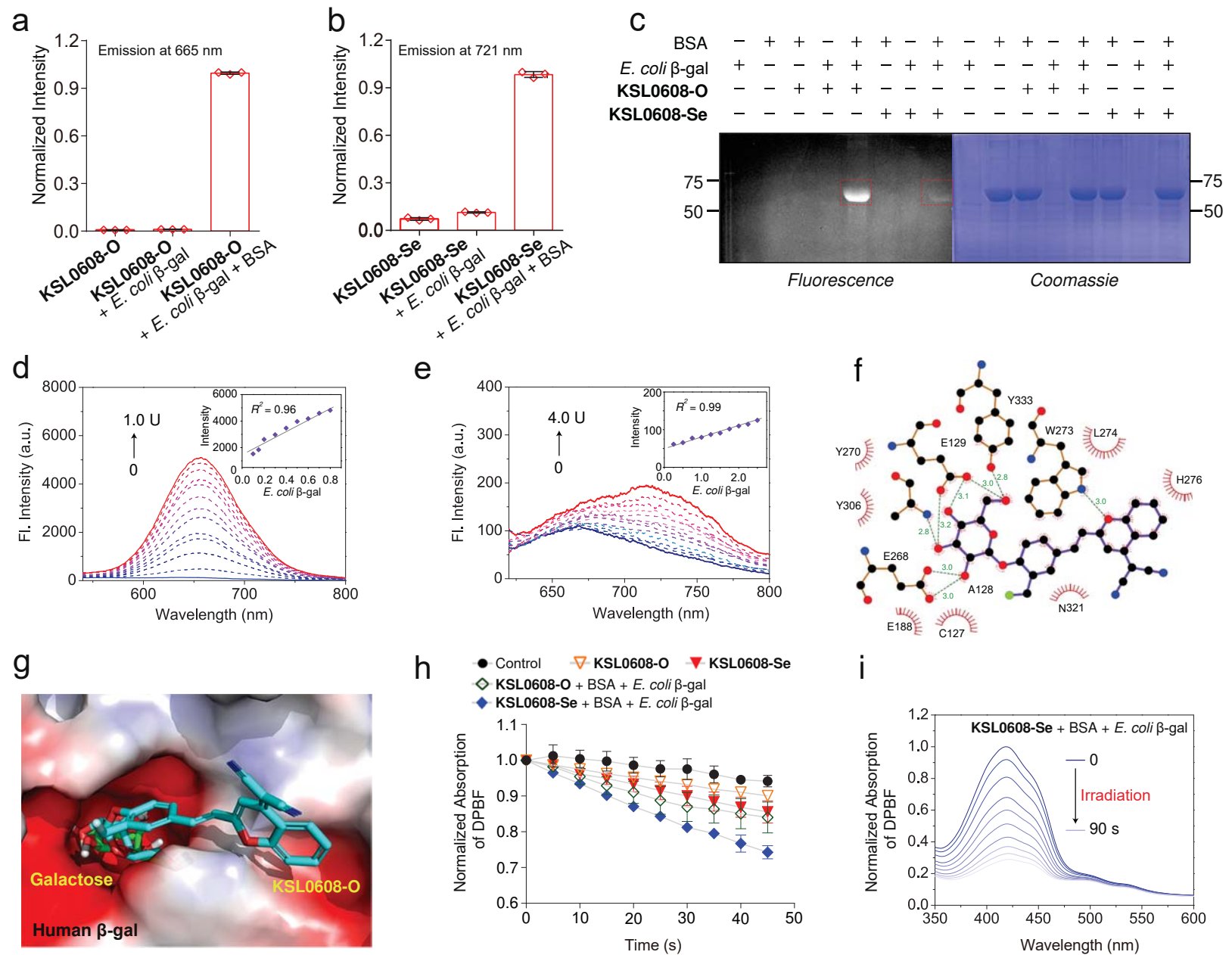
759 **Fig. 8 | The expression of SASP factors, evaluation of physical function in mice and RNA sequencing analysis.**
760 **a-g**, The expression levels of CXCL1 (**a**), CXCL3 (**b**), IL-1 β (**c**), IL-6 (**d**), MMP-1 (**e**), MMP-7 (**f**) and TNF- α (**g**) in blood
761 serum of mice in different groups (young control, n = 12; aged control, n = 9; **KSL0608-Se**, n = 11; **KSL0608-Se** +
762 irradiation, n = 11). **h**, Quantification of the ratio of grip strength to weight from mice in different groups. **i**, Quantification of
763 maximal time in rotarod from mice in different groups. **j**, Quantification of hanging endurance from mice in different
764 groups. **k**, The alteration (left) and total arm entries (right) in a Y-maze test from mice in different groups. For **h-k**, young
765 control, n = 12; aged control, n = 9; **KSL0608-Se**, n = 11; **KSL0608-Se** + irradiation, n = 10. Error bars (**a-k**) represent as
766 the mean values (\pm S.D.). Significant differences (**a-k**) (ns, not significant) were obtained by analysis with one-way
767 ANOVA followed by Tukey's multiple comparisons test (**a-g**) and two-sided Student's t-test (**h-k**). **l**, Venn diagram
768 showing the number of differential genes and overlapped genes between A and B (A, aged control group vs. young
769 control group; B, **KSL0608-Se** + irradiation group vs. aged control group). And the representative GO processes

