Supplementary Information

Therapeutic senescence via GPCR activation in synovial fibroblasts facilitates resolution of arthritis

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Supplementary Table 1. Summary of clinical details of patients (n=20) at the time of fibroblasts sample collection (mean±SD).

	Patients
Age -years-	50 ± 14.55
Female, n (%)	10 (50%)
ESR -mm/h-	29 ± 22.8
CRP -mg/l-	24 ± 23.4
DAS28ESR	5 ± 1.5
Symptom duration -weeks-	342 ± 559
IgM-RF$^+$, n (%)	9 (45%)
CCP$^+$, n (%)	5 (25%)
Ethnicity, n (%)	White British: 17 (85%) Caribbean: 2 (10%) Indian: 1 (5%)

Supplementary Table 2. Primers used in this study.

PCR primers		
Hs_ADAMTS2_1_SG QuantiTect Primer Assay	QIAGEN	QT00020083
Hs_AGRP_1_SG QuantiTect Primer Assay	QIAGEN	QT00204477
Hs_ASIP_1_SG QuantiTect Primer Assay	QIAGEN	QT00061509
Hs_BMP2_1_SG QuantiTect Primer Assay	QIAGEN	QT00012544
Hs_CD74_1_SG QuantiTect Primer Assay	QIAGEN	QT00059402
Hs_CDH11_1_SG QuantiTect Primer Assay	QIAGEN	QT00019418
Hs_CDKN1A_1_SG QuantiTect Primer Assay	QIAGEN	QT00062090
Hs_CDKN2A_1_SG QuantiTect Primer Assay	QIAGEN	QT00089964
Hs_COL3A1_1_SG QuantiTect Primer Assay	QIAGEN	QT00058233
Hs_COL3A1_1_SG QuantiTect Primer Assay	QIAGEN	QT00058233
Hs_COL5A1_1_SG QuantiTect Primer Assay	QIAGEN	QT00044527
Hs_CTSK_1_SG QuantiTect Primer Assay	QIAGEN	QT00093856
Hs_CTSL_1_SG QuantiTect Primer Assay	QIAGEN	QT01664978
Hs_DMKN_1_SG QuantiTect Primer Assay	QIAGEN	QT00057897
Hs_FGF2_1_SG QuantiTect Primer Assay	QIAGEN	QT00047579
Hs_FTL_1_SG QuantiTect Primer Assay	QIAGEN	QT00055860
Hs_GLB1_1_SG QuantiTect Primer Assay	QIAGEN	QT00066206
Hs_GPBAR1_1_SG QuantiTect Primer Assay	QIAGEN	QT00209594
Hs_GHSR_1_SG QuantiTect Primer Assay	QIAGEN	QT00040901
Hs_HMGCR_1_SG QuantiTect Primer Assay	QIAGEN	QT00004081
Hs_HMOX1_1_SG QuantiTect Primer Assay	QIAGEN	QT00092645
Hs_HPRT1_1_SG QuantiTect Primer Assay	QIAGEN	QT00059066
Hs_HRAS_2_SG QuantiTect Primer Assay	QIAGEN	QT01668338
Hs_HSD3B7_2_SG QuantiTect Primer Assay	QIAGEN	QT02316300
Hs_ICAM1_1_SG QuantiTect Primer Assay	QIAGEN	QT00074900
Hs_INSIG1_1_SG QuantiTect Primer Assay	QIAGEN	QT00090314
Hs_LDLR_1_SG QuantiTect Primer Assay	QIAGEN	QT00045864
Hs_MC1R_1_SG QuantiTect Primer Assay	QIAGEN	QT01004241
Hs_MC2R_2_SG QuantiTect Primer Assay	QIAGEN	QT01155007
Hs_MC3R_1_SG QuantiTect Primer Assay	QIAGEN	QT00209895
Hs_MC4R_1_SG QuantiTect Primer Assay	QIAGEN	QT00245595
Hs_MC5R_1_SG QuantiTect Primer Assay	QIAGEN	QT00211960
Hs_MMP11_1_SG QuantiTect Primer Assay	QIAGEN	QT00024031

