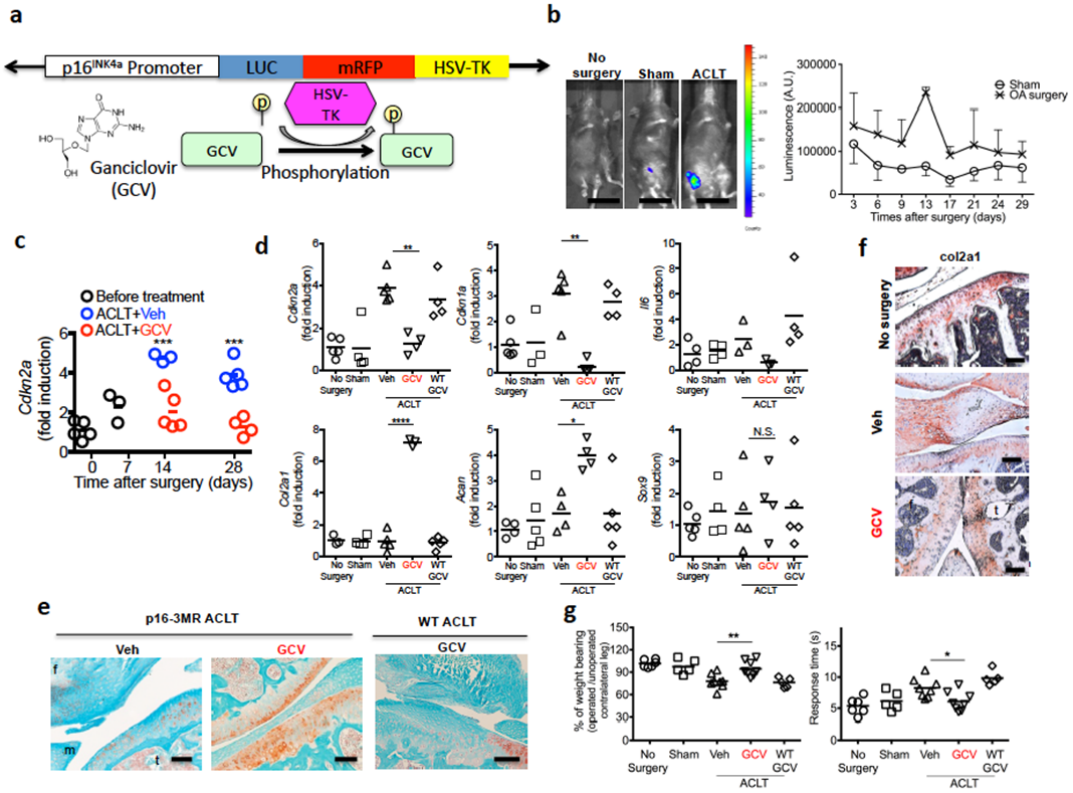

Supplementary Figure 1. Kinetics of SnCs development in surgically-induced OA and effect of GCV-induced SnC clearance on OA disease progression in C57BL and p16-3MR mice. (**a**) Diagram depicting the p16-3MR transgene. (**b**) p16-3MR mice received vehicle (Veh, 10 µl saline) to the ACLT-operated or sham-operated knee by IA injection 8 days after the surgery for 5 consecutive days, with a 2nd treatment 21 days after post-surgery for 5 consecutive days. Shown are representative images of mice 28 days post-surgery and quantification of luminescence (in arbitrary units, A.U.) at the indicated time. $n = 4$ for each group. Scale bars, 2 cm. In **c**–**g**, we administered two cycles of GCV for 5 consecutive days (1 mM in 10 µl saline) starting at day 8 after surgery in p16-3MR or non-transgenic C57BL mice. (**c**) Quantification of mRNA expression for *Cdkn2a* at indicated time and (**d**) *Cdkn2a, Cdkn1a, Il6, Col2a1*, *Acan* and *Sox9* normalized to *β-actin* day 28 after surgery from p16-3MR mice articular joints that received Veh or GCV and non-transgenic C57BL mice that received GCV. (**e**) Representative images of Safranin-O/methyl green staining; Veh-treated p16-3MR ACLT, $n = 1$; GCV-treated p16-3MR ACLT or C57BL ACLT, $n = 3$ and (**f**) col2a1 immunohistochemistry; No surgery, $n = 2$; Veh-treated ACLT, $n = 6$; GCV-treated ACLT, $n = 3$, Scale bars, 100 µm and (**g**) the percentage of weight placed on the operated limb versus contralateral control limb and response time of mice after placement onto a 55°C hotplate. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and N.S. (Not Significant); one-way ANOVA (Tukey's multiple comparison test) for **c**, **d**; unpaired t-test (two-tailed) for **g**. All data are expressed as mean and each data point represents an individual mouse.

