Supplementary Material

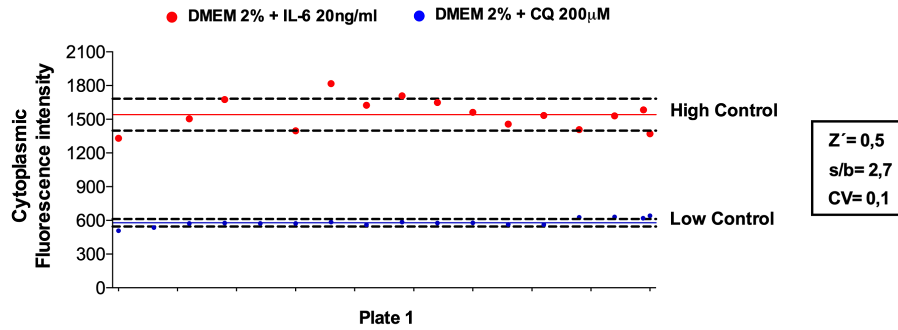
Materials and Methods

IMR90 lung fibroblasts. Human IMR90 lung fibroblasts were obtained from American Type Culture Collection (ATCC®, Cat# CCL-186, RRID:CVCL 0347) and cultured in EMEM medium with 10% FBS, 1% P/S antibiotics and 1% Glutamax. To induce senescence, cells were treated for 48h with Etoposide (20 µM). Two days after Etoposide removal, about 70% of IMR90 cells were SA-β-Gal positive. Cells were treated for 48h with PPARα agonists (Fenofibrate, CP775146, GW7647) at concentrations indicated and with navitoclax (2.5µM) and Rapamycin (200nM). The medium was removed and bafilomycin (100 nM) was added. Cells were incubated for 1h at 37ºC. Then, C_{12}FDG were added and incubated for 90 min at 37ºC. Next, Bafilomycin and C_{12}FDG were removed and Hoechst (2.5µl/ml) was added for 20min at 37ºC. Finally, cells were washed with HBSS and resuspended in EMEM to read the fluorescence in IN Cell Analyzer. The percentage of SA-β-Gal-positive cells was determined using C_{12}FDG-based senescence assay.

Ercc1 deficient mice fibroblasts. Ercc1 deficient mice (*Ercc1*$^{-/-}$*)* fibroblasts (MEFs) are embryonic mouse fibroblasts with low repair capacity of DNA due to lack of DNA repair endonuclease Ercc1-XPF[55]. *Ercc1*$^{-/-}$ *MEFs* were provided by Paul D. Robbins (Institute on the Biology of Aging and Metabolism and Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA). *Ercc1*$^{-/-}$ *MEFs* were cultured in a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F10 with 10% fetal bovine serum, 1X nonessential amino acids, penicillin, and streptomycin and incubated at 3% O2 initially, followed by a shift to 20 % for 5 passages to induce senescence[56]. Then, cells were treated for 48h with PPARα agonists (CP775146, GW7647) at concentrations indicated and with Navitoclax (2.5µM) and Rapamycin (200nM) and incubated at 20% O_2, 5% CO_2 and 37°C and the staining was carried out with the fluorescent substrate C_{12}FDG. The medium was removed and Bafilomycin (100 nM) was added. Cells were incubated for 1h at 37ºC. Then, C_{12}FDG at 20µM were added and incubated for 30min at 37ºC. Next, Bafilomycin and C_{12}FDG were removed and Hoechst (2.5µl/ml) was added for 90min at 37ºC. Finally, cell fluorescence was analyzed in IN Cell Analyzer.