Hs_MRAP_1_SG QuantiTect Primer Assay	QIAGEN	QT00103866
Hs_MRAP2_1_SG QuantiTect Primer Assay	QIAGEN	QT00493150
Hs_PCSK1_1_SG QuantiTect Primer Assay	QIAGEN	QT00013853
Hs_PCSK2_1_SG QuantiTect Primer Assay	QIAGEN	QT00054754
Hs_POMC_1_SG QuantiTect Primer Assay	QIAGEN	QT00001204
Hs_POSTN_1_SG QuantiTect Primer Assay	QIAGEN	QT00023800
Hs_PTEN_1_SG QuantiTect Primer Assay	QIAGEN	QT00086933
Hs_TNC_1_SG QuantiTect Primer Assay	QIAGEN	QT00024409
Hs_TP53_1_SG QuantiTect Primer Assay	QIAGEN	QT00060235
MC1R-Forward: 5’-GAAGAACTGTGGGGACCTGG-3’	Sigma-Aldrich	In-house designed
MC1R-Reverse: 5’-GGGTCACACAGGAACCAGAC-3’	Sigma-Aldrich	In-house designed

Supplementary Table 3. Extended information on genes identified by RNAseq.

Symbol	Gene name	FC	Refseq ID	Locus
qPCR validation (Related to Fig3A,B)				
ADAMTS2	ADAM metallopeptidase with thrombospondin type 1 motif 2	0.79	NM_014244	chr5 : 179110850 - 179345430
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	0.74	NM_004355	chr5 : 150401636 - 150412936
CDH11	cadherin 11, type 2, OB-cadherin (osteoblast)	0.84	NM_001797	chr16 : 64943752 - 65122137
CDKN2A	cyclin-dependent kinase inhibitor 2A	1.09	NM_000077	chr9 : 21967751 - 21975133
COL3A1	collagen, type III, alpha 1	0.65	NM_000090	chr2 : 188974372 - 189012746
COL5A1	collagen, type V, alpha 1	0.72	NM_000093	chr9 : 134641804 - 134844842
CTSK	cathepsin K	1.99	NM_000396	chr1 : 150796207 - 150808441
DMKN	dermokine	1.79	NM_001035516	chr19 : 35497216 - 35501911
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	1.53	NM_001130996	chr5 : 75337167 - 75362101
HMOX1	heme oxygenase 1	1.58	NM_002133	chr22 : 35381066 - 35394214
HRAS	Harvey rat sarcoma viral oncogene homolog	1.12	NM_001130442	chr11 : 532241 - 535567
HSD3B7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	1.41	NM_001142777	chr16 : 30985197 - 30989152
INSIG1	insulin induced gene 1	1.98	NM_005542	chr7 : 155297775 - 155310235
LDLR	low density lipoprotein receptor	2.04	NM_001195799	chr19 : 11089361 - 11133829
MMP11	matrix metallopeptidase 11	1.29	NM_005940	chr22 : 23772818 - 23784316
POSTN	periostin, osteoblast specific factor	0.80	NM_006475	chr13 : 37562581 - 37598844
PTEN	phosphatase and tensin homolog	0.89	NM_000314	chr10 : 87863437 - 87971930
TNC	tenascin C	0.75	NM_002160	chr9 : 115019575 - 115118257

Senescence profile (Related to Figure 3C)

BAG1	BCL2 associated athanogene 1	1.12	NM_001172415	chr9 : 33252470 - 33264761
BCL2	B-cell CLL/lymphoma 2	1.58	NM_000633	chr18 : 63123345 - 63319380
BCL2L1	BCL2-like 1	1.39	NM_001191	chr20 : 31664451 - 31723098
CCND2	cyclin D2	0.91	NM_001759	chr12 : 4273735 - 4305356
CCNI	cyclin I	0.91	NM_006835	chr4 : 77048020 - 77075972
CDC7	cell division cycle 7	0.79	NM_003503	chr1 : 91500846 - 91525764
CDK6	cyclin-dependent kinase 6	0.83	NM_001259	chr7 : 92604920 - 92833917
CDK5R1	cyclin-dependent kinase 5, regulatory subunit 1 (p35)	0.81	NM_003885	chr17 : 32487086 - 32491253
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.39	NM_001291549	chr6 : 36676536 - 36687339
CDKN2A	cyclin-dependent kinase inhibitor 2A (p16)	1.09	NM_000077	chr9 : 21967751 - 21975133
CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	1.29	NM_004936	chr9 : 22002902 - 22009313
E2F7	E2F transcription factor 7	1.43	NM_203394	chr12 : 77021245 - 77065580
PTEN	phosphatase and tensin homolog	0.89	NM_000314	chr10 : 87863437 - 87971930
RBBP9	retinoblastoma binding protein 9	1.13	NM_006606	chr20 : 18486540 - 18497243

