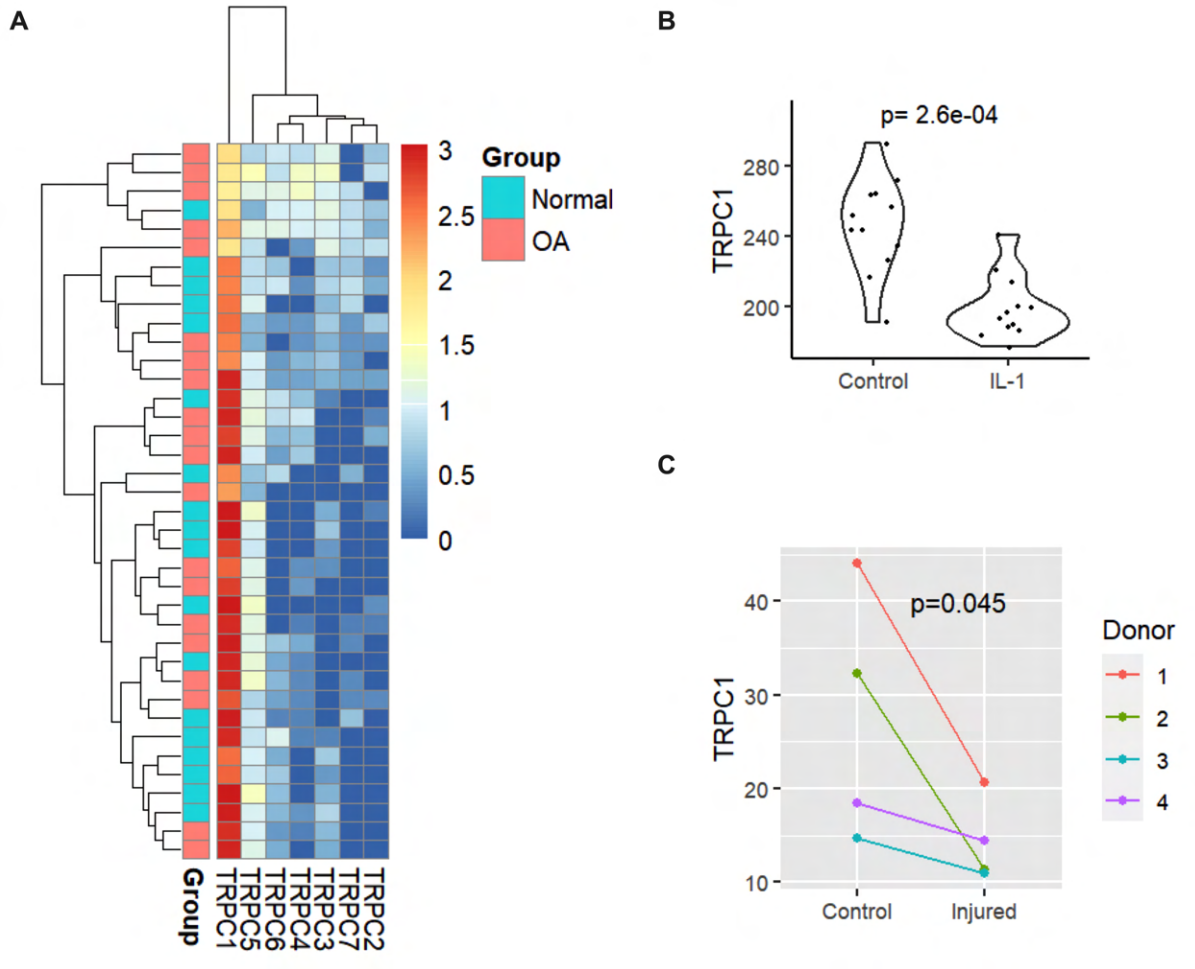
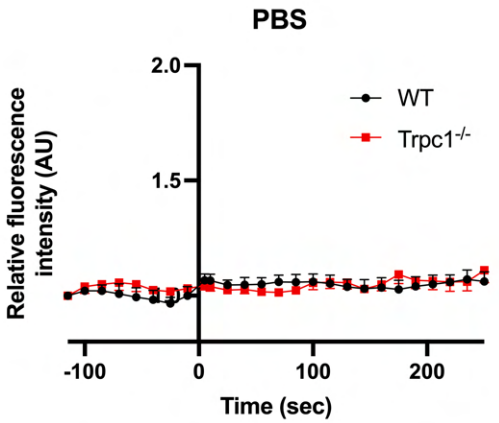
**Transient receptor potential channel 1 (TRPC1) links intracellular calcium signaling to cellular senescence and is required for protection against post-traumatic osteoarthritis.**