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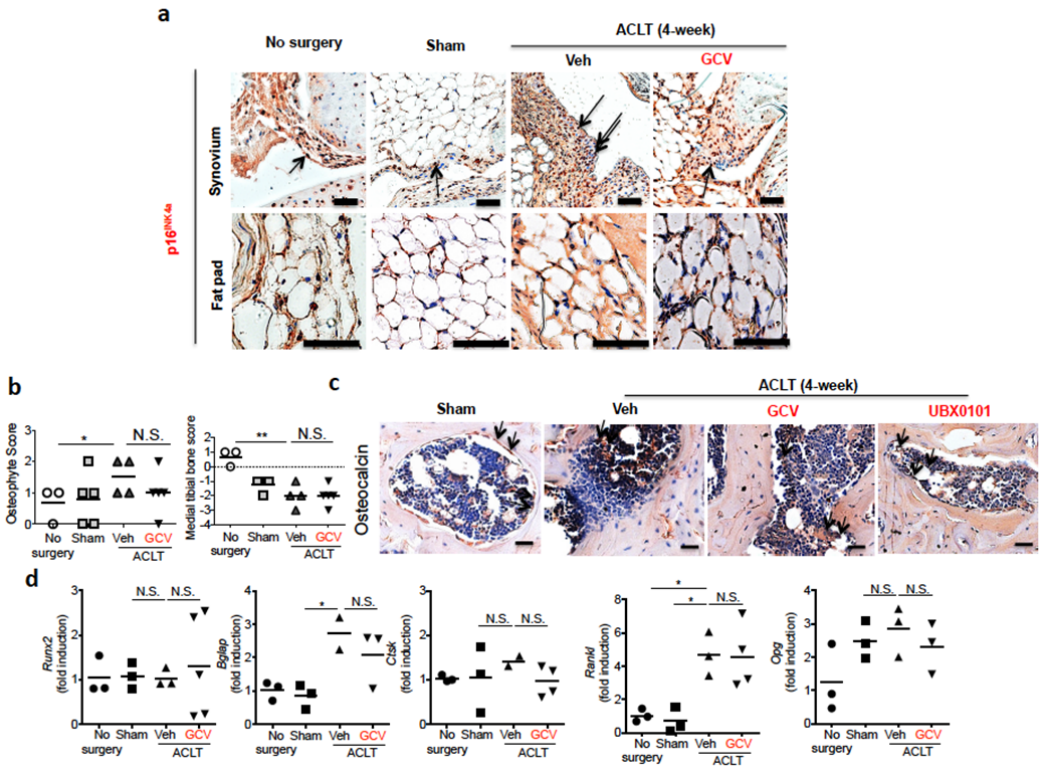
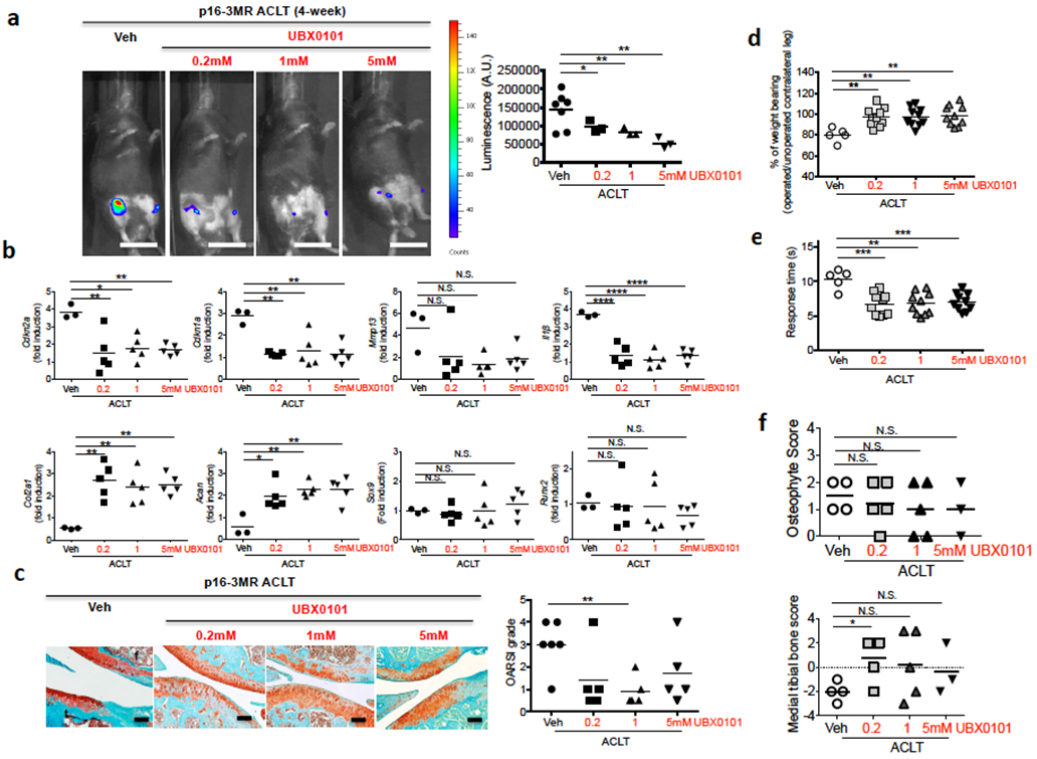
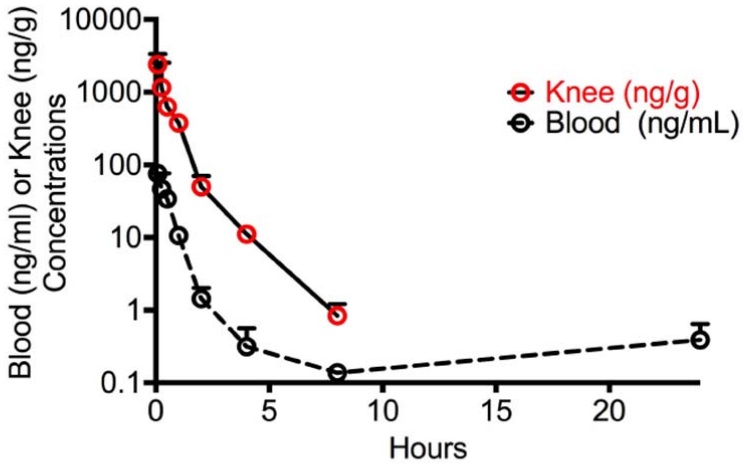