Extracellular matrix (Related to Figure 3F)

ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif 1	0.71	NM_006988	chr21 : 26836286 - 26845409
ADAMTS12	ADAM metallopeptidase with thrombospondin type 1 motif 12	0.80	NM_030955	chr5 : 33527180 - 33892019
ADAMTS15	ADAM metallopeptidase with thrombospondin type 1 motif 15	0.67	NM_139055	chr11 : 130448973 - 130476644
ADAMTS16	ADAM metallopeptidase with thrombospondin type 1 motif 16	0.77	NM_139056	chr5 : 5140329 - 5320299
ADAMTS2	ADAM metallopeptidase with thrombospondin type 1 motif 2	0.79	NM_014244	chr5 : 179110850 - 179345430
ADAMTS3	ADAM metallopeptidase with thrombospondin type 1 motif 3	0.64	NM_014243	chr4 : 72280968 - 72568799
B4GAT1	beta-1,4-glucuronyltransferase 1	0.90	NM_006876	chr11 : 66345371 - 66347690
COL1A1	collagen, type I, alpha 1	0.80	NM_000088	chr17 : 50184095 - 50201639
COL3A1	collagen, type III, alpha 1	0.65	NM_000090	chr2 : 188974372 - 189012746

COL5A1	collagen, type V, alpha 1	0.72	NM_000093	chr9 : 134641804 - 134844842
COL5A3	collagen, type V, alpha 3	0.67	NM_015719	chr19 : 9959560 - 10010471
COL6A1	collagen, type VI, alpha 1	0.78	NM_001848	chr21 : 45981748 - 46005049
COL14A1	collagen, type XIV, alpha 1	0.74	NM_021110	chr8 : 120125107 - 120372034
DPP4	dipeptidyl-peptidase 4	1.27	NM_001935	chr2 : 161992244 - 162074542
GALNT12	polypeptide N-acetylgalactosaminyltransferase 12	1.53	NM_024642	chr9 : 98807698 - 98850081
GLT8D1	glycosyltransferase 8 domain containing 1	0.90	NM_152932	chr3 : 52694483 - 52703698
LAMA2	laminin subunit alpha 2	0.87	NM_001079823	chr6 : 128883140 - 129516565
LARGE	like-glycosyltransferase	1.21	NM_004737	chr22 : 33273075 - 33920428
LOXL2	lysyl oxidase like 2	0.86	NM_002318	chr8 : 23296896 - 23404209
MME	membrane metallo-endopeptidase	1.76	NM_007288	chr3 : 155080163 - 155183729
MMP11	matrix metallopeptidase 11	1.29	NM_005940	chr22 : 23772818 - 23784316
MMP3	matrix metallopeptidase 3	1.48	NM_002422	chr11 : 102835796 - 102843689

Cell cycle regulation (Related to Figure 3F)

CCND2	cyclin D2	0.91	NM_001759	chr12 : 4273735 - 4305356
CDC7	cell division cycle 7	0.79	NM_003503	chr1 : 91500846 - 91525764
CDIP1	cell death-inducing p53 target 1	1.17	NM_013399	chr16 : 4510675 - 4538815
CDK6	cyclin-dependent kinase 6	0.83	NM_001259	chr7 : 92604920 - 92833917
CNTRL	centriolin	0.84	NM_007018	chr9 : 121088295 - 121177608
FIGN	fidgetin	0.88	NM_018086	chr2 : 163607607 - 163736003
G2E3	G2/M-phase specific E3 ubiquitin protein ligase	0.93	NM_017769	chr14 : 30559122 - 30620061
GAS1	growth arrest specific 1	0.69	NM_002048	chr9 : 86944361 - 86947189
ING1	inhibitor of growth family member 1	0.85	NM_005537	chr13 : 110715011 - 110721074
RBBP9	retinoblastoma binding protein 9	1.13	NM_006606	chr20 : 18486540 - 18497243

Lysosome markers (Related to Figure 3F)