Figures

a



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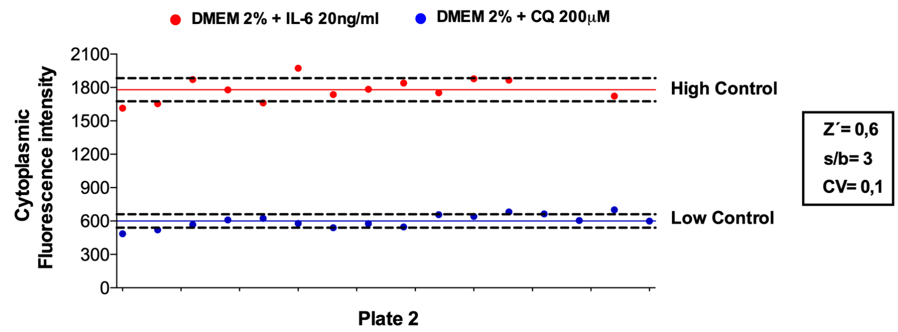

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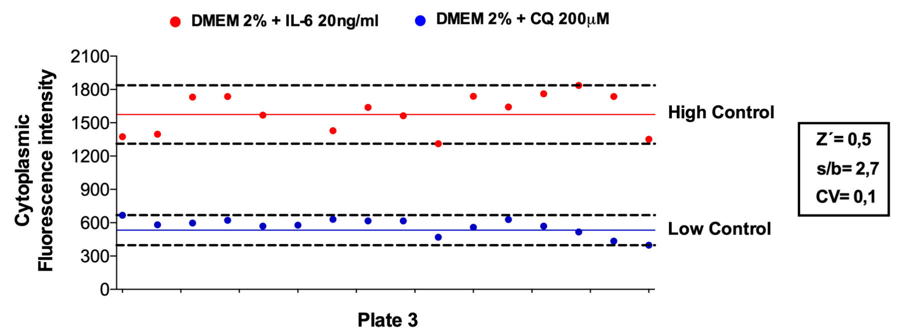

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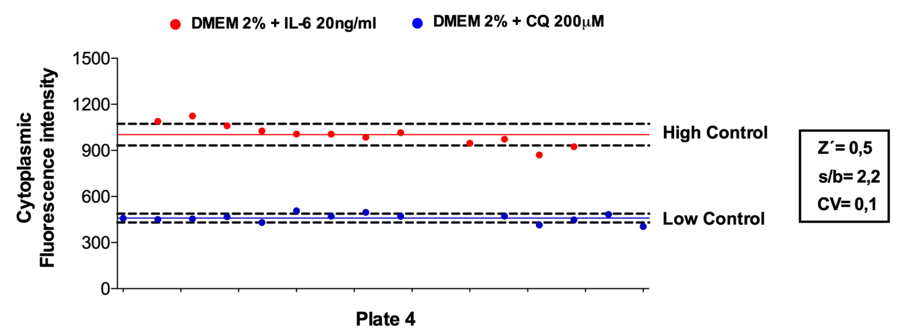
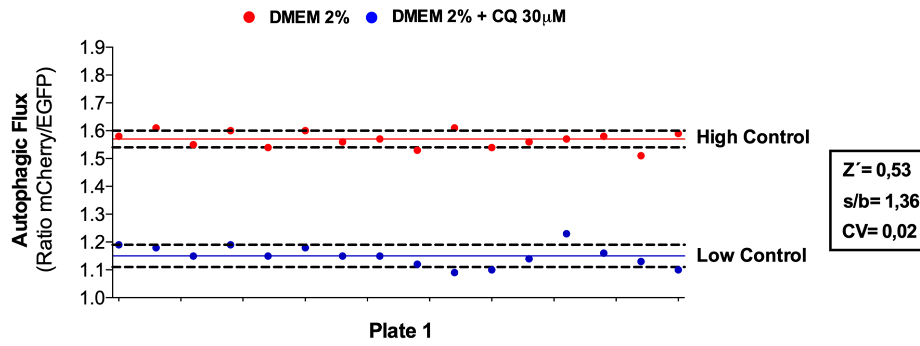
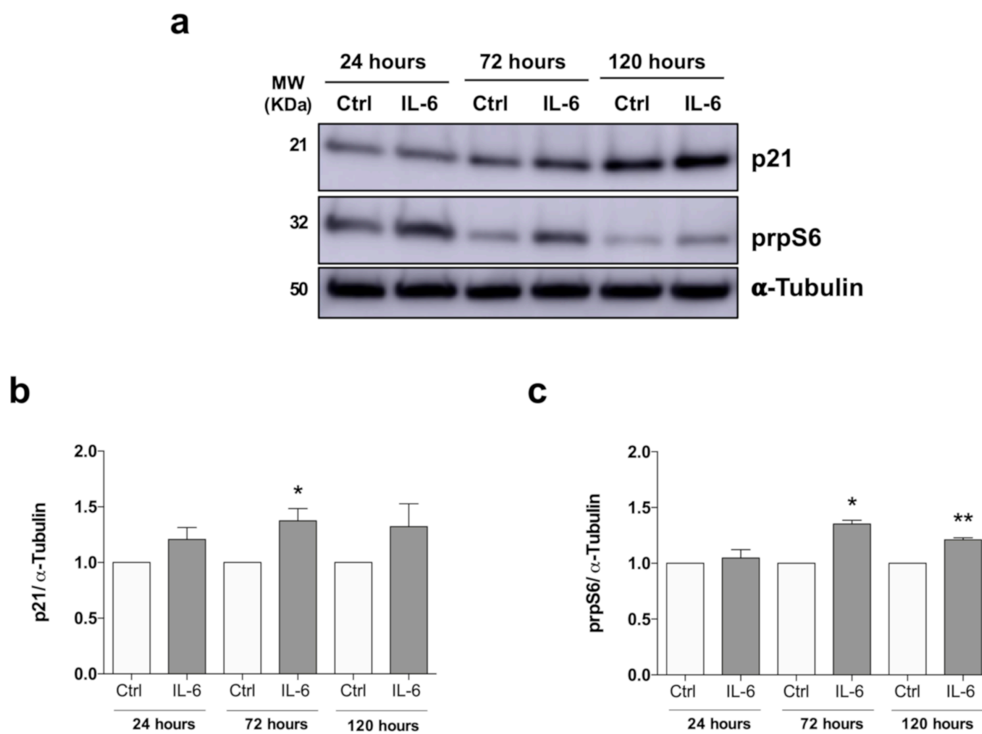