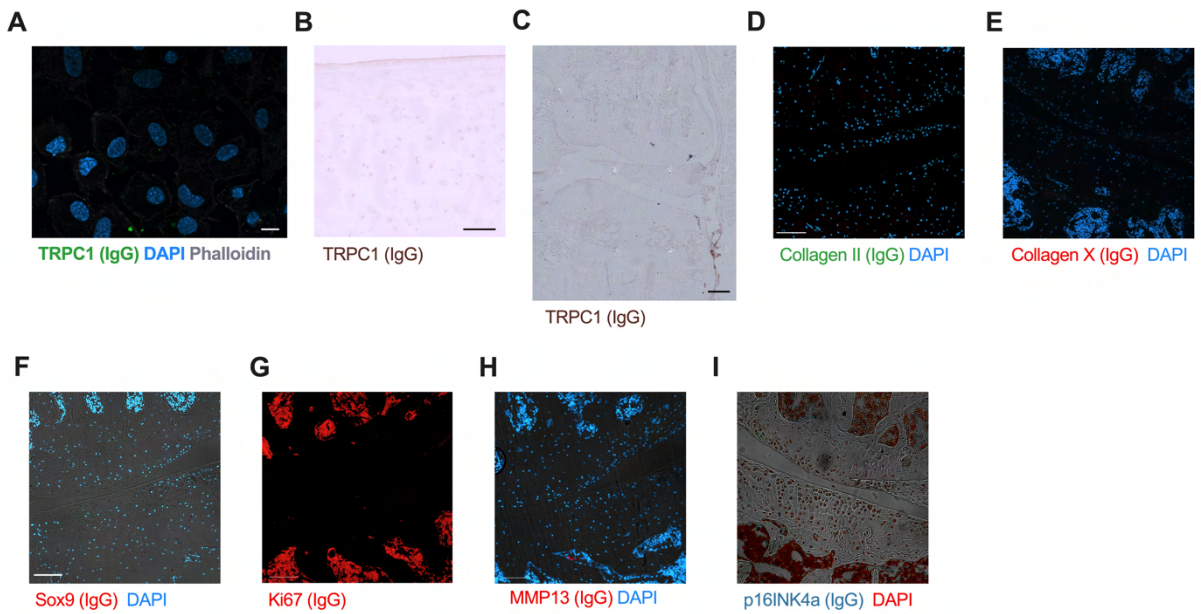
**Supplemental materials**



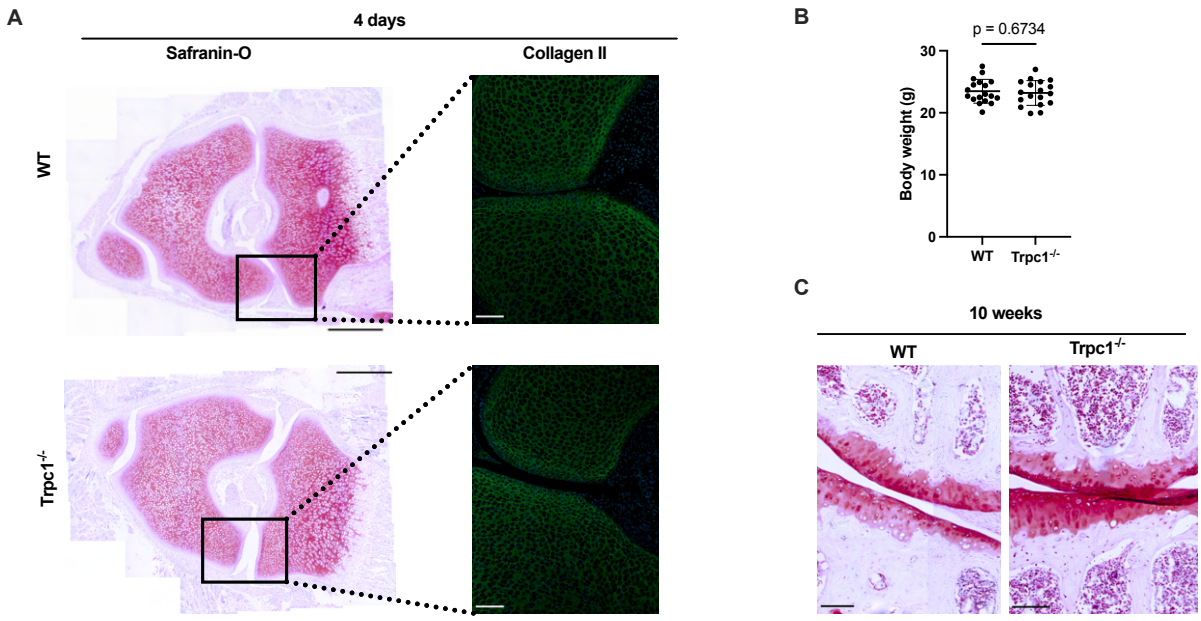

**Supplemental Figure 1.** (A) Post-hoc analysis of RNAseq data for TRPC channel expression in human normal and OA cartilage (GEO: GSE114007) (1) (B) Post-hoc analysis of microarray data from IL-1β treated human OA chondrocytes showing downregulation of TRPC1 gene expression following IL-1β application (GEO: GSE75181) (2). (C) Microarray gene expression data comparing TRPC1 expression in human cartilage before and following cutting injury (3) P-value: paired t-test after reciprocal transformation (n = 4).