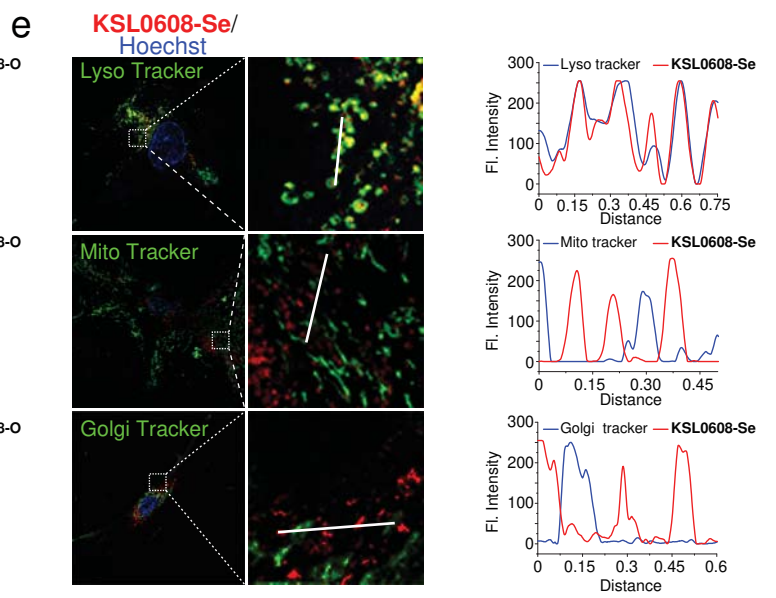
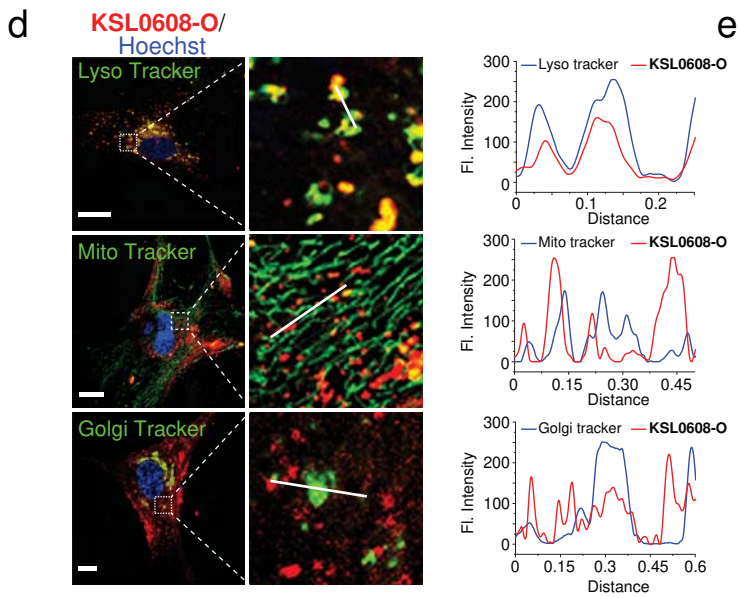
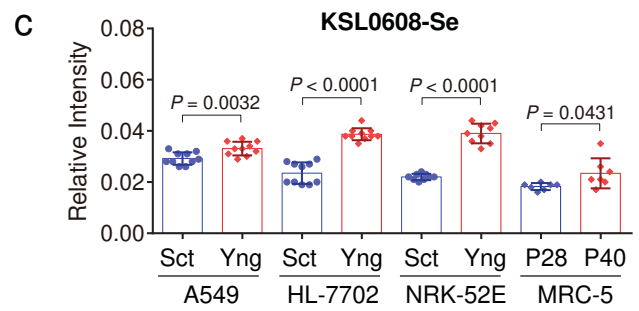
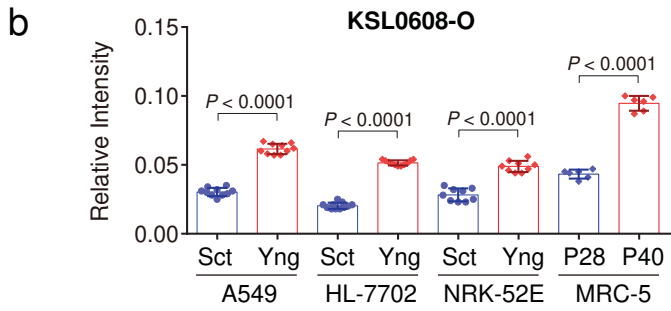
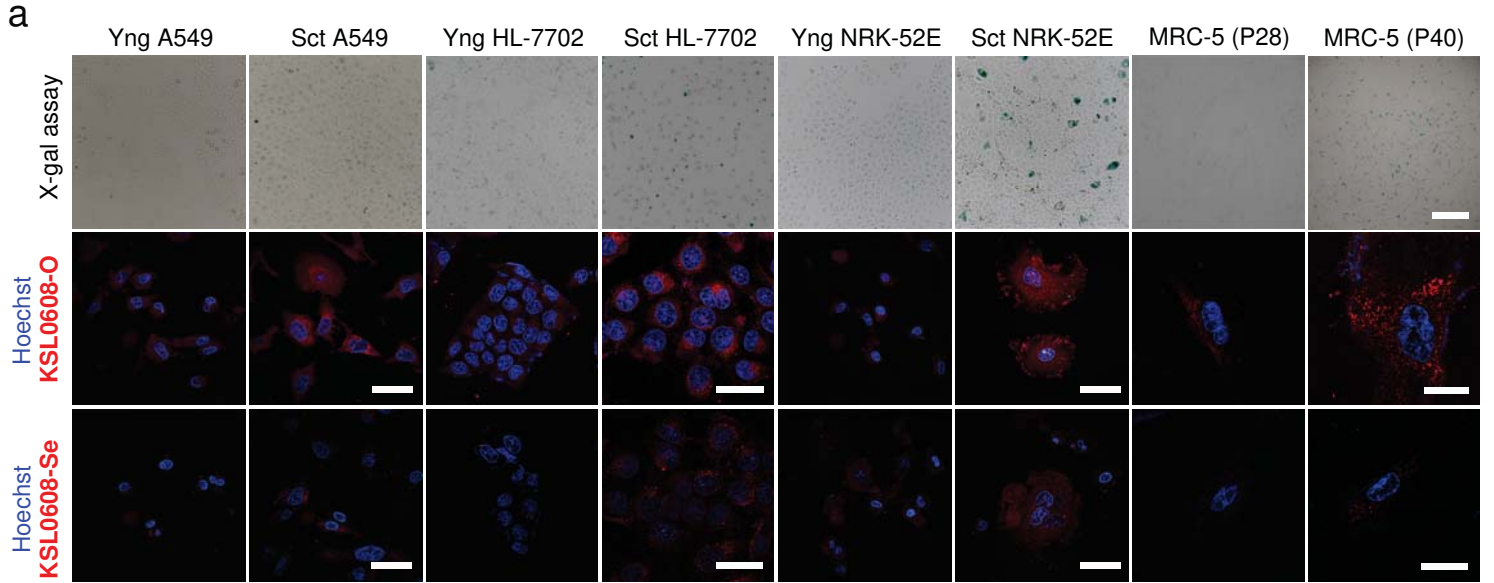
770 associated with the 146 common differentially expressed genes. **m,n**, A volcano plot of gene expression in the aged
771 control group compared to the young control group (**m**), and that in the **KSL0608-Se** + irradiation group compared to the
772 aged control group (**n**) (more than twofold, $P < 0.05$, calculated by raw count value). **o**, Heatmap of age-related genes of
773 livers in different groups, where '#' represents no significance genes in livers of all mice. n = 5 for each group in RNA
774 sequencing analysis. 'n' stands for the number of mice.
775