Supplementary Figure 2. The presence of SnCs in the synovium and infrapatellar fat pad, and characterization of subchondral bone changes in p16-3MR mice after vehicle or GCV-treatment. (**a**) Representative images of $p16^{INK4a}$-positive SnCs in the synovium and infrapatellar fat pad; No surgery and Sham-operated controls, $n = 2$; Veh- or GCV treated ACLT, $n = 3$. Scale bars, 100 μm (**b**) Scores for osteophyte formation and medial tibial bone loss. Marked osteophyte formation and subchondral bone loss were observed in veh-treated mice 28 days post-surgery, which did not significantly decrease with GCV treatment. **(c**) Representative images of osteocalcin. There was an increase in abnormal osteocalcin-positive osteoblasts (arrows) in the marrow area of the tibial subchondral bone are increased in abnormal in Veh, GCV and UBX0101-treated p16-3MR with ACLT surgery. In contrast, these cells were located on the bone surface of sham-operated controls. Sham-operated controls, $n = 2$; Veh-treated, $n = 4$; GCV-treated, $n = 2$; UBX0101-treated ACLT, $n = 5$. Scale bars, 100 μm (**d**) Quantification of mRNA expression for *Runx2* and *Bglap* (osteocalcin) as bone formation markers and *Ctsk*, *Rankl*, and *Opg* (osteoprotegerin) as bone turnover markers normalized to *β-actin* in joints. ACLT surgery resulted in an increase in expression of *Runx2* and *Bglap* and accelerated *Ctsk*, *Rankl*, and *Opg*, that did not change with GCV treatment on day 28 after surgery. In (**b**) and (**d**), $*P < 0.05$, $**P < 0.01$, and N.S. (Not Significant); unpaired t-test (two-tailed) for **b**; one-way ANOVA (Tukey's multiple comparisons test) for **d**. All data are expressed as mean and each data point represents an individual mouse.

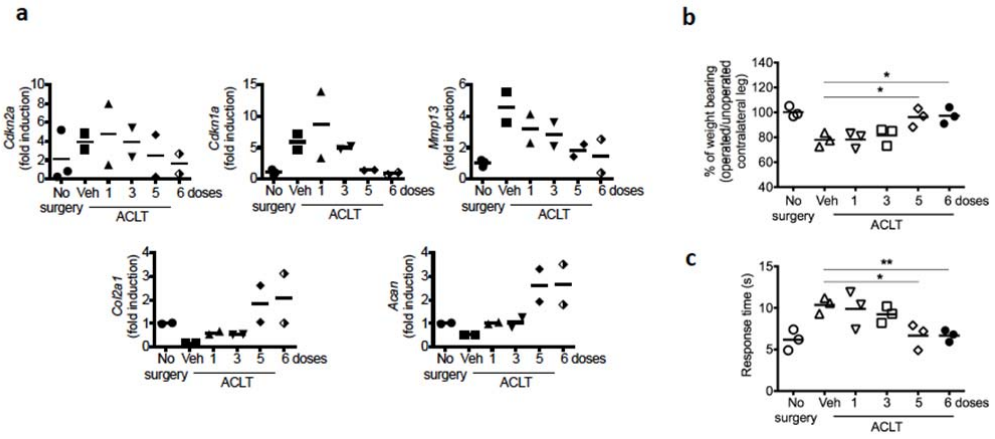
Nature Medicine: doi:10.1038/nm.4324



Supplementary Figure 3. UBX0101 dose–dependent elimination of SnCs induced by post-traumatic OA in p16-3MR mice and resulting changes in progression of post-traumatic OA. (**a**) Representative whole body luminescent images of vehicle (Veh) or 0.2, 1, and 5 mM of UBX0101-treated p16–3MR mice 28 days following IA injection once every two days over 2 weeks starting 14 days post-surgery (left). Quantification of luminescence (right). No surgery, $n = 7$; 0.2, 1 or 5 mM UBX0101-treated ACLT mice, $n = 3$. Scale bars, 2 cm. (**b**) Quantification of mRNAs expression for *Cdkn2a, Cdkn1a, Mmp13, Il1β, Col2a1, Acan, Sox9* and *Runx2* normalized to *β-actin*. (**c**) Representative images of Safranin-O/methyl green and comparison of Veh or 0.2, 1, and 5 mM of UBX0101-treated p16–3MR mice knee joints by OARSI grade. No surgery, $n = 6$; 0.2, 1 or 5 mM UBX0101-treated ACLT mice, $n = 5$. Scale bars, 100 μm. (**d**) Weight bearing test and (**e**) hotplate analysis upon UBX0101 treatment. (**f**) Scores for osteophyte formation and local medial tibial bone density on day 28 after surgery. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ and N.S. (Not Significant); one-way ANOVA (Tukey's multiple comparisons test) for **b**; unpaired t-test (two-tailed) for **a** and **c**–**f**. All data are expressed as mean and each data point represents an individual mouse.



Supplementary Figure 4. Local and blood pharmacokinetics (PK) of UBX0101 after IA injection. C57BL mice were injected IA with 1 mM UBX0101 (in 10 µl of saline per knee, n = 2 mice per time point). The initial dose of UBX0101 falls below the IC50 1.5 hours following IA injection. Approximately 1/30th of the initial dose reaches the circulation and the IC50 is never reached in the circulation.



Supplementary Figure 5. Efficacy of increasing UBX0101 injections on OA progression. C57BL mice that underwent the ACLT in one rear limb to induce OA were treated every other day with vehicle (Veh) or different numbers of UBX0101 IA injections (1 mM in 10 µl saline, 1 to 6 injections) for 2 weeks starting 14 days post-surgery. (a) Quantification of mRNA expression for *Cdkn2a*, *Cdkn1a Mmp13*, *Il1β, Col2a1* and *Acan* normalized to *β-actin* in joints 28 days after ACLT; $n = 2$ for each group. No statistical analysis. (b) The percentage of weight placed on the operated limb versus the contralateral control limb and (c) response time after placement on a 55°C platform on day 28 after ACLT surgery. *$P < 0.05$ and **$P < 0.01$; two-tailed t tests (unpaired). All data are expressed as mean and each data point represents an individual mouse.