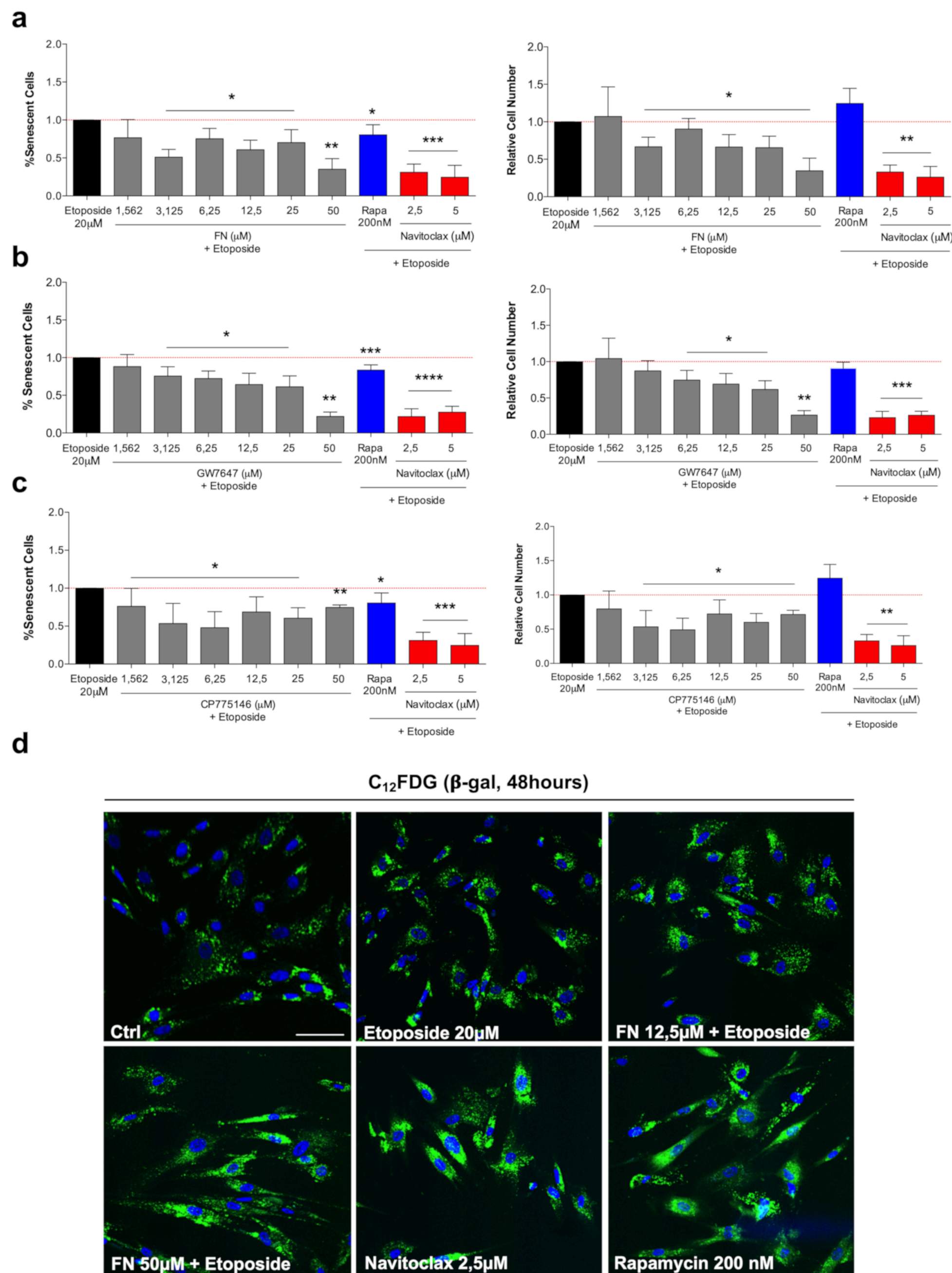
Supplementary Fig. 1 Analysis of cell-based chondrocyte senescence assay quality. Negative and positive controls are used to determine Z´ factor. Signal to background (s/b) ratio and coefficient of variation (CV) for each plate.