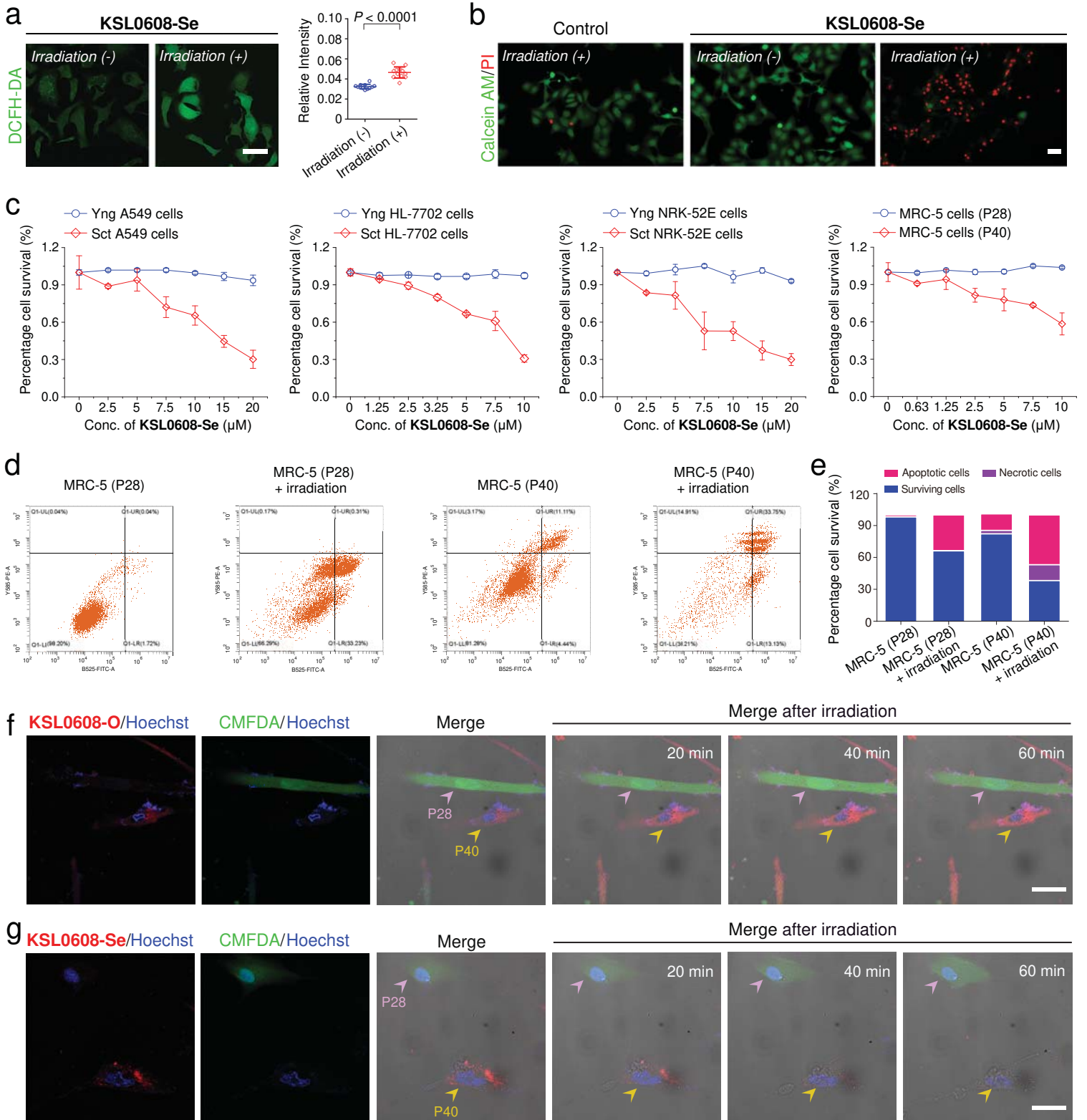


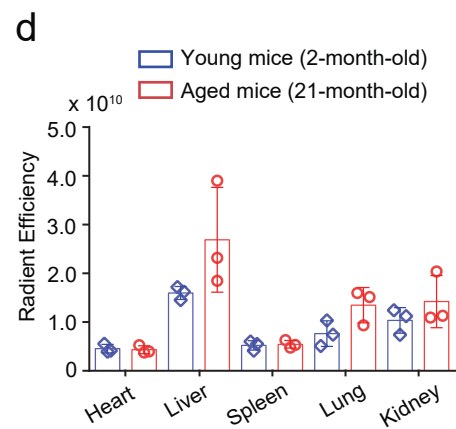
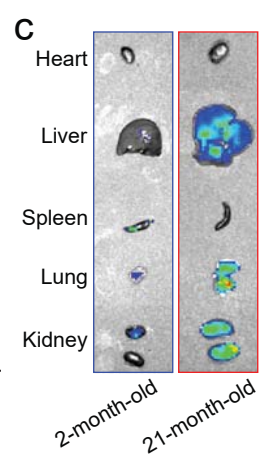
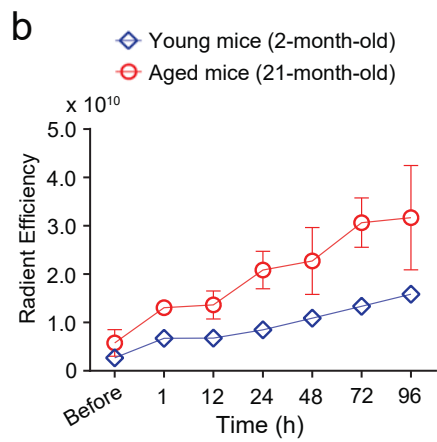
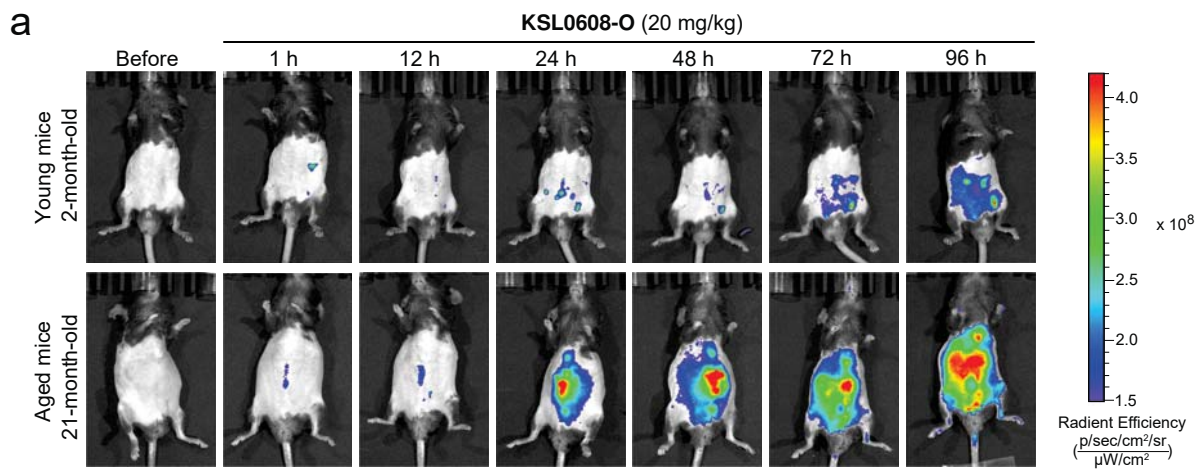
Reagents and conditions: a: NaH, H₂SO₄/CH₃COOH, r.t.; b: Malononitrile, Ac₂O, 140 °C; c: CH₃CCMgBr, THF; NaOCl, TEMPO, NaHCO₃, DCM; d: NaBH₄, Se, DMF, 100 °C; e: Malononitrile, Ac₂O, 140 °C; f: Acetone, 2,2-dimethoxypropane, *p*-TsOH, Na₂SO₄, 40 °C; g: THF, *n*-BuLi, DMF, -78 °C; h: CH₃CN/H₂O, (cat.) HCl; i: K₂CO₃, DMF, tetra-O-acetyl- α -D-galactopyranosyl-1-bromide, r.t.; j: Piperidine, CH₃CN, 85 °C; k: DAST, DCM, 0 °C; l: MeOH, MeONa, r.t.; m: Piperidine, CH₃CN, 85 °C; n: DAST, DCM, 0 °C; o: MeOH, MeONa, r.t..

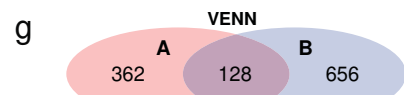
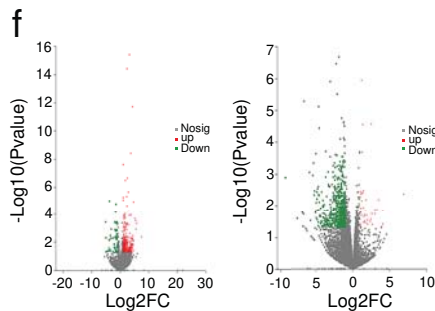
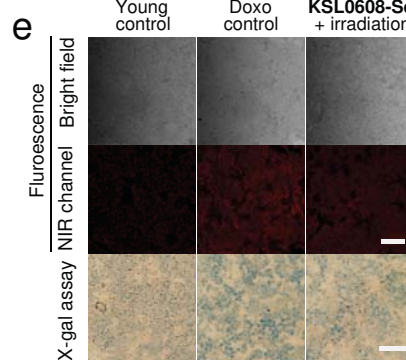
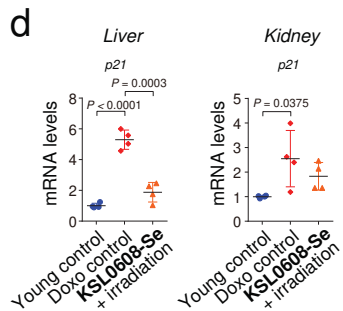
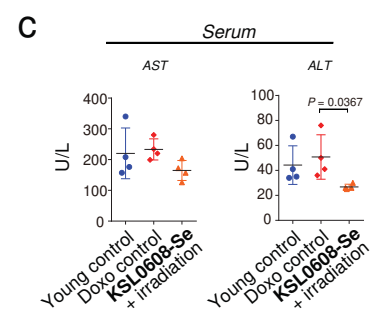
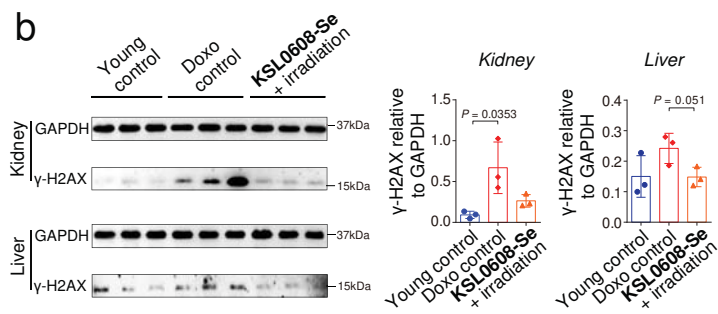