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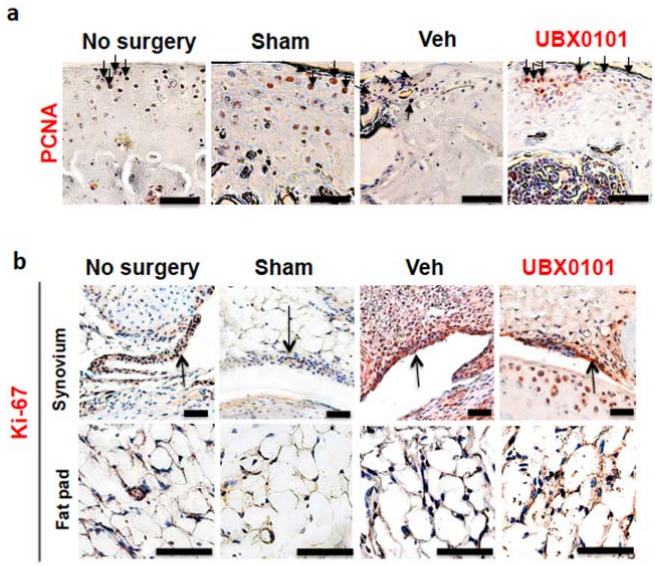
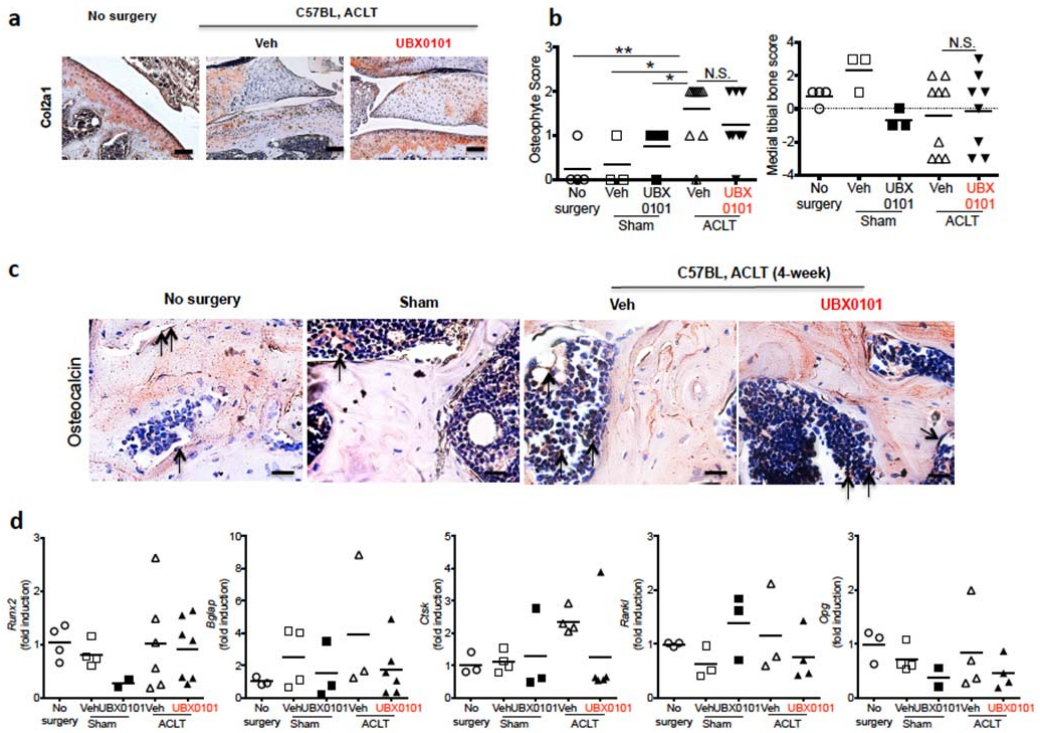
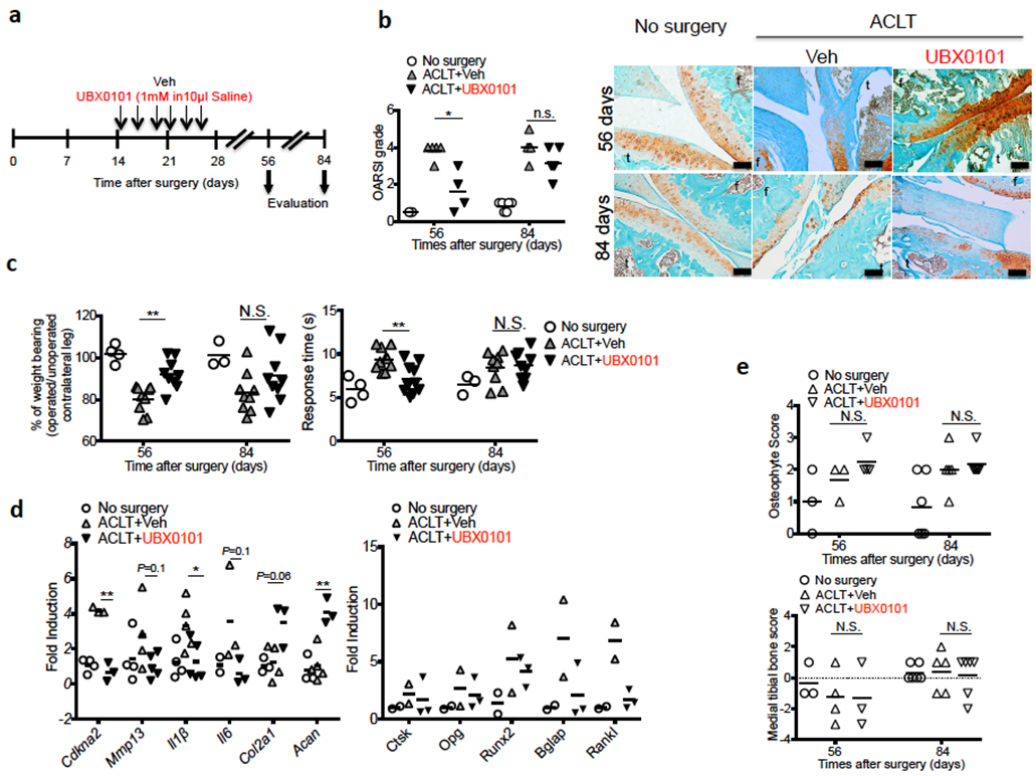