ARSG	arylsulfatase G	1.36	NM_001267727	chr17 : 68291494 - 68420859
ATP6V0A1	ATPase, H+ transporting, lysosomal V0 subunit a1	1.22	NM_005177	chr17 : 42458843 - 42522579
ATP6V1H	ATPase, H+ transporting, lysosomal 50/57kDa, V1 subunit H	1.25	NM_213620	chr8 : 53715542 - 53843042
CTSK	cathepsin K	1.99	NM_000396	chr1 : 150796207 - 150808441
CTSL	cathepsin L	1.23	NM_001912	chr9 : 87726058 - 87731469
CTSS	cathepsin S	1.33	NM_001199739	chr1 : 150730195 - 150765957
LIPA	lipase A, lysosomal acid, cholesterol esterase	1.19	NM_000235	chr10 : 89213568 - 89252039
M6PR	mannose-6-phosphate receptor (cation dependent)	1.21	NM_001207024	chr12 : 8940360 - 8949761
NPC1	Niemann-Pick disease, type C1	1.56	NM_000271	chr18 : 23531498 - 23586617
NPC2	Niemann-Pick disease, type C2	1.25	NM_006432	chr14 : 74479939 - 74493381

Synovial Fibroblast aggressiveness (Related to Figure 3G)

CCL2	chemokine (C-C motif) ligand 2	0.77	NM_002982	chr17 : 34255276 - 34257201
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	0.74	NM_004355	chr5 : 150401636 - 150412936
CXCL16	chemokine (C-X-C motif) ligand 16	0.85	NM_022059	chr17 : 4733528 - 4739928
IL17RC	interleukin 17 receptor C	0.92	NM_001203263	chr3 : 9917073 - 9933621
IL18R1	interleukin 18 receptor 1	0.75	NM_003855	chr2 : 102356282 - 102398775
ITGA9	integrin subunit alpha 9	0.65	NM_002207	chr3 : 37452321 - 37819790
ITGA11	integrin subunit alpha 11	0.87	NM_001004439	chr15 : 68301703 - 68432153
MSTN	myostatin	0.50	NM_005259	chr2 : 190055699 - 190062729
PTGS2	prostaglandin-endoperoxide synthase 2	0.82	NM_000963	chr1 : 186671811 - 186680427
SENP1	SUMO1/sentrin specific peptidase 1	0.90	NM_001267595	chr12 : 48042897 - 48105992
THBS4	thrombospondin 4	0.76	NM_001306212	chr5 : 79991295 - 80083284
TNC	tenascin C	0.75	NM_002160	chr9 : 115019575 - 115118257
VCAM1	vascular cell adhesion molecule 1	0.65	NM_080682	chr1 : 100719639 - 100739046

VEGFA	vascular endothelial growth factor A	0.85	NM_001204385	chr6 : 43770208 - 43786486

Identified from Cell Age (Related to Figure 3H)

ACLY	ATP citrate lyase	1.15	NM_001303275	chr17 : 41866916 - 41919022
BHLHE40	basic helix-loop-helix family member e40	1.17	NM_003670	chr3 : 4979411 - 4985180
BTG3	BTG family member 3	1.16	NM_006806	chr21 : 17593649 - 17612950
CCND1	cyclin D1	1.27	NM_053056	chr11 : 69641104 - 69654474
CDK6	cyclin-dependent kinase 6	0.83	NM_001259	chr7 : 92604920 - 92833917
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	1.39	NM_001291549	chr6 : 36676536 - 36687339
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.86	NM_004064	chr12 : 12717269 - 12722382
CDKN2A	cyclin-dependent kinase inhibitor 2A (p16)	1.09	NM_000077	chr9 : 21967751 - 21975133
CDKN2B	cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	1.29	NM_004936	chr9 : 22002902 - 22009313
CPEB1	cytoplasmic polyadenylation element binding protein 1	1.28	NM_001288819	chr15 : 82543200 - 82647605
CXCL1	chemokine (C-X-C motif) ligand 1	0.53	NM_001511	chr4 : 73869391 - 73871302
DHCR24	24-dehydrocholesterol reductase	1.48	NM_014762	chr1 : 54849626 - 54887248
DUSP3	dual specificity phosphatase 3	1.19	NM_004090	chr17 : 43766120 - 43779000
HDAC4	histone deacetylase 4	0.89	NM_006037	chr2 : 239048167 - 239400949
HRAS	Harvey rat sarcoma viral oncogene homolog	1.12	NM_001130442	chr11 : 532241 - 535567
HSPB2	heat shock protein family B (small) member 2	1.23	NM_001541	chr11 : 111912735 - 111914093
ID1	inhibitor of DNA binding 1, dominant negative helix-loop-helix protein	0.79	NM_002165	chr20 : 31605282 - 31606514
ING1	inhibitor of growth family member 1	0.85	NM_005537	chr13 : 110715011 - 110721074
KDM5B	lysine (K)-specific demethylase 5B	0.91	NM_006618	chr1 : 202725184 - 202809470
LIMK1	LIM domain kinase 1	1.26	NM_001204426	chr7 : 74093155 - 74122525
MAP2K3	mitogen-activated protein kinase kinase 3	1.15	NM_002756	chr17 : 21288035 - 21315239
MVK	mevalonate kinase	1.53	NM_000431	chr12 : 109573694 - 109597270
NOTCH3	notch 3	0.84	NM_000435	chr19 : 15159632 - 15200981