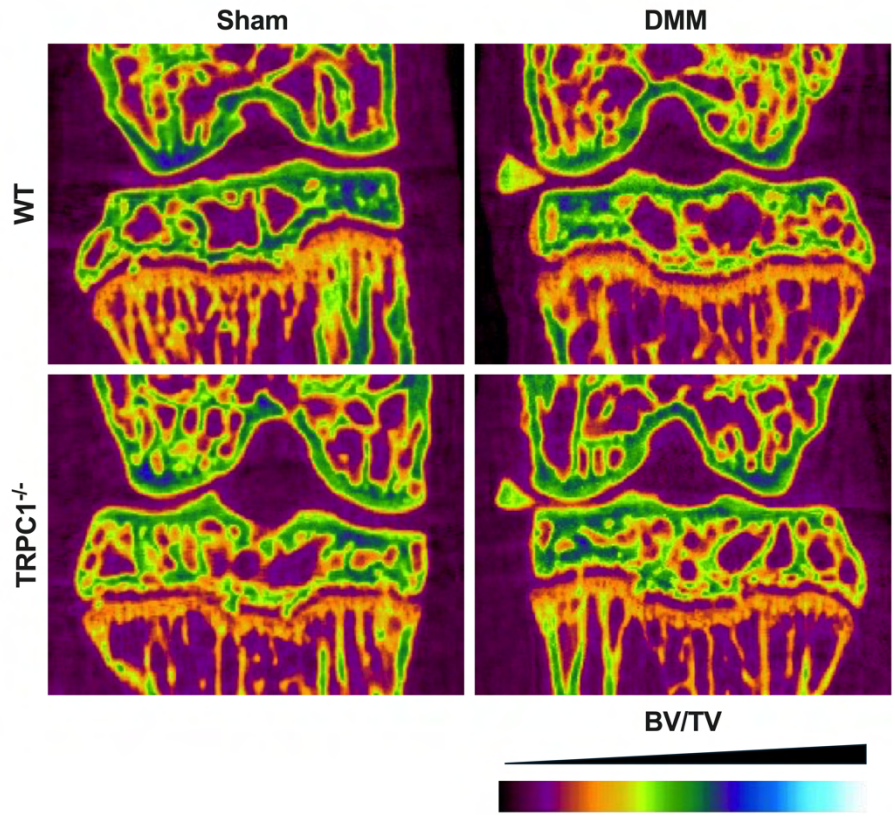

**Supplemental Figure 2.** Time course analysis of intracellular $Ca^{2+}$ levels as measured by fluorescence intensity of loaded Fluo-4 $Ca^{2+}$ indicator in wild type and $Trpc1^{-/-}$ chondrocytes during stimulation with PBS (negative control)