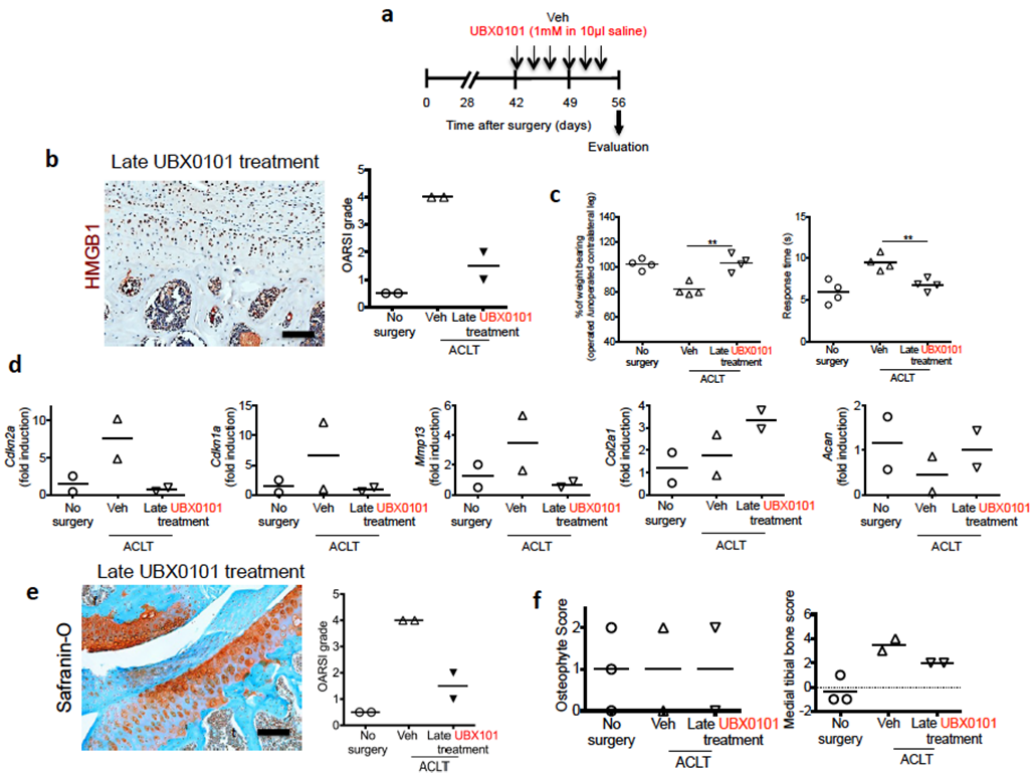
Supplementary Figure 6. The PCNA-positive non-SnCs in cartilage and Ki67 positive non-SnCs in synovium and infrapatellar fat pad after vehicle or UBX0101-treated C57BL mice. (**a**) Representative images of PCNA expression by immunohistochemistry (brown staining at arrows). There were fewer PCNA-expressing non-SnCs in the articular cartilage of vehicle (Veh)-treated C57BL mice, whereas increased number of PCNA positive proliferating cells in those of UBX0101-treated mice 4 weeks after ACLT. n = 3 for each group. Scale bars, 100 μm. (**b**) Representative images of Ki-67 expression (brown staining at arrows). In Veh-treated C57BL mice, fewer Ki-67-expressing cells (hyperplastic synovial membrane, brown staining at arrows) were detected in the synovium, consistent with the presence of SnCs, which was abrogated by UBX0101 treatment. n = 3 for each group. Scale bars, 100 μm.



Supplementary Figure 7. UBX0101 treatment increased type II collagen protein but did not significantly reduce subchondral bone changes in ACLT C57BL mice 28 days after the injury. (**a**) Immunohistochemical analyses showed that in UBX0101-treated C57BL mice, increased type II collagen protein was detected relative to vehicle (Veh)-treated ACLT mice. No surgery, $n = 4$; Veh-treated ACLT, $n = 6$; UBX0101-treated ACLT, $n = 4$. Scale bars, 100 μm (**b**) UBX0101 treatment did not significantly suppress osteophyte formation and the extent of subchondral bone loss in ACLT mice. (**c**) Osteocalcin-positive cells (brown staining at arrows) in tibial subchondral bone marrow collected 28 days after ACLT surgery and treatment with Veh or UBX0101. No surgery, Sham-operated and Veh-treated ACLT, $n = 2$; UBX0101-treated ACLT, $n = 3$. Scale bars, 100 μm (**d**) Quantification of the levels of mRNAs encoding *Runx2, Bglap, Ctsk*, *Rankl* and *Opg* (which encode runt related transcription factor 2, osteocalcin, cathepsin K, Receptor activator of nuclear factor kappa-B ligand and osteoprotegerin, individually) normalized to *β-actin* in joints from C57BL mice that received Veh or UBX0101 on day 28 after surgery.