NTN4	netrin 4	1.13	NM_021229	chr12 : 95657806 - 95790758
P3H1	prolyl 3-hydroxylase 1	0.84	NM_022356	chr1 : 42746334 - 42767084
PRKCH	protein kinase C, eta	0.81	NM_006255	chr14 : 61321442 - 61550980
PSMD14	proteasome 26S subunit, non-ATPase 14	1.13	NM_005805	chr2 : 161308274 - 161411717
RNASEL	ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent)	0.88	NM_021133	chr1 : 182573633 - 182589259
SENP1	SUMO1/sentrin specific peptidase 1	0.90	NM_001267595	chr12 : 48042897 - 48105992
SENP7	SUMO1/sentrin specific peptidase 7	0.91	NM_001077203	chr3 : 101324188 - 101513241
SIX1	SIX homeobox 1	0.89	NM_005982	chr14 : 60644698 - 60649437
SMG1	SMG1 phosphatidylinositol 3-kinase-related kinase	0.93	NM_015092	chr16 : 18804852 - 18926404
SREBF1	sterol regulatory element binding transcription factor 1	1.77	NM_001005291	chr17 : 17811348 - 17837011
TXN	thioredoxin	1.10	NM_003329	chr9 : 110243811 - 110256640
VEGFA	vascular endothelial growth factor A	0.85	NM_001204385	chr6 : 43770208 - 43786486
WNT2	wingless-type MMTV integration site family member 2	0.56	NR_024047	chr7 : 117276630 - 117323289
ZFP36	ZFP36 ring finger protein	0.92	NM_003407	chr19 : 39406846 - 39409412

Identified from TiRe (Related to Figure 3H)

ADAMTS1	ADAM metallopeptidase with thrombospondin type 1 motif 1	0.71	NM_006988	chr21 : 26836286 - 26845409
ADRA2A	adrenoceptor alpha 2A	0.75	NM_000681	chr10 : 111077031 - 111080904
AHR	aryl hydrocarbon receptor	0.75	NM_001621	chr7 : 17298651 - 17346151
ANGPTL4	angiopoietin like 4	0.76	NR_104213	chr19 : 8364126 - 8374375
ARHGAP1	Rho GTPase activating protein 1	1.14	NM_004308	chr11 : 46677074 - 46700665
B4GALNT1	beta-1,4-N-acetyl-galactosaminyl transferase 1	1.20	NM_001276469	chr12 : 57629330 - 57633239
CCL2	chemokine (C-C motif) ligand 2	0.77	NM_002982	chr17 : 34255276 - 34257201
CD151	CD151 molecule (Raph blood group)	1.07	NM_139030	chr11 : 832951 - 838835
CDKN1B	cyclin-dependent kinase inhibitor 1B (p27, Kip1)	0.86	NM_004064	chr12 : 12717269 - 12722382
COL1A1	collagen, type I, alpha 1	0.80	NM_000088	chr17 : 50184095 - 50201639