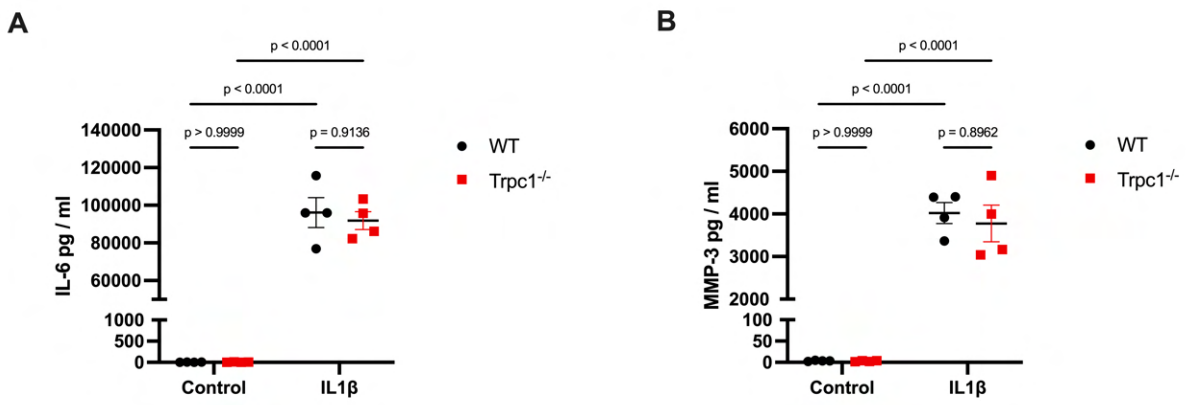

**Supplemental Figure 3.** (A) IgG isotype negative control for TRPC1 immunofluorescence staining on P0 murine chondrocytes. Scale bar: 20 µm. (B) IgG isotype negative control for TRPC1 immunohistochemical staining on human healthy cartilage paraffin section. Scale bar: 100 µm. (C) IgG isotype negative control for TRPC1 immunohistochemical staining on murine sham operated knee joint section. Scale bar: 100 µm. (D) IgG isotype negative control for type II collagen immunofluorescence staining on murine sham operated knee joint section. Scale bar: 100 µm. (E) IgG isotype negative control for type X collagen immunofluorescence staining on murine 2 weeks post-DMM knee joint section. Scale bar: 100 µm. (F) IgG isotype negative control for Sox9 immunofluorescence staining on murine sham operated knee joint section. Scale bar: 100 µm. (G) IgG isotype negative control for Ki67 immunofluorescence staining on murine 2 weeks post-DMM knee joint section. Scale bar: 100 µm. (H) IgG isotype negative control for MMP-13 immunofluorescence staining on murine 2 weeks post-DMM knee joint section. Scale bar: 100 µm. (I) IgG isotype negative control for p16INK4a immunofluorescence staining on murine 2 weeks post-DMM knee joint section. Scale bar: 100 µm. Where present, DAPI (blue) was used to label nuclei.




**Supplemental Figure 4.** (A) Safranin-O histology and type II collagen immunofluorescence staining of murine 4 day old wild type and *Trpc1*$^{-/-}$ knee joint sections demonstrating no obvious abnormalities caused by Trpc1 deficiency during development. Scale bars: 500 µm (Safranin-O) and 100 µm (type II collagen, green). (B) Comparison of total body weights of 10 weeks old male wild type and *Trpc1*$^{-/-}$ mice at time of DMM. P value: unpaired t-test (n = 18). (C) Safranin-o staining of 10-week old unchallenged male murine knee joint sections. Scale bar: 100 µm.




**Supplemental Figure 5.** Representative frontal cross section images taken from microCT analyses of wild type and *Trpc1*$^{-/-}$ knee joints 2 weeks post-DMM. Colour spectrum illustrates changes in BV/TV.




**Supplemental Figure 6.** Protein concentration analyses of murine IL-6 and MMP-3 within supernatants of wild type and *Trpc1*$^{-/-}$ chondrocytes after 24 hours stimulation with 10 ng/ml IL-1β and 48 hours further culture in control medium measured by ELISA (n = 4). P values: 2-way ANOVA with multiple comparisons.

**Supplemental Table 1.** Top 100 differentially expressed genes found within RNA sequencing comparison articular cartilage collected from wild type and *Trpc1*$^{-/-}$ mice 2 weeks post-DMM.