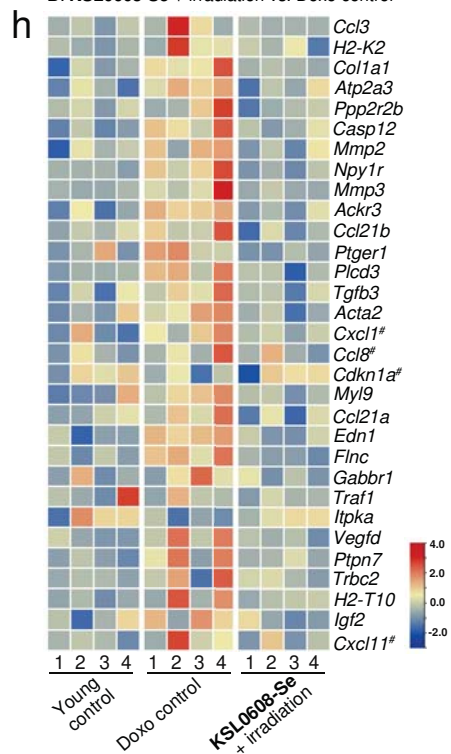


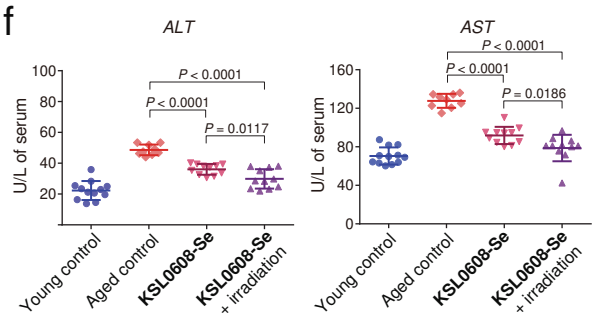
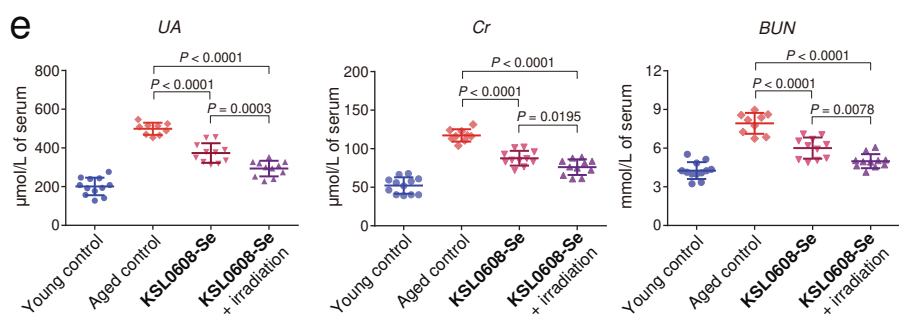
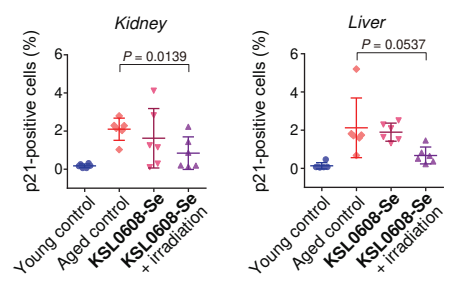
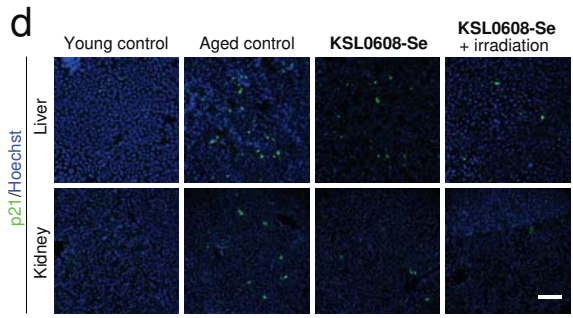
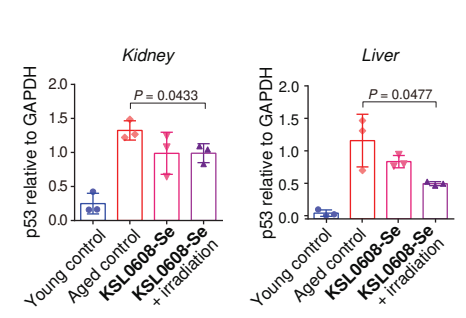
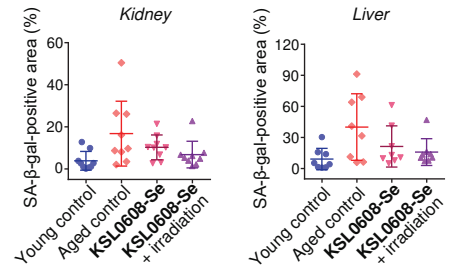
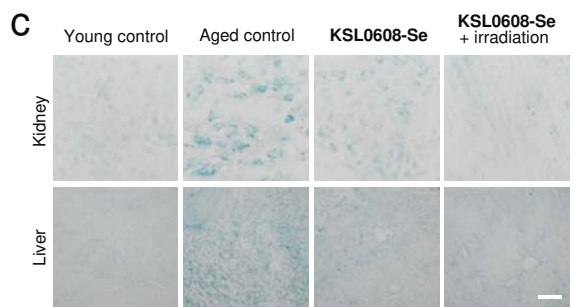
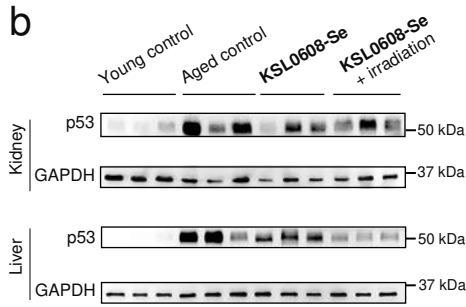
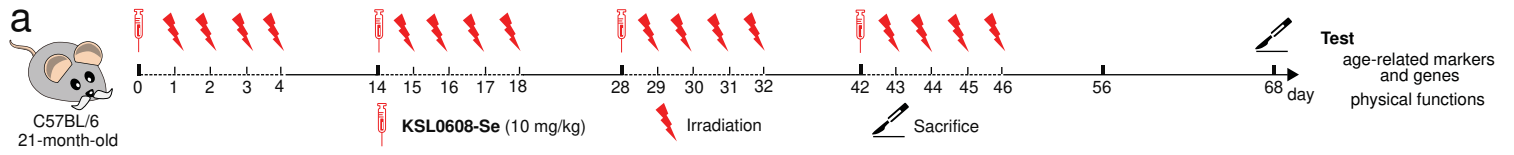