CXXC5	CXXC finger protein 5	0.88	NM_001317204	chr5 : 139648924 - 139683885
ESR1	estrogen receptor 1	1.16	NM_000125	chr6 : 151807678 - 152103273
FGF2	fibroblast growth factor 2 (basic)	1.21	NM_002006	chr4 : 122826707 - 122898235
HMOX1	heme oxygenase 1	1.58	NM_002133	chr22 : 35381066 - 35394214
ICAM1	intercellular adhesion molecule 1	1.27	NM_000201	chr19 : 10270840 - 10286615
ITGA11	integrin subunit alpha 11	0.87	NM_001004439	chr15 : 68301703 - 68432153
ITGA9	integrin subunit alpha 9	0.65	NM_002207	chr3 : 37452321 - 37819790
LSP1	lymphocyte-specific protein 1	0.86	NM_001013255	chr11 : 1870923 - 1892263
MRC2	mannose receptor, C type 2	0.87	NM_006039	chr17 : 62627400 - 62693601
MSTN	myostatin	0.50	NM_005259	chr2 : 190055699 - 190062729
NFIC	nuclear factor I/C (CCAAT-binding transcription factor)	0.86	NM_005597	chr19 : 3366566 - 3469217
NID1	nidogen 1	0.82	NM_002508	chr1 : 235975831 - 236065181
NR4A1	nuclear receptor subfamily 4 group A member 1	1.16	NM_001202233	chr12 : 52022831 - 52059507
PGF	placental growth factor	0.66	NM_001207012	chr14 : 74941829 - 74955764
PLAUR	plasminogen activator, urokinase receptor	1.16	NM_001005376	chr19 : 43646094 - 43670346
POSTN	periostin, osteoblast specific factor	0.80	NM_006475	chr13 : 37562581 - 37598844
PPARA	peroxisome proliferator-activated receptor alpha	0.92	NM_001001928	chr22 : 46150595 - 46243756
PPARG	peroxisome proliferator-activated receptor gamma	1.25	NM_138711	chr3 : 12288936 - 12434356
PRKCH	protein kinase C, eta	0.81	NM_006255	chr14 : 61321442 - 61550980
PTEN	phosphatase and tensin homolog	0.89	NM_000314	chr10 : 87863437 - 87971930
PTGS2	prostaglandin-endoperoxide synthase 2	0.82	NM_000963	chr1 : 186671811 - 186680427
SNAI2	snail family zinc finger 2	0.80	NM_003068	chr8 : 48917680 - 48921440
TGFB3	transforming growth factor beta 3	0.83	NM_003239	chr14 : 75958096 - 75982022
THBD	thrombomodulin	1.55	NM_000361	chr20 : 23045632 - 23049664
TNFRSF1A	tumor necrosis factor receptor superfamily member 1A	0.89	NM_001065	chr12 : 6328756 - 6342117
VAV3	vav guanine nucleotide exchange factor 3	1.36	NM_006113	chr1 : 107571159 - 107964923

VEGFA	vascular endothelial growth factor A	0.85	NM_001204385	chr6 : 43770208 - 43786486
VTN	vitronectin	0.70	NM_000638	chr17 : 28367277 - 28370352