| gene_id | geneSymbol | baseMean | log2FoldChange | lfcSE | stat | pvalue | padj |
|---|---|---|---|---|---|---|---|
| ENSMUSG00000050550 | Gm11868 | 654,54 | 2,90671818 | 0,3121 | 9,3119 | 1,255E-20 | 1,0302E-16 |
| ENSMUSG00000094497 | Gm8210 | 1133,71 | 2,216069522 | 0,4597 | 4,8212 | 1,427E-06 | 0,00060075 |
| ENSMUSG00000083833 | Gm13841 | 1202,01 | 2,153237441 | 0,4226 | 5,0949 | 3,489E-07 | 0,00020456 |
| ENSMUSG00000096632 | Igkv9-124 | 161,49 | 2,091116993 | 0,3786 | 5,5236 | 3,322E-08 | 2,479E-05 |
| ENSMUSG00000096712 | Gm15454 | 204,10 | 1,535250393 | 0,2301 | 6,6726 | 2,513E-11 | 6,8747E-08 |
| ENSMUSG00000096715 | Igkv3-4 | 143,83 | 1,43307761 | 0,3245 | 4,4161 | 1,005E-05 | 0,002578 |
| ENSMUSG00000094546 | Ighv1-26 | 754,58 | 1,362296619 | 0,2149 | 6,3397 | 2,302E-10 | 3,7795E-07 |
| ENSMUSG00000116637 | Gm8130 | 79,63 | 1,230868486 | 0,2595 | 4,7432 | 2,104E-06 | 0,00082225 |
| ENSMUSG00000116875 | Morf4l1-ps1 | 258,74 | 1,041319953 | 0,1467 | 7,0997 | 1,251E-12 | 4,106E-09 |
| ENSMUSG00000094446 | Gm6344 | 356,97 | 0,991965094 | 0,1766 | 5,6175 | 1,938E-08 | 1,5801E-05 |
| ENSMUSG00000062611 | Rps3a2 | 4418,44 | 0,958697538 | 0,1786 | 5,3682 | 7,953E-08 | 5,2225E-05 |
| ENSMUSG00000058126 | Tpm3-rs7 | 3720,32 | 0,922204706 | 0,1909 | 4,8304 | 1,362E-06 | 0,00058858 |
| ENSMUSG00000099974 | Bcl2a1d | 129,08 | 0,831627005 | 0,1933 | 4,3028 | 1,686E-05 | 0,00369128 |
| ENSMUSG00000035692 | Isg15 | 1243,57 | 0,790102163 | 0,1355 | 5,8315 | 5,492E-09 | 4,7453E-06 |
| ENSMUSG00000066361 | Serpina3c | 403,62 | 0,746323368 | 0,1578 | 4,7292 | 2,254E-06 | 0,00084341 |
| ENSMUSG00000038871 | Bpgm | 24042,67 | 0,719360606 | 0,1665 | 4,3194 | 1,565E-05 | 0,00347116 |
| ENSMUSG00000036594 | H2-Aa | 10198,99 | 0,627216499 | 0,1138 | 5,5103 | 3,583E-08 | 2,5576E-05 |
| ENSMUSG00000044468 | Tent5c | 47083,11 | 0,61527824 | 0,0625 | 9,8451 | 7,193E-23 | 1,1809E-18 |
| ENSMUSG00000090113 | Nhlrc4 | 373,58 | 0,600002707 | 0,1305 | 4,5985 | 4,256E-06 | 0,00124781 |
| ENSMUSG00000060586 | H2-Eb1 | 5694,52 | 0,590545487 | 0,1053 | 5,6102 | 2,021E-08 | 1,5801E-05 |
| ENSMUSG00000020641 | Rsad2 | 24839,68 | 0,57672145 | 0,1317 | 4,3795 | 1,19E-05 | 0,00278983 |
| ENSMUSG00000022748 | Cmss1 | 367,51 | 0,56726312 | 0,1200 | 4,7286 | 2,26E-06 | 0,00084341 |
| ENSMUSG00000081992 | Gm13408 | 985,20 | 0,553932197 | 0,1298 | 4,2666 | 1,984E-05 | 0,00408861 |
| ENSMUSG00000040569 | Slc26a7 | 841,57 | 0,544000441 | 0,1238 | 4,3939 | 1,113E-05 | 0,00269637 |
| ENSMUSG00000073421 | H2-Ab1 | 6743,88 | 0,532691776 | 0,1220 | 4,3658 | 1,267E-05 | 0,00292853 |
| ENSMUSG00000026348 | Acmsd | 595,91 | 0,515277168 | 0,1222 | 4,2168 | 2,478E-05 | 0,00478686 |
| ENSMUSG00000034127 | Tspan8 | 1926,78 | 0,501010638 | 0,1073 | 4,6675 | 3,049E-06 | 0,0010309 |
| ENSMUSG00000032411 | Tfdp2 | 21571,66 | 0,497572187 | 0,0647 | 7,6953 | 1,411E-14 | 7,7219E-11 |
| ENSMUSG00000022051 | Bnip3l | 22669,13 | 0,495603975 | 0,1160 | 4,2720 | 1,937E-05 | 0,00407664 |
| ENSMUSG00000078139 | AK157302 | 1509,73 | 0,485021183 | 0,1038 | 4,6723 | 2,979E-06 | 0,0010309 |
| ENSMUSG00000044792 | Isca1 | 11732,58 | 0,481712056 | 0,0952 | 5,0622 | 4,144E-07 | 0,00022678 |
| ENSMUSG00000040435 | Ppp1r15a | 10355,53 | 0,476060159 | 0,1138 | 4,1840 | 2,864E-05 | 0,00528342 |
| ENSMUSG00000035472 | Slc25a21 | 1277,46 | 0,469971541 | 0,0797 | 5,8975 | 3,69E-09 | 4,1602E-06 |