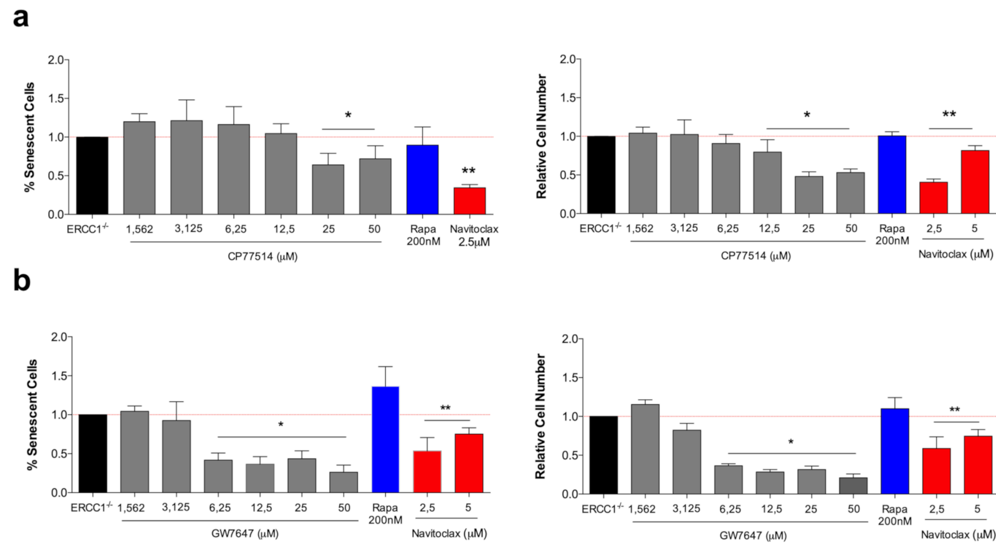
Supplementary Fig. 2 Analysis of cell-based autophagic flux assay quality. Negative and positive controls are used to determine Z´ factor. Signal to background (s/b) ratio and coefficient of variation (CV) for each plate.