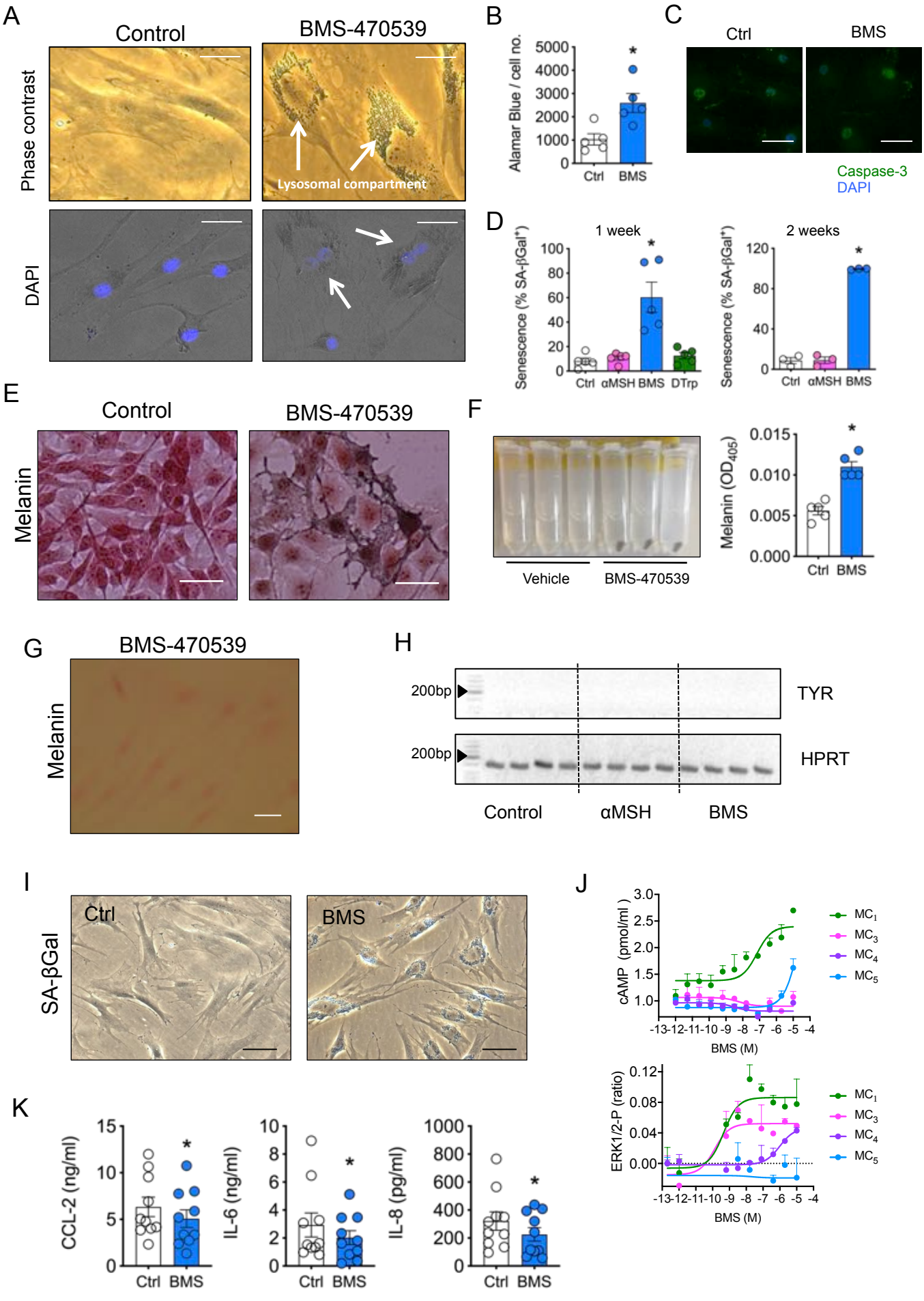
Notch pathway (Related to Figure 4)

CREBBP	CREB binding protein	0.92	NM_004380	chr16 : 3725054 - 3880120
DTX3L	deltex 3 like, E3 ubiquitin ligase	0.89	NM_138287	chr3 : 122564337 - 122575202
DTX4	deltex 4, E3 ubiquitin ligase	0.64	NM_015177	chr11 : 59172338 - 59208587
EP300	E1A binding protein p300	0.90	NM_001429	chr22 : 41092609 - 41180077
HES1	hes family bHLH transcription factor 1	0.81	NM_005524	chr3 : 194136141 - 194138612
JAG1	jagged 1	0.78	NM_000214	chr20 : 10637683 - 10674046
MAML2	mastermind like transcriptional coactivator 2	0.87	NM_032427	chr11 : 95976592 - 96343180
MAML3	mastermind like transcriptional coactivator 3	0.79	NM_018717	chr4 : 139716390 - 140154079
MIB1	mindbomb E3 ubiquitin protein ligase 1	0.93	NM_020774	chr18 : 21741328 - 21870957
NCOR2	nuclear receptor corepressor 2	0.93	NM_001206654	chr12 : 124324410 - 124567464
NOTCH3	notch 3	0.84	NM_000435	chr19 : 15159632 - 15200981

Cholesterol pathway (Related to Figure 5)

ABCA1	ATP binding cassette subfamily A member 1	1.26	NM_005502	chr9 : 104781002 - 104928246
DHCR24	24-dehydrocholesterol reductase	1.48	NM_014762	chr1 : 54849626 - 54887248
DHCR7	7-dehydrocholesterol reductase	1.97	NM_001360	chr11 : 71434410 - 71448431
FDFT1	farnesyl-diphosphate farnesyltransferase 1	1.25	NM_001287744	chr8 : 11795572 - 11839309
FDPS	farnesyl diphosphate synthase	1.57	NM_002004	chr1 : 155308747 - 155320666
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	1.53	NM_001130996	chr5 : 75337167 - 75362101
HMGCS1	3-hydroxy-3-methylglutaryl-CoA synthase 1	1.95	NM_001098272	chr5 : 43287469 - 43313512
HSD3B7	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	1.41	NM_001142777	chr16 : 30985197 - 30989152
IDI1	isopentenyl-diphosphate delta isomerase 1	1.57	NM_004508	chr10 : 1039419 - 1049366