Supplementary Figure 8. Long-term efficacy of UBX0101 in attenuating post-traumatic OA development. (**a**) Schematic of time course for experiments in **b** **e**. C57BL mice that underwent the ACLT of one rear limb were treated with vehicle (Veh) or UBX0101 (1 mM in 10 µl saline) once every two days over 2 weeks starting 14 days post-surgery. (**b**) Left: Comparison of vehicle-treated and UBX0101-treated knee joints 56 or 84 days after surgery by OARSI grade. Right: Representative images of Safranin-O/methyl green staining of the medial compartment as indicated time. No surgery, $n = 3$; Veh-treated, $n = 5$; UBX0101-treated, $n = 4$ for 56 days time point. No surgery and UBX0101-treated, $n = 6$; Veh-treated, $n = 5$ for 84 days time point. Scale bars, 100 µm. (**c**) Percentage of weight placed on the operated limb versus contralateral control limb (left) and response time after placement onto a 55° C platform (right). (**d**) Quantification of mRNA expression for *Cdkn2a*, *Mmp13*, *Il1β, Il6, Col2a1, Acan, Ctsk*, *Opg*, *Runx2, Bglap* and *Rankl* normalized to *β-actin* in joints 56 days after ACLT. (**e**) Histological analysis of subchondral bone changes as confirmed by osteophyte formation and bone sclerosis score. All data are expressed as mean and each data point represents an individual mouse. $*P < 0.05$, $**P < 0.01$, and N.S. (Not Significant); one-way ANOVA (Tukey's multiple comparison test) for **d**; two-tailed t tests (unpaired) for **b, c,** and **e**.



147
148 **Supplementary Figure 9. Efficacy of UBX0101 for treating advanced post-**
149 **traumatic OA.** (**a**) Schematic of experiment for **b**–**e**. C57BL mice underwent ACLT
150 of one rear limb to induce OA and were injected IA once every two days with vehicle
151 (Veh) or UBX0101 (1 mM in 10 µl saline) during 6 and 7 weeks post-surgery for 2
152 weeks. (**b**) Representative images of cells positive for nuclear HMGB1 by
153 immunohistochemistry (left) and quantification of HMGB1-positive non-SnCs in
154 articular cartilage (right). *n* = 2. Scale bar, 100 µm. (**c**) OA-induced joint pain was
155 alleviated by UBX0101 as monitored by weight bearing and hot plate analyses on day
156 56 after ACLT surgery; *n* = 4 for each group. (**d**) Quantification of mRNAs
157 expression *Cdkn2a, Cdkn1a, Mmp13, Il1β, Col2a1* and *Acan* normalized to *β-actin* in
158 joints as indicated; *n* = 2 for each group. (**e**) Representative images of Safranin-
159 O/methyl green staining of late UBX0101 treated joint (left, *n* = 2) and OARSI scores.
160 Scale bar, 100 µm. (**f**) Histological analysis of parameters of subchondral bone
161 changes at 56 days post ACLT surgery: osteophyte formation and medial tibial bone
162 score as indicated for trabecular bone sclerosis. All data are expressed as mean and
163 each data point represents an individual mouse. No statistical analysis.
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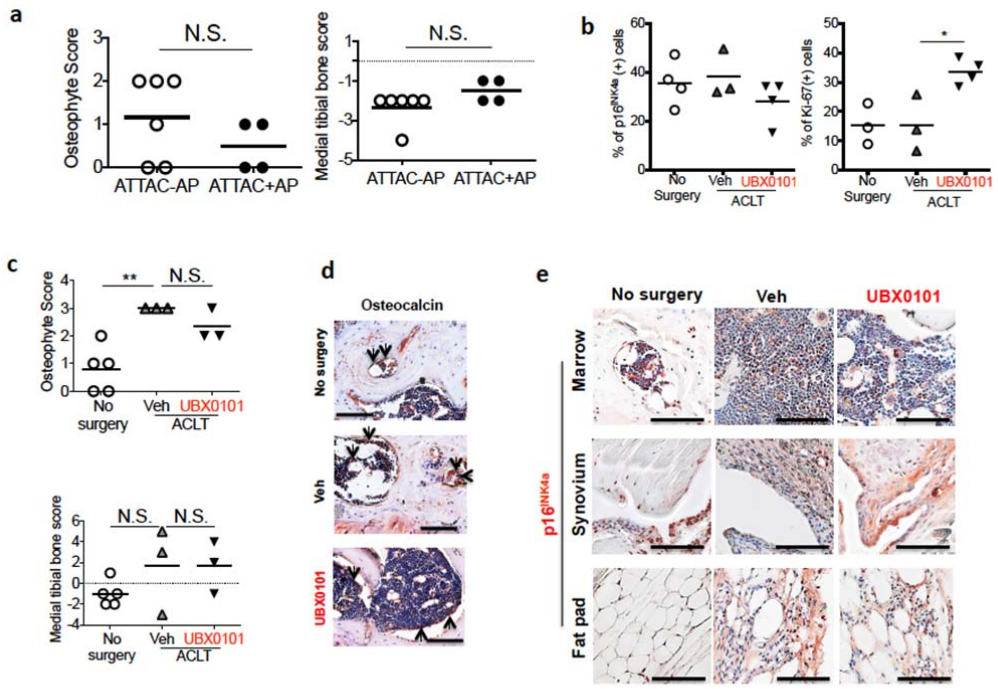
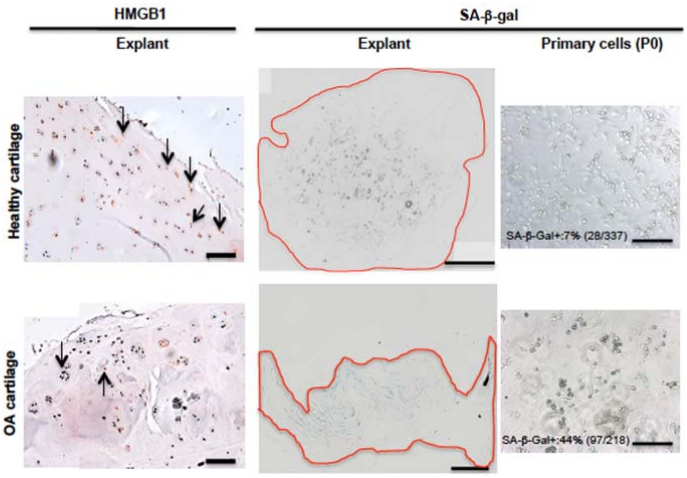
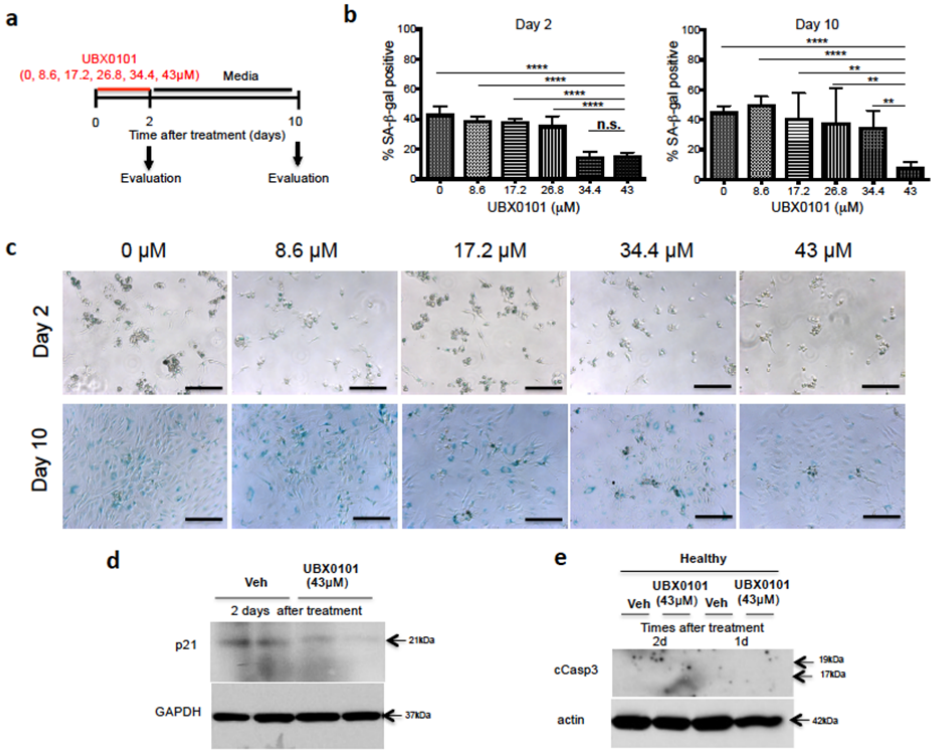