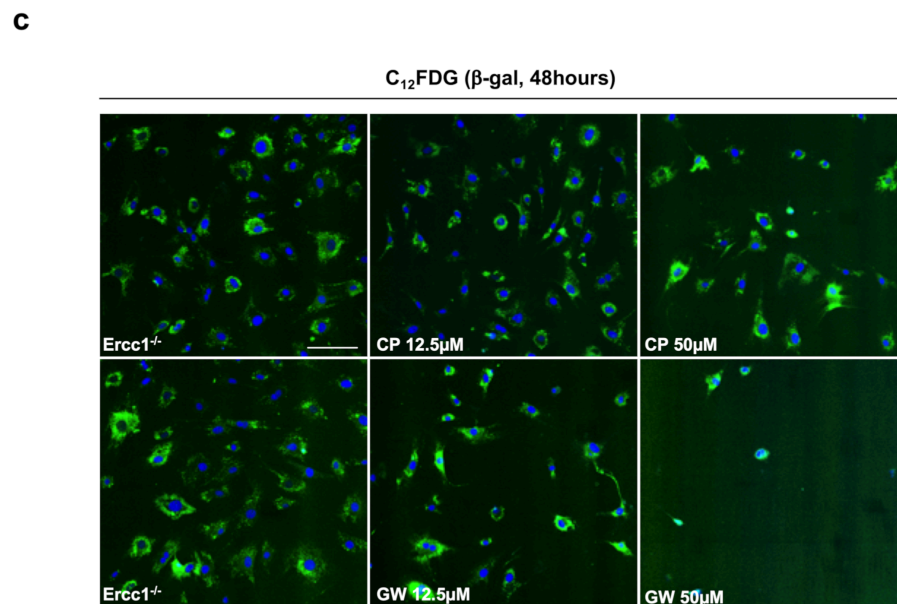


Supplementary Fig. 3 IL-6 induces senescence and mTOR markers in human chondrocytes. a. Western blot of p21 and prpS6 in T/C28a2 human chondrocytes treated with IL-6 (20ng/ml) for 24, 72 and 120h. α-tubulin was employed as a loading control **b.** Densitometric analysis of p21. Values are mean ± SEM of *n=3* independent experiments, **p < .001 vs. Ctrl 72h*, two-tailed unpaired Student's t-test. **c.** Densitometric analysis of prbS6. Values are mean ± SEM of *n=3* independent experiments, **p < .001 vs. Ctrl 72h and **p < .001 vs. Ctrl 120h*, two-tailed unpaired Student's t-test.