| ENSMUSG00000042770 | Hebp1 | 2974,00 | 0,466525391 | 0,1062 | 4,3932 | 1,117E-05 | 0,00269637 |
| ENSMUSG00000028906 | Epb41 | 55559,35 | 0,450217808 | 0,0767 | 5,8722 | 4,299E-09 | 4,1602E-06 |
| ENSMUSG00000011179 | Odc1 | 22000,69 | 0,446787085 | 0,1003 | 4,4529 | 8,474E-06 | 0,00220813 |
| ENSMUSG00000028124 | Gclm | 9047,81 | 0,444875212 | 0,0700 | 6,3560 | 2,071E-10 | 3,7783E-07 |
| ENSMUSG00000023926 | Rhag | 7621,03 | 0,439430617 | 0,0865 | 5,0784 | 3,806E-07 | 0,00021547 |
| ENSMUSG00000090946 | Ccdc71l | 4615,42 | 0,40475105 | 0,0968 | 4,1801 | 2,914E-05 | 0,00531572 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ENSMUSG00000027322 | Siglec1 | 2895,53 | 0,399967298 | 0,0863 | 4,6320 | 3,621E-06 | 0,0011009 |
| ENSMUSG00000024588 | Fech | 34158,88 | 0,39649454 | 0,0732 | 5,4138 | 6,172E-08 | 4,2216E-05 |
| ENSMUSG00000040675 | Mthfd1l | 3352,66 | 0,387897236 | 0,0862 | 4,5022 | 6,726E-06 | 0,0017811 |
| ENSMUSG00000029802 | Abcg2 | 5581,07 | 0,384118904 | 0,0654 | 5,8715 | 4,319E-09 | 4,1602E-06 |
| ENSMUSG00000042066 | Tmcc2 | 32119,83 | 0,374662968 | 0,0854 | 4,3863 | 1,153E-05 | 0,00274291 |
| ENSMUSG00000042225 | Ammecr1 | 3543,00 | 0,358534661 | 0,0589 | 6,0821 | 1,186E-09 | 1,4978E-06 |
| ENSMUSG00000006574 | Slc4a1 | 107387,75 | 0,343172096 | 0,0818 | 4,1970 | 2,704E-05 | 0,00504513 |
| ENSMUSG00000028436 | Dcaf12 | 14430,42 | 0,33953874 | 0,0729 | 4,6546 | 3,246E-06 | 0,0010309 |
| ENSMUSG00000027115 | Kif18a | 4817,40 | 0,338442336 | 0,0738 | 4,5838 | 4,567E-06 | 0,0013154 |
| ENSMUSG00000028587 | Orc1 | 1859,93 | 0,33362834 | 0,0694 | 4,8040 | 1,555E-06 | 0,00063822 |
| ENSMUSG00000024726 | Carnmt1 | 2297,05 | 0,327255356 | 0,0662 | 4,9462 | 7,567E-07 | 0,00037644 |
| ENSMUSG00000026311 | Asb1 | 9027,54 | 0,326437974 | 0,0723 | 4,5160 | 6,301E-06 | 0,00172405 |
| ENSMUSG00000024014 | Pim1 | 5470,22 | 0,323694296 | 0,0758 | 4,2730 | 1,929E-05 | 0,00407664 |
| ENSMUSG00000041147 | Brca2 | 6849,03 | 0,315660183 | 0,0680 | 4,6417 | 3,455E-06 | 0,00107015 |
| ENSMUSG00000037458 | Azin1 | 22638,39 | 0,313299149 | 0,0713 | 4,3964 | 1,101E-05 | 0,00269637 |
| ENSMUSG00000014956 | Ppp1cb | 29803,94 | 0,311409223 | 0,0591 | 5,2715 | 1,353E-07 | 8,5437E-05 |
| ENSMUSG00000042029 | Ncapg2 | 13627,50 | 0,306160104 | 0,0663 | 4,6146 | 3,939E-06 | 0,00117582 |
| ENSMUSG00000078652 | Psme3 | 11512,23 | 0,294962666 | 0,0645 | 4,5732 | 4,803E-06 | 0,00135938 |
| ENSMUSG00000044763 | Trmt10c | 1609,37 | 0,285364572 | 0,0630 | 4,5317 | 5,852E-06 | 0,00162836 |
| ENSMUSG00000022100 | Xpo7 | 46144,25 | 0,283446098 | 0,0581 | 4,8791 | 1,065E-06 | 0,00051446 |
| ENSMUSG00000020124 | Usp15 | 19152,63 | 0,266303671 | 0,0646 | 4,1214 | 3,765E-05 | 0,00650402 |
| ENSMUSG00000018983 | E2f2 | 16239,36 | 0,251422501 | 0,0578 | 4,3521 | 1,348E-05 | 0,00307457 |
| ENSMUSG00000027452 | Acss1 | 4400,92 | 0,234692666 | 0,0570 | 4,1191 | 3,803E-05 | 0,00650402 |
| ENSMUSG00000002222 | Rmnd5a | 12160,35 | 0,176302913 | 0,0431 | 4,0889 | 4,334E-05 | 0,00711561 |
| ENSMUSG00000064147 | Rab44 | 9871,82 | -0,274455612 | 0,0650 | -4,2238 | 2,402E-05 | 0,00469484 |
| ENSMUSG00000062031 | Pgghg | 1793,80 | -0,291288317 | 0,0709 | -4,1108 | 3,943E-05 | 0,00667388 |
| ENSMUSG00000001248 | Gramd1a | 5759,92 | -0,338672236 | 0,0727 | -4,6567 | 3,214E-06 | 0,0010309 |
| ENSMUSG00000026335 | Pam | 18845,73 | -0,366101523 | 0,0865 | -4,2321 | 2,315E-05 | 0,00457892 |