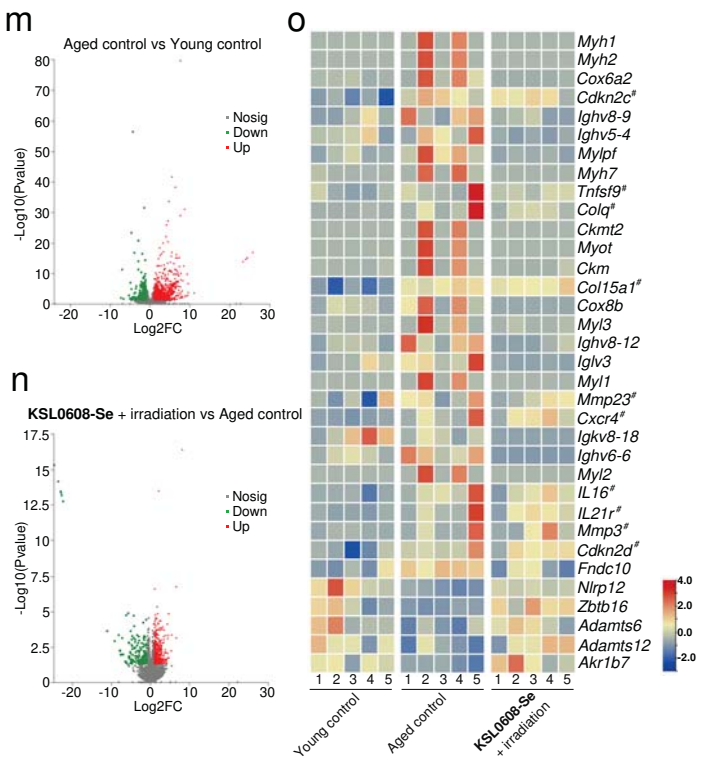
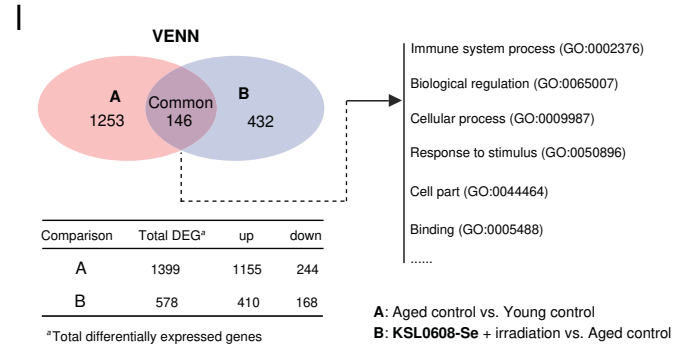
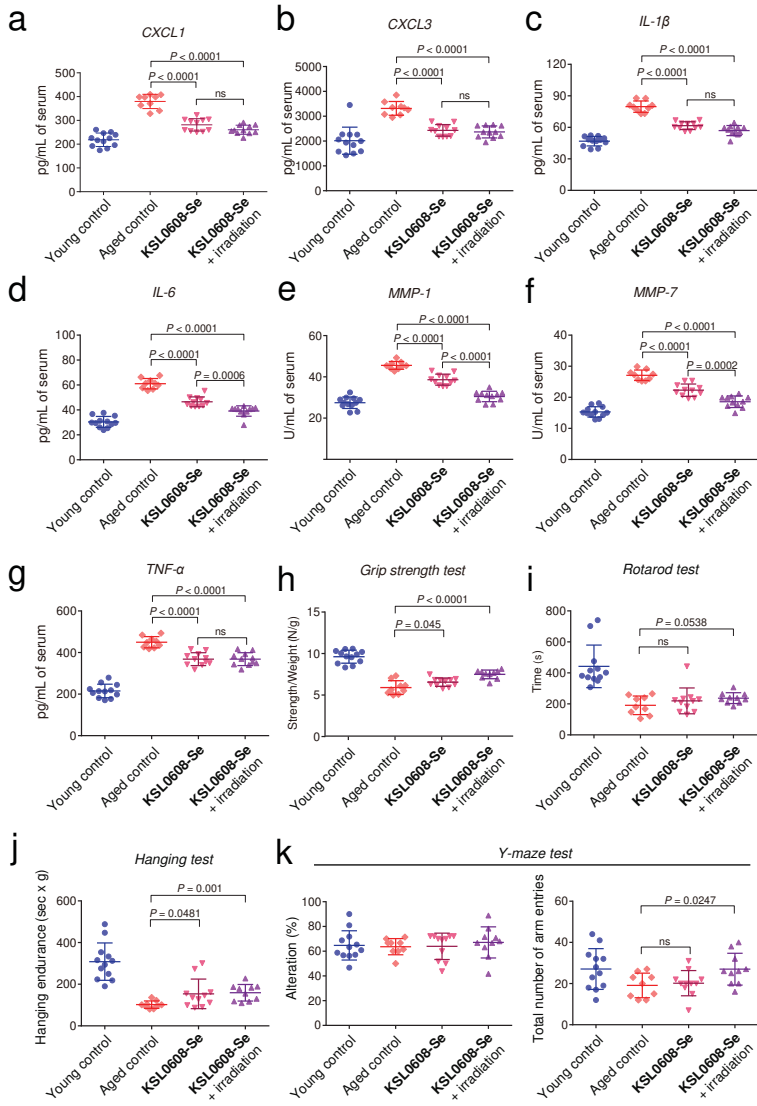


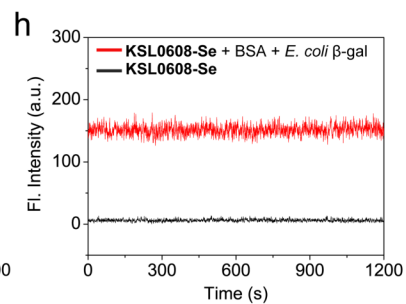
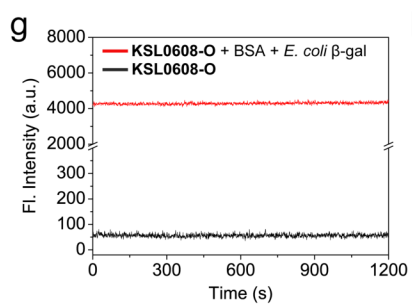
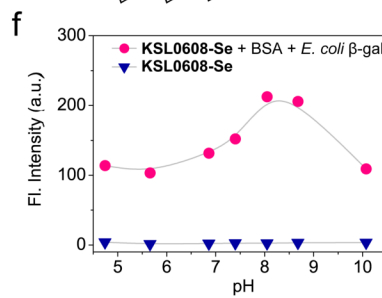
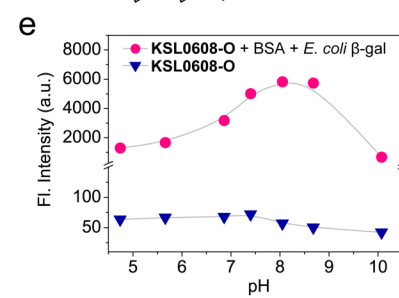
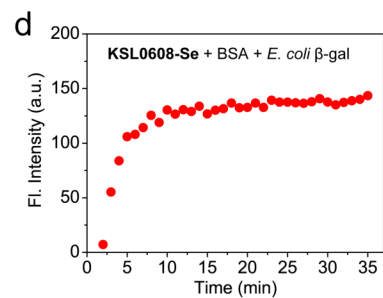
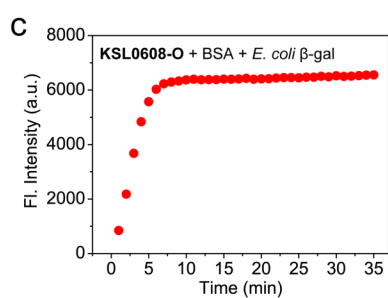
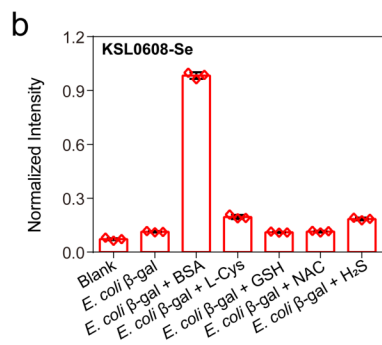
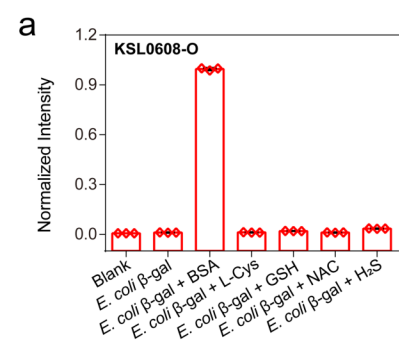
A: Doxo control vs. Young control