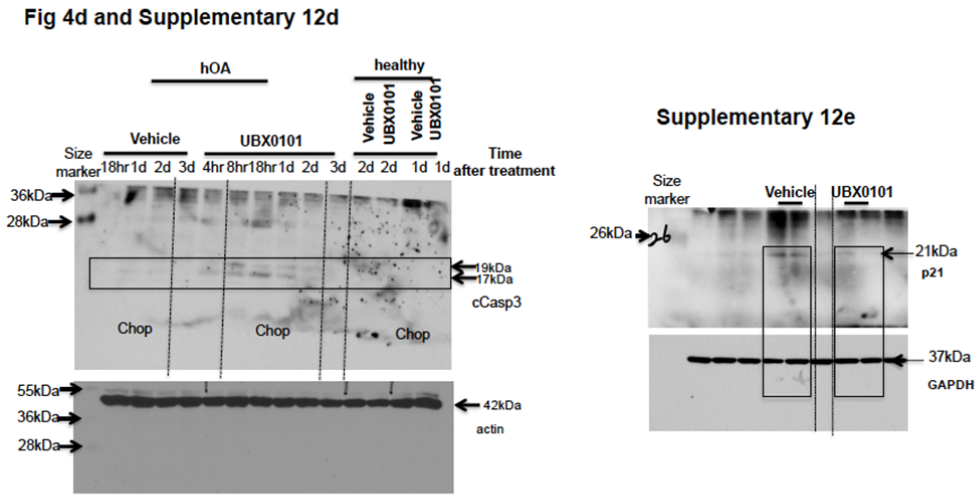
Supplementary Figure 10. Characterization of bone and other joint tissue changes after SnC clearance in naturally-occurring or surgically-induced INK-ATTAC or p16-3MR aged mice. (**a**) Subchondral bone damage scores for osteophyte formation and medial tibial bone sclerosis in INK-ATTAC female mice that received vehicle (-AP, $n = 6$) or AP (+AP, $n = 4$) according to the scheme shown in Figure 3a. (**b**) Quantification of $p16^{INK4a}$-positive SnCs and Ki-67-positive non-SnCs staining in articular cartilage. (**c**) Subchondral bone damage scores for osteophyte formation and medial tibial bone sclerosis and (**d**) osteocalcin-positive cells in subchondral bone marrows (brown staining at arrows) by immunohistochemistry of 19- to 20-month-old p16-3MR male mice with ACLT surgery treated with vehicle (Veh) or UBX0101 (1 mM in 10 µl saline, once every 2 days over 2 weeks starting 14 days post-surgery). $n = 2$ for each group. All data are expressed as mean and each data point represents an individual mouse. Scale bars, 100 µm. (**e**) The presence of $p16^{INK4a}$-positive cells in the subchondral bone marrow, synovium and fat pad (brown) of p16-3MR mice shown by immunostaining and their changes after UBX0101 treatment. $n = 3$ for each group. Scale bars, 100 µm. $^*P < 0.05$, $^{**}P < 0.01$ and N.S. (Not Significant); two-tailed t tests (unpaired).