| ENSMUSG00000028041 | Adam15 | 3528,81 | -0,367155974 | 0,0789 | -4,6534 | 3,265E-06 | 0,0010309 |
| ENSMUSG00000019970 | Sgk1 | 5005,05 | -0,375885954 | 0,0918 | -4,0942 | 4,236E-05 | 0,00709679 |
| ENSMUSG00000038168 | P3h2 | 1500,45 | -0,425216887 | 0,1029 | -4,1326 | 3,587E-05 | 0,00626479 |
| ENSMUSG00000040964 | Arhgef10l | 2422,98 | -0,436914994 | 0,0903 | -4,8368 | 1,319E-06 | 0,00058536 |
| ENSMUSG00000024529 | Lox | 12761,01 | -0,485552744 | 0,1002 | -4,8454 | 1,264E-06 | 0,00057632 |
| ENSMUSG00000018906 | P4ha2 | 1956,33 | -0,530675282 | 0,1298 | -4,0890 | 4,333E-05 | 0,00711561 |
| ENSMUSG00000070407 | Hs3st3b1 | 563,22 | -0,534602048 | 0,1074 | -4,9781 | 6,42E-07 | 0,00032936 |
| ENSMUSG00000082286 | Pisd-ps1 | 1200,78 | -0,542514183 | 0,0758 | -7,1580 | 8,188E-13 | 3,3604E-09 |
| ENSMUSG00000027848 | Olfml3 | 6558,00 | -0,557740206 | 0,1236 | -4,5125 | 6,406E-06 | 0,00172405 |
| ENSMUSG00000006369 | Fbln1 | 805,99 | -0,5642298 | 0,0883 | -6,3875 | 1,686E-10 | 3,4596E-07 |
| ENSMUSG00000024247 | Pkdcc | 1194,26 | -0,582029945 | 0,0993 | -5,8624 | 4,561E-09 | 4,1602E-06 |
| ENSMUSG00000036412 | Arsi | 588,12 | -0,606909589 | 0,1416 | -4,2855 | 1,823E-05 | 0,00393819 |
| ENSMUSG00000022780 | Meltf | 3915,07 | -0,610547105 | 0,1255 | -4,8630 | 1,156E-06 | 0,00054222 |
| ENSMUSG00000023249 | Parp3 | 1750,57 | -0,619348953 | 0,1473 | -4,2040 | 2,622E-05 | 0,00500555 |
| ENSMUSG00000023800 | Tiam2 | 3401,10 | -0,621360012 | 0,1480 | -4,1976 | 2,698E-05 | 0,00504513 |
| ENSMUSG00000025504 | Eps8l2 | 1417,50 | -0,621590052 | 0,1463 | -4,2496 | 2,141E-05 | 0,00434025 |
| ENSMUSG00000056919 | Cep162 | 1812,98 | -0,630199781 | 0,1226 | -5,1410 | 2,733E-07 | 0,00016621 |
| ENSMUSG00000015709 | Arnt2 | 382,37 | -0,658474586 | 0,1582 | -4,1610 | 3,168E-05 | 0,00565377 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ENSMUSG00000054252 | Fgfr3 | 3326,62 | -0,663173742 | 0,1603 | -4,1364 | 3,529E-05 | 0,00622878 |
| ENSMUSG00000016200 | Syt14 | 492,44 | -0,678769167 | 0,1457 | -4,6592 | 3,175E-06 | 0,0010309 |
| ENSMUSG00000061718 | Ppp1r1b | 488,73 | -0,700324759 | 0,1500 | -4,6693 | 3,022E-06 | 0,0010309 |
| ENSMUSG00000031952 | Chst5 | 300,22 | -0,710824866 | 0,1422 | -4,9976 | 5,805E-07 | 0,00030742 |
| ENSMUSG00000039084 | Chad | 13815,97 | -0,737723134 | 0,1675 | -4,4051 | 1,057E-05 | 0,00267026 |
| ENSMUSG00000001494 | Sost | 2717,25 | -0,739783454 | 0,1707 | -4,3338 | 1,466E-05 | 0,00329594 |
| ENSMUSG00000042073 | Abhd14b | 399,44 | -0,742321486 | 0,1740 | -4,2657 | 1,992E-05 | 0,00408861 |
| ENSMUSG00000044006 | Cilp2 | 3998,14 | -0,787026677 | 0,1640 | -4,7982 | 1,601E-06 | 0,0006412 |
| ENSMUSG00000006403 | Adamts4 | 350,19 | -0,862034626 | 0,1392 | -6,1943 | 5,855E-10 | 8,7376E-07 |
| ENSMUSG00000032872 | Cyb5r4 | 6144,96 | -0,891695898 | 0,2102 | -4,2421 | 2,215E-05 | 0,0044341 |
| ENSMUSG00000042254 | Cilp | 40930,26 | -0,937919323 | 0,1593 | -5,8887 | 3,894E-09 | 4,1602E-06 |
| ENSMUSG00000101429 | BC055402 | 490,11 | -1,067626246 | 0,2287 | -4,6689 | 3,028E-06 | 0,0010309 |
| ENSMUSG00000026077 | Npas2 | 225,78 | -1,248982415 | 0,3001 | -4,1625 | 3,148E-05 | 0,00565377 |
| ENSMUSG00000098975 | Gm27177 | 363,36 | -7,324938074 | 1,2034 | -6,0870 | 1,151E-09 | 1,4978E-06 |
| ENSMUSG00000105790 | Gm24105 | 102,88 | -23,94383664 | 3,6786 | -6,5090 | 7,565E-11 | 1,7741E-07 |