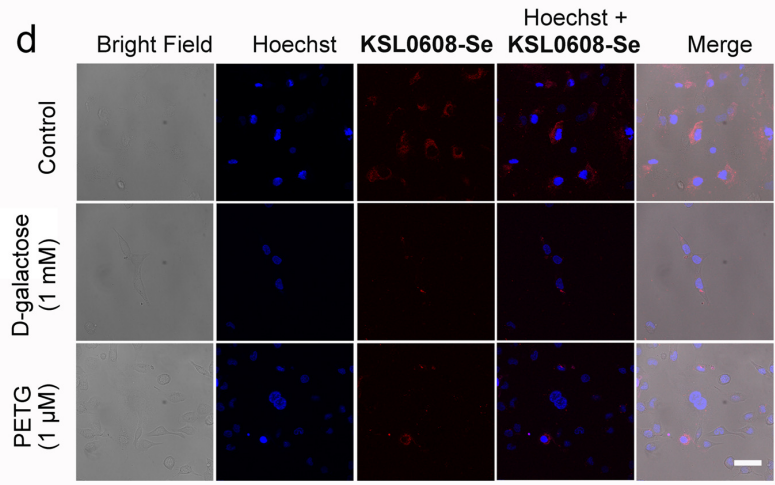
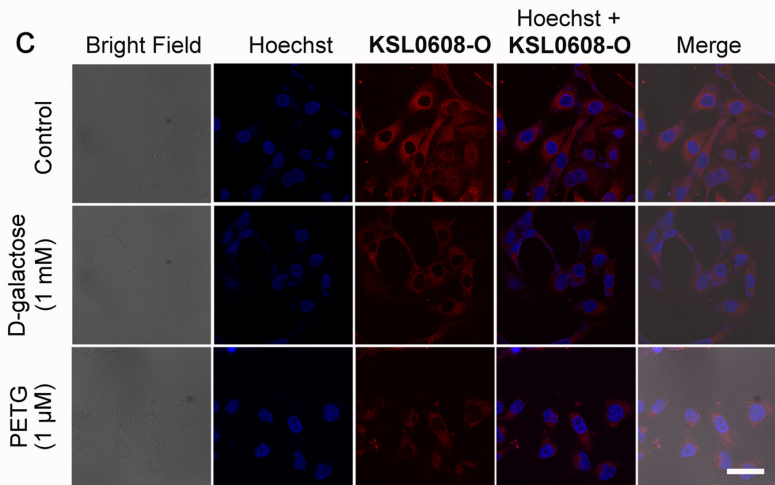
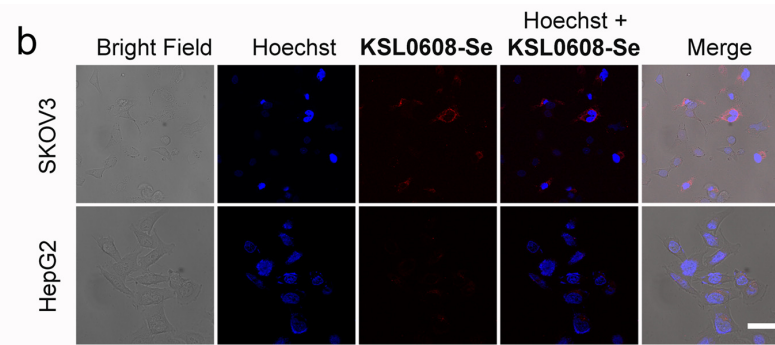
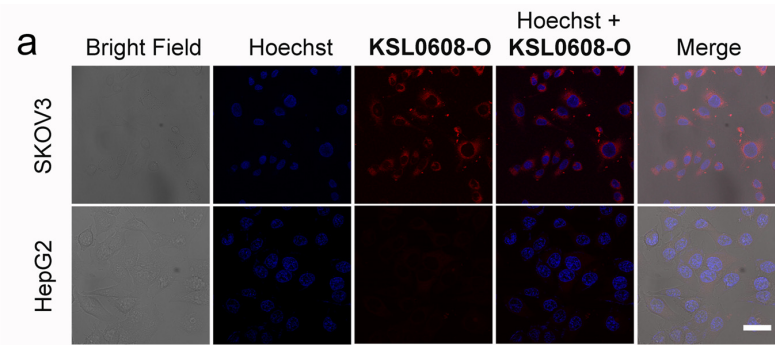
B: KSL0608-Se + irradiation vs. Doxo control