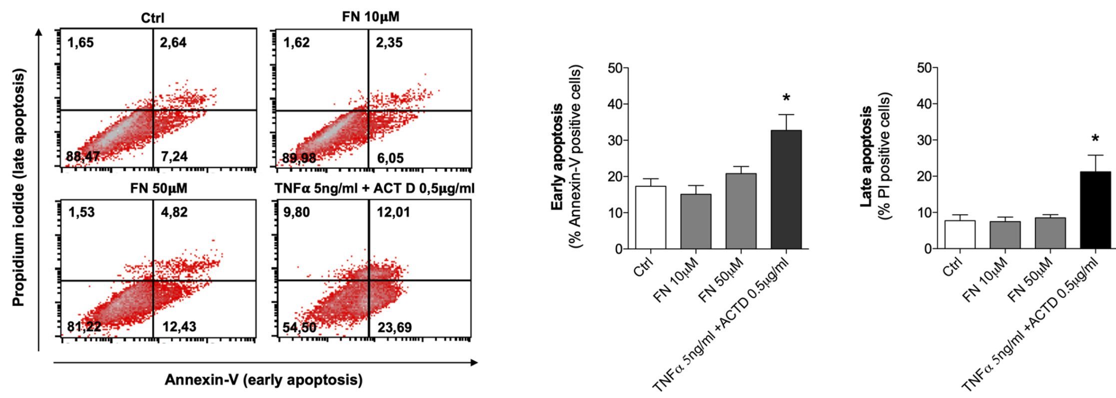


Supplementary Fig. 4 PPARα agonists are senolytics in IMR90 normal lung human cells. a. IMR90 human cells were treated with Etoposide (20µM) and in combination with fenofibrate, FN (1,582-50µM), Rapa (200nM), or Navitoclax (2.5µM) for 48h. Relative senescence cells: values are mean ± SEM of 4 well/condition, **p < .05, **p < .001, ***p < .0001 vs. Etoposide*. Relative cell number: values are mean ± SEM of 4 well/condition, **p < .01, **p < .0001 vs. Etoposide*, two-tailed unpaired Student's t-test. **b.** IMR90 human cells were treated with Etoposide (20µM) and in combination with GW7647, GW (1,582 - 50µM), Rapa (200nM), or Navitoclax (2.5 and 5µM) for 48h. Relative senescence cells: values are mean ± SEM of 4 well/condition, **p < .01, **p < .0001, ***p < .05 and ****p < .0001 vs. Etoposide*. Relative cell number: values are mean ± SEM of 4 well/condition, **p < .01, **p < .001 and ***p < .0001 vs. Etoposide*, two-tailed unpaired Student's t-test. **c.** IMR90 human cells were treated with Etoposide (20µM) and in combination with CP775146, CP (1,582-50µM), Rapa (200nM), or Navitoclax (2.5 and 5µM) for 48h. Relative senescence cells: values are mean ± SEM of 4 well/condition, **p < .01, **p < .001, ***p < .0001 vs. Etoposide*. Relative cell number: values are mean ± SEM of 4 well/condition **p < .01, **p < .0001 vs. Etoposide*, two-tailed unpaired Student's t-test. **d.** Representative images of SA-β-Gal activity from T/C28a2 human chondrocytes treated with FN in response to Etoposide treatment. Scale bar, 200µm.