**Supplemental Table 2** Clinical parameters of patient donors providing cartilage samples within this study

| Group | OARSI Score | Age (years) | Sex (m/f) | Synovitis (y/n) | BMI (kg/m$^2$) |
|---|---|---|---|---|---|
| Healthy | 0 | 60 +/- 11.3 | 2/3 | 1/4 | 30.4 +/- 4.21 |
| Mild | 1.0 - 2.5 | 65 +/- 9.2 | 2/3 | 1/4 | 33.4 +/- 5.12 |
| Advanced | > 3.0 | 69 +/- 9.9 | 5/3 | 1/7 | 26.2 +/- 4.30 |

**Supplemental Table 3** List of antibodies used within this study

| Antibody | Application | Manufacturer | Cat. No. | Conjugation |
|---|---|---|---|---|
| TRPC1 | IHC-DAB | Alomone | a018 | unconjugated |
| TRPC1 | IF | Alomone | a010 | unconjugated |
| Type II collagen | IHC-F | Merck | MAB8887 | unconjugated |
| Type X collagen | IHC-F | Quartett | CO097-05 | unconjugated |
| Sox9 | IHC-F | Merck | MAB5535 | unconjugated |
| Ki67 | IHC-F | Leica Biosystems | Ki67P-CE | unconjugated |
| p16INK4a | WB | Abcam | ab211542 | unconjugated |
| GAPDH | WB | Cell Signaling | 5174 | unconjugated |
| p16INK4a | IHC-F | Abcam | ab54210 | unconjugated |
| Phalloidin | IF | ThermoFisher | AB22287 | Alexa Fluor 647 |
| Goat anti-rabbit IgG (H+L) | WB | DAKO | P0448 | Horseradish peroxidase |
| Rabbit anti-mouse IgG (H+L) | IHC-F | ThermoFisher | A11059 | Alexa Fluor 488 |
| Donkey anti-mouse IgG (H+L) | IHC-F | ThermoFisher | A31570 | Alexa Fluor 555 |
| Donkey anti-rabbit IgG (H+L) | IHC-F | ThermoFisher | A10040 | Alexa Fluor 546 |

**Supplemental Table 4** List of primers using within this study

| Gene | Taqman Probe | FW sequence | RV sequence |
|---|---|---|---|
| *Actb* | Mm02619580_g1 | - | - |
| *Aggrecan* | Mm00545798_m1 | - | - |
| *B2M* | Mm00437762_m1 | - | - |
| *Col1a1* | Mm00801666_g1 | - | - |
| *Col2a1* | Mm01309565_m1 | - | - |
| *Gapdh* | Mm99999916_g1 | - | - |
| *Sox9* | Mm00448840_m1 | - | - |
| *Actb* | - | TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA | CTA GAA GCA TTT GCG GTG GAC GAT GGA GG |
| *B2M* | - | AGA GGT CCT TTT CAC CAG CA | TCA GTC TCA GTG GGG GTG AA |
| *Gapdh* | - | AGC AAG GAC ACT GAG CAA GAG AGG | GGG TCT GGG ATG GAA ATT GTG AGG |
| *p16INK4a* | - | CCC AAC GCC CCG AAC T | GCA GAA GAG CTG CTA CGT GAA |