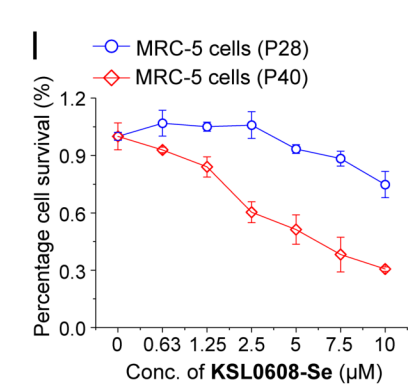
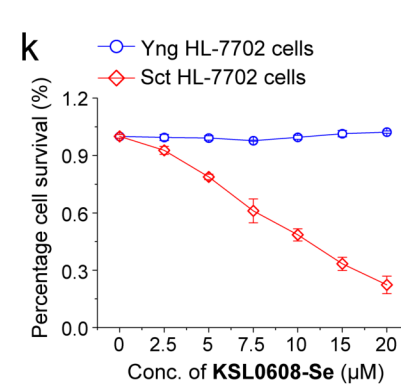
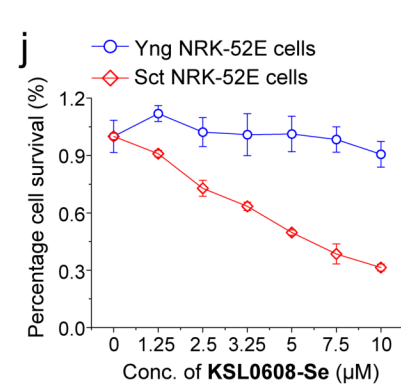
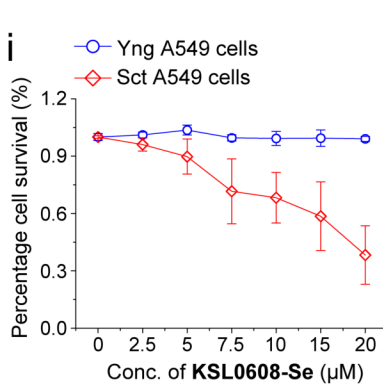
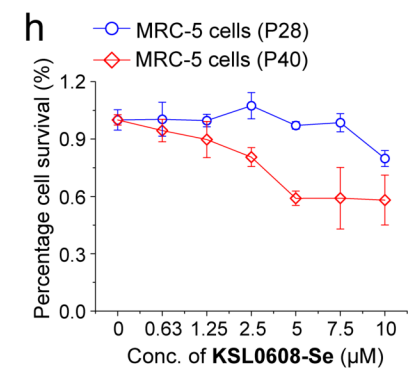
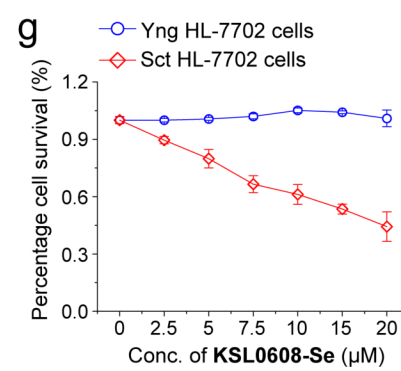
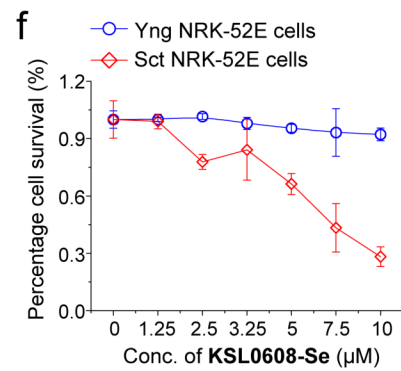
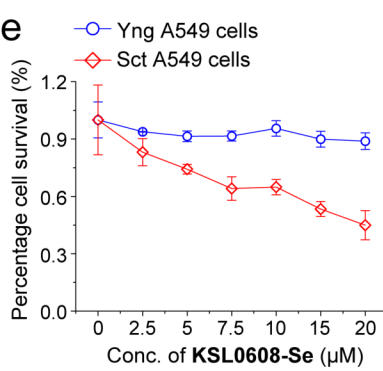
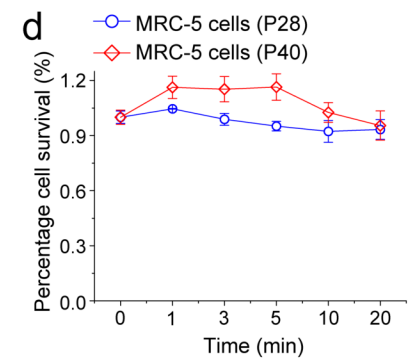
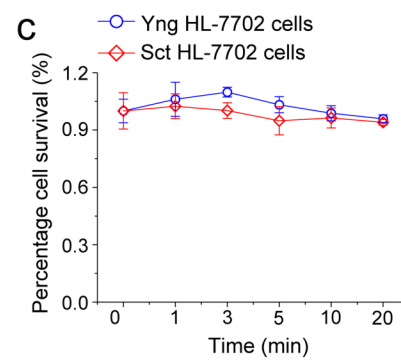
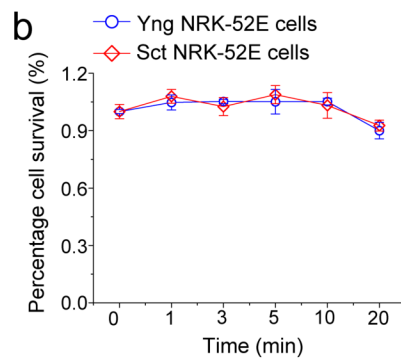
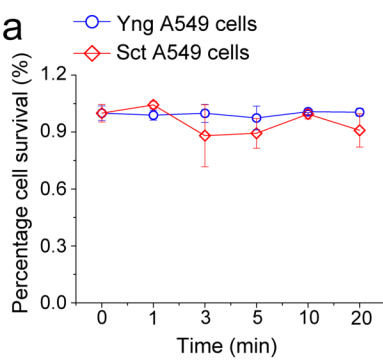


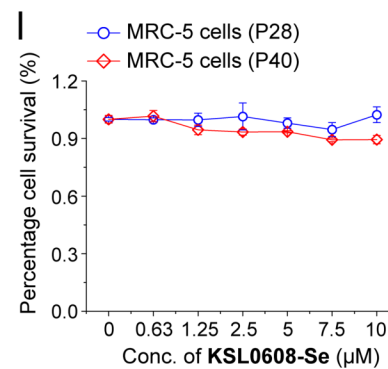
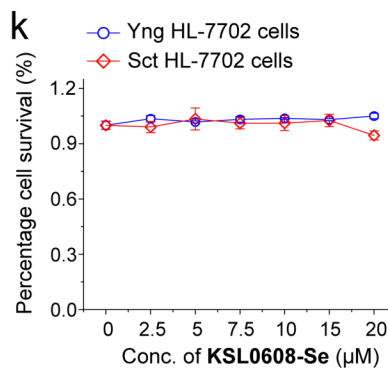
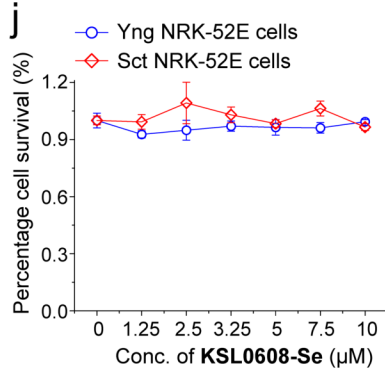
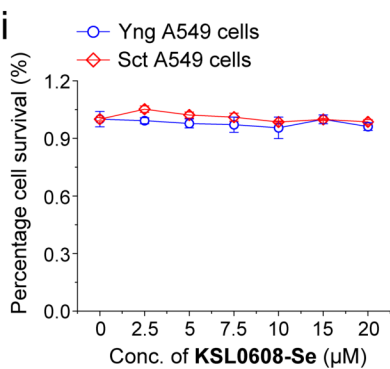
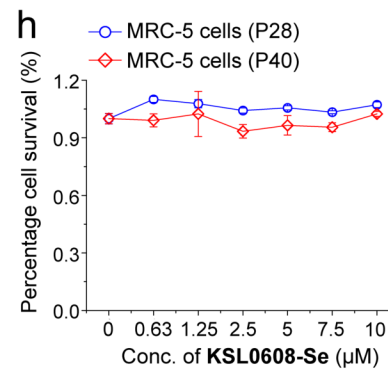
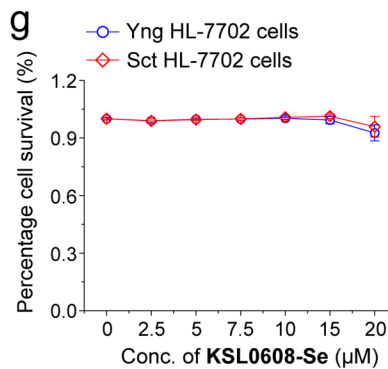
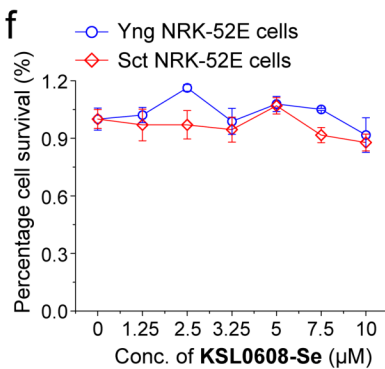
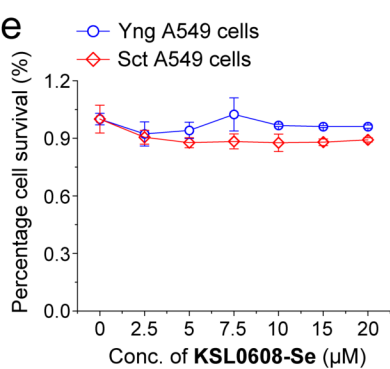
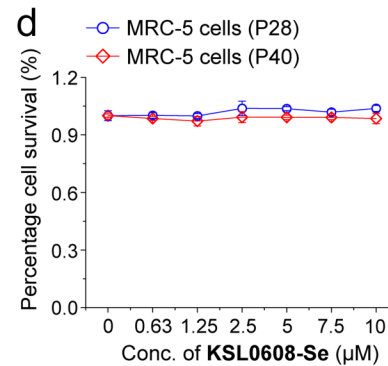
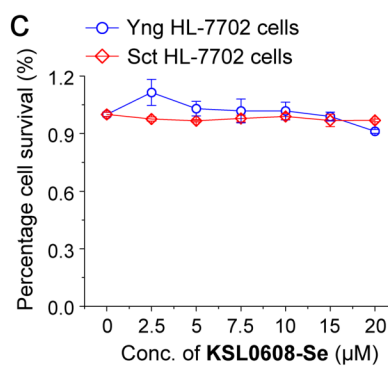
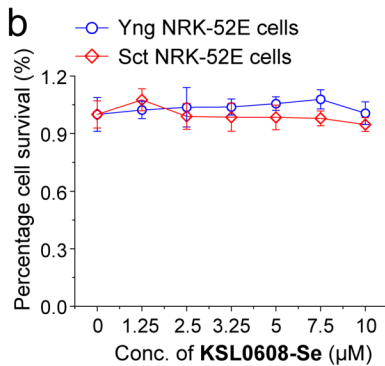
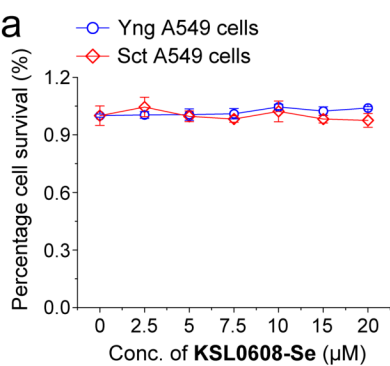