Supplementary Figure 11. Presence of SnCs in human healthy and osteoarthritic articular cartilage. Representative images of HMGB1-positive non-SnCs (brown staining at arrows, Scale bars, 100 μm) by immunohistochemistry and SA-β-gal-positive SnCs expression on the articular cartilage explant. SA-β-gal-positive SnCs were observed throughout the depth of osteoarthritic cartilage (donor: 65-years-old, Male), but were sparsely present in healthy cartilage (donor: 62-years-old, Male). Scale bars, 500 μm. A range of 7-15% SA-β-gal-positive SnCs were observed in primary chondrocytes isolated from healthy cartilage and 41–50% SA-β-gal-positive SnCs were detected in the chondrocytes from osteoarthritic cartilage (passage 0). Scale bars, 100 μm.



Supplementary Figure 12. Dose-dependent elimination of senescent chondrocytes isolated from human OA tissue by UBX0101 treatment. (**a**) Schematic of experiment for **b**–**c**. (**b**) SA-β-gal positive cells were quantified 2 and 8 days following treatment with increasing concentrations of UBX0101. A dose of 43 μM and a short treatment time (2 days) selectively eliminated SA-β-gal positive senescent chondrocytes. Data are averages ± S.D. $^{**}P < 0.01$, $^{****}P < 0.0001$ and n.s. (Not Significant); One-way ANOVA (Tukey's multiple comparison test). $n = 5$ for 0, 8.6 and 12.2 μM; $n = 6$ for 34.4 and 43 μM. (**c**) Representative images of cells with SA-β-gal staining. $n = 5$ for each group. Scale bars, 100 μm. (**d**) Western blot analysis of p21 and GAPDH in OA and 2 days after treatment with vehicle or 43 μM UBX0101. $n = 2$ for each group. (**e**) Western blot analysis of activated caspase-3 (cCasp-3) and actin in healthy chondrocytes (Non-SnCs) 1 or 2 days after treatment with vehicle or 43 μM UBX0101 from 2 independent experiments. All uncropped immunoblots in Supplementary Fig. 13.



Supplementary Figure 13. Uncropped immunoblots from indicated figures.

Supplementary Table 1. Primers sequences used for RT-PCR

Gene (Mice)	Forward primer (5'-3')	Reverse primer (5'-3')
Cdkn2a	AATCTCCGCGAGGAAAGC	GTCTGCAGCGGACTCCATS
Cdkn1a	ATTCCATAGGCGTGGGACCT	TCCTGGGCATTTCGGTCAC
Il6	GCTACCAAACTGGATATAATCAGGA	CCAGGTAGCTATGGTACTCCAGAA
Mmp13	GGAGCCCTGATGTTTCCCAT	GTCTTCATCGCCTGGACCATA
Il1β	GTATGGGCTGGACTGTTTC	GCTGTCTGCTCATTCACG
Sox9	ACCCACAGCTCCCCTGAAG	CTCACCTTCAGTGGCAAGAGC
Col2a1	CCTCCGTCTACTGTCCACTGA	ATTGGAGCCCTGGATGAGCA
Acan	CGTTGCAGACCAGGAGCAAT	CGGTCATGAAAGTGGCGGTA
Runx2	GCCGGGAATGATGAGAACTA	GGTGAAACTCTTGCCTCGTC
Bglap	GGCGCTACCTTGGGTAAGTG	GACCACTCCAGCACAACTCC
Ctsk	GCACCCTTAGTCTTCCGCTC	ACCCACATCCTGCTGTTGAG
Rankl	GAGCACGAAAAACTGGTCGG	AGGGTTGGACACCTGAATGC
Opg	CAGCCATTTGCACACCTCAC	TTAGAGATCTTGGCCCAGCC
β-actin	CAACCGTGAAAAGATGACCC	GTAGATGGGCACAGTGTGGG

Gene (human)	Forward primer (5'-3')	Reverse primer (5'-3')
cdkn2a	AGCTGGAATTACACAGCTGC	GGACTGGCTTGCAATCTTGT
mmp3	CACTCACAGACCTGACTCGG	GAGTCAGGGGGAGGTCCATA
il6	CCCCTGACCCAACCACAAAT	ATTTGCCGAAGAGCCCTCAG
mmp13	TGGTCCAGGAGATGAAGACC	TCCTCGGAGACTGGTAATGG
il1β	GGACAAGCTGAGGAAGATGC	TCGTTATCCCATGTGTCGAA
col2a1	CGCCGCTGTCCTTCGGTGTC	AGGGCTCCGGCTTCCACACAT
acan	TGGGAACCAGCCTATACCCCAG	CAGTTGCAGAAGGGCCTTCTGTAC
β-actin	GCTCCTCCTGAGCGCAAGTAC	GGACTCGTCATACTCCTGCTTGC