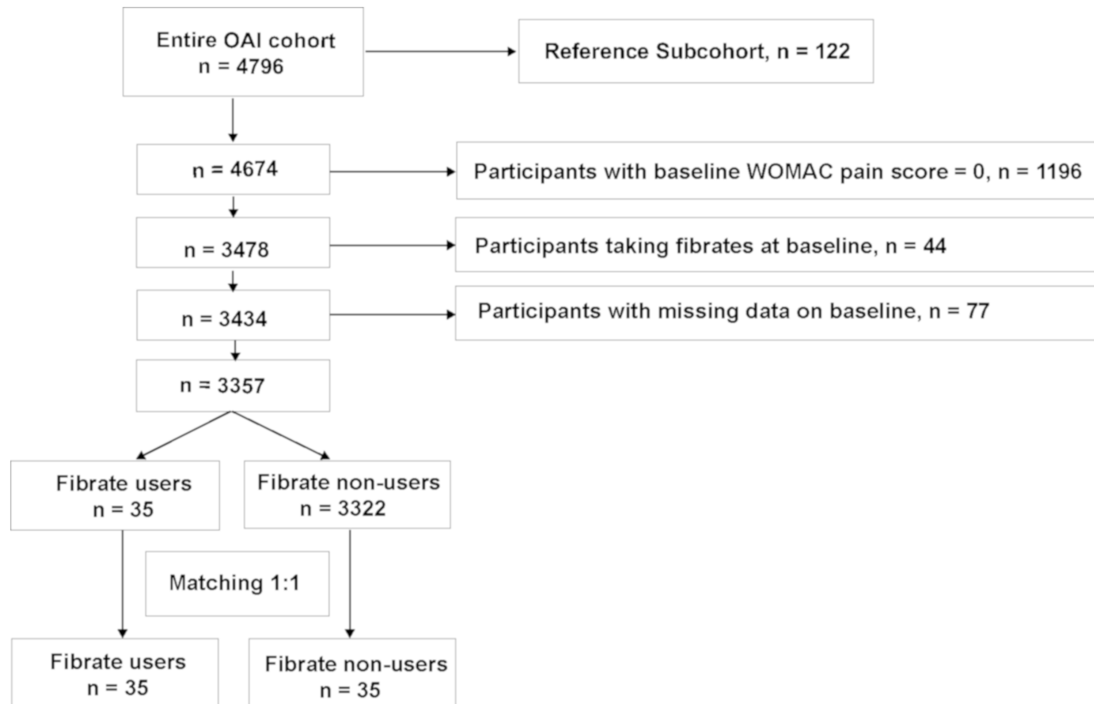




Supplementary Fig. 5 PPARα agonists are senolytics in Ercc1 deficient fibroblast. a. Ercc1$^{-/-}$ MEFs were treated with CP775146, CP (1,582 - 50µM), Rapa (200nM), or Navitoclax (2.5µM) for 48h. Relative senescence cells: values are mean ± SEM of 4 well/condition, $*p < .01$, $**p < .0001$ *vs. Ercc1*$^{-/-}$. Relative cell number: values are mean ± SEM of 4 well/condition, $*p < .001$, $**p < .05$ *vs. Ercc1*$^{-/-}$, two-tailed unpaired Student's t-test. **b.** Ercc1$^{-/-}$ MEFs were treated with GW7647, GW (1,582 - 50µM), Rapa (200 nM), or Navitoclax (2.5µM) for 48h. Relative senescence cells: values are mean ± SEM of 4 well/condition, $*p < .001$, $**p < .05$ *vs. Ercc1*$^{-/-}$. Relative cell number: values are mean ± SEM of 4 well/condition, $*p < .0001$, $**p < .05$ *vs. Ercc1*$^{-/-}$, two-tailed unpaired Student's t-test. **c.** Representative images of SA-β-Gal activity from Ercc1$^{-/-}$ MEFs treated with CP and GW. Scale bar, 200µm.



Supplementary Fig. 6 Fenofibrate does not affect to chondrocyte viability. Quantitative analysis of chondrocyte death by Annexin-V and PI staining in human aging chondrocytes treated with FN (10, 50 μM) and TNFα (5ng/ml) + Actinomycin D (0.5μg/ml) as positive control for apoptosis, for 18h in a 12 well plate human chondrocytes. Values are mean ± SEM of *n=3* human aging chondrocytes. **p < .05 vs. Ctrl*, two-tailed unpaired Student's t-test.



Supplementary Fig. 7 Stratification of fibrate users from Osteoarthritis initiative (OAI) cohort.

Supplementary Table 1. Patient demographics and characteristics before and after group matching

	Before matching			After matching		
	Fibrate users (n = 35)	**Control (n=3322)**	**SMD**	**Fibrate users (n = 35)**	**Control (n=3322)**	**SMD**
Age (years)	62,1429	61, 0135	0,1294	2,1429	62, 3429	-0,0229
BMI, Kg/m2	30,3657	29, 0563	0,3354	30,3657	30, 4114	-0,0117
Female	0,6286	0,3977	0,471	0,6286	0,6286	0
Male	0,3714	0,6023	-0,471	0,3714	0,3714	0
History of knee surgery	0,3714	0,2474	0,2529	0,3714	0,3714	0
WOMAC pain	5,7429	4,7562	0.2284	5,7429	5,6857	0,0132
WOMAC function	16,6311	14,3494	0,189	16,6311	16, 9363	-0,0253
WOMAC stiffnness	3,2	2,5093	0,3919	3,2	3,2286	-0,0162
PASE	155,2575	159,8893	-0,0715	155,2575	156,2857	-0,0159

Data are presented as the mean (continuous variables), proportion (dichotomous varaiables); **SMD**, standardized mean difference; **BMI**, Body Mass Index; **PASE**, Physical Activity Scale for the Elderly; **WOMAC**, Western Ontario and McMaster Universities OA index; Possible ranges for WOMAC pain score are 0-20, possible ranges for WOMAC function score are 0-68, possible ranges for WOMAC stiffness are 0-8, possible ranges for PASE are 0-400.