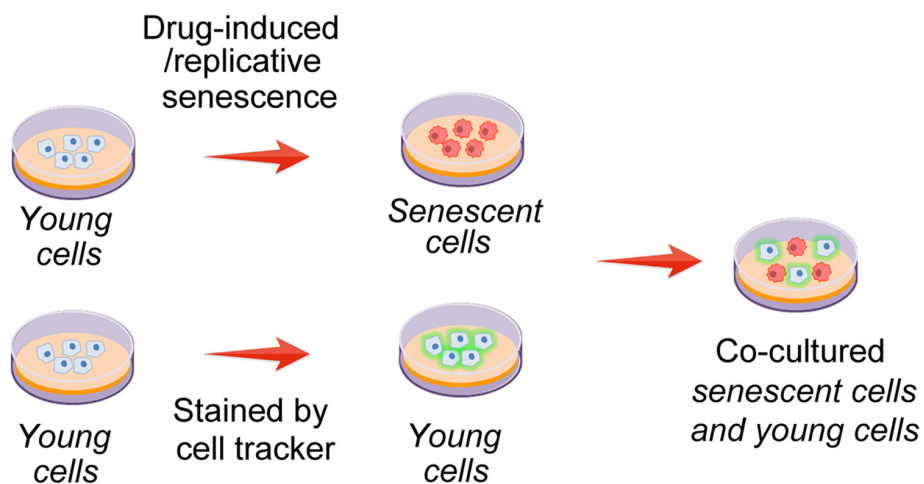




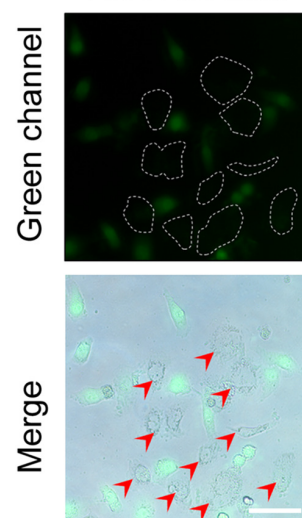
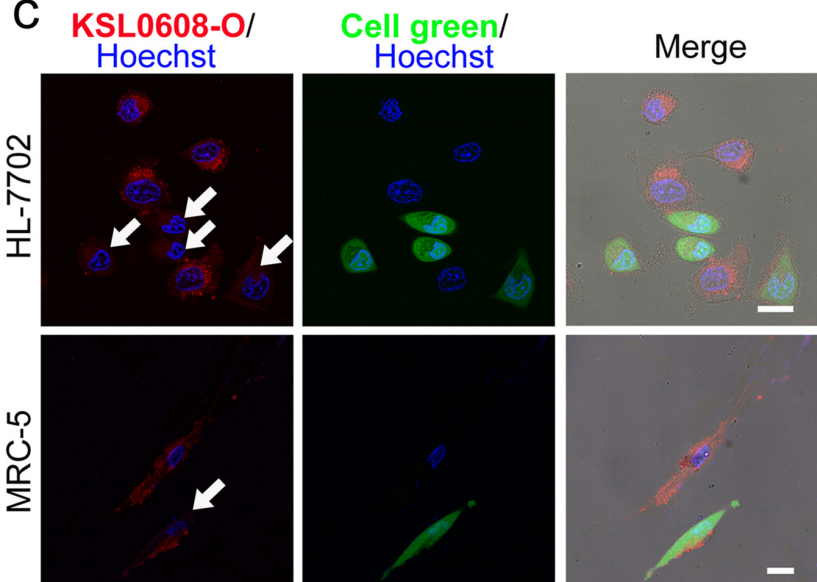
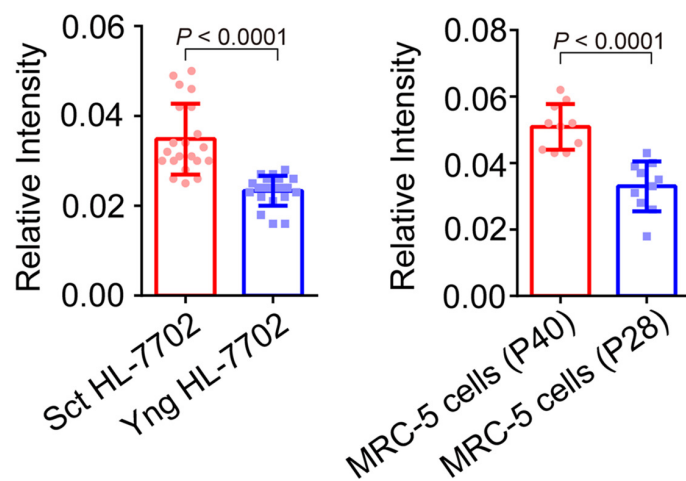
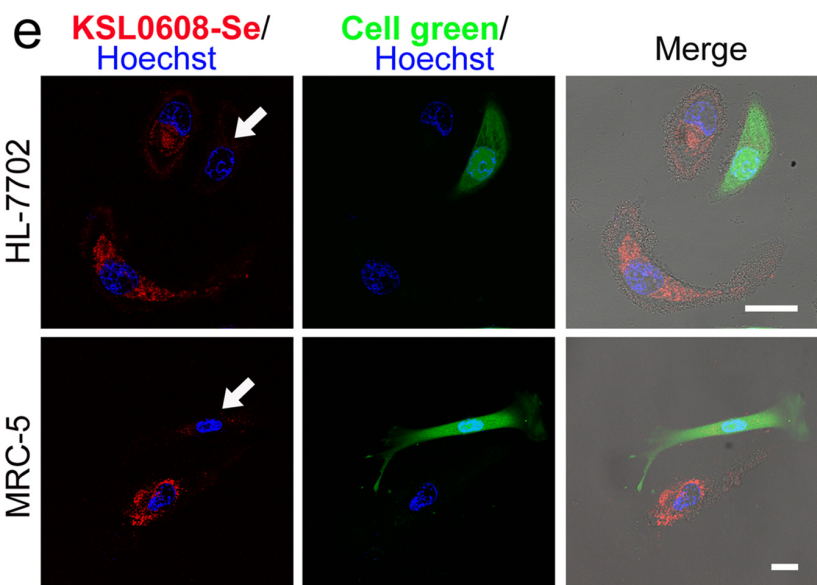






a**b**

Young and senescent
HL-7702 cells

**c****d****e****f**