INSIG1	insulin induced gene 1	1.98	NM_005542	chr7 : 155297775 - 155310235
LDLR	low density lipoprotein receptor	2.04	NM_001195799	chr19 : 11089361 - 11133829
LSS	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)	1.27	NM_001145437	chr21 : 46189123 - 46228824
MVK	mevalonate kinase	1.53	NM_000431	chr12 : 109573694 - 109597270
NPC1	Niemann-Pick disease, type C1	1.56	NM_000271	chr18 : 23531498 - 23586617
NPC2	Niemann-Pick disease, type C2	1.25	NM_006432	chr14 : 74479939 - 74493381
NSDHL	NAD(P) dependent steroid dehydrogenase-like	1.21	NM_001129765	chrX : 152830966 - 152869363
PCSK9	proprotein convertase subtilisin/kexin type 9	2.85	NM_174936	chr1 : 55039475 - 55064853
SQLE	squalene epoxidase	1.48	NM_003129	chr8 : 124998477 - 125022283
SREBF1	sterol regulatory element binding transcription factor 1	1.77	NM_001005291	chr17 : 17811348 - 17837011
SREBF2	sterol regulatory element binding transcription factor 2	1.37	NR_103834	chr22 : 41833078 - 41907308


A
Control
BMS-470539
Phase contrast
DAPI
Lysosomal compartment
B
Alamar Blue / cell no.
5000
4000
3000
2000
1000
0
Ctrl
BMS
C
Ctrl
BMS
Caspase-3
DAPI
D
1 week
2 weeks
Senescence (% SA-βGal+)
100
80
60
40
20
0
120
Ctrl
αMSH
BMS
DTrp
E
Control
BMS-470539
Melanin
F
Vehicle
BMS-470539
Melanin (OD405)
0.015
0.010
0.005
0.000
G
BMS-470539
Melanin
H
200bp
TYR
HPRT
Control
αMSH
BMS
I
SA-βGal
Ctrl
BMS
J
cAMP (pmol/ml)
3.0
2.5
2.0
1.5
1.0
-13 -12 -11 -10 -9 -8 -7 -6 -5 -4
BMS (M)
MC1
MC3
MC4
MC5
ERK1/2-P (ratio)
0.12
0.08
0.04
0.00
K
CCL-2 (ng/ml)
15
10
5
0
IL-6 (ng/ml)
10
8
6
4
2
0
IL-8 (pg/ml)
1000
800
600
400
200
0

Supplementary Figure 1. Effect of BMS on fibroblast senescence, melanin production and signalling.

(A) Images of SF (control or treated with 1μM BMS) showing expansion of lysosomal compartment and presence of bi-nucleated cells (DAPI staining). Scale bars indicate 100μm.

(B) SF were treated with BMS (1μM) for 7 days. Alamar blue assay was used as an indicator of cell health and metabolic activity. Data represent mean values ±SE (n=5, Student's t-test vs. control).

(C) Cleaved caspase-3 expression was assessed by immunofluorescence on SF (control, or treated with 1μM BMS for 7 days). Scale bars indicate 200μm.

(D) SA-βGal was determined 7 or 14 days after drug treatment with 1μM BMS, 10μM αMSH and 10μM [D-Trp^{8}]-γMSH. Senescence was analyzed by SA-βGal^{+} staining. Data represent mean values ±SE (n=3-5, one-way ANOVA vs. control).

(E) Melanin synthesis was analyzed on B16-F10 mouse melanocytes treated with 1μM BMS for 4 days using Fontana-Masson staining. Scale bars indicate 1000μm.

(F) B16-F10 melanocytes treated with 1μM BMS for 4 days were centrifuged to visualize pellet colour denoting melanin synthesis. Melanin was further quantified on supernatants by measuring absorbance at 405nm. Data represent mean values ±SE (n=5, Student's t-test vs. control).

(G) SF were stained with Fontana-Masson after treatment with 1μM BMS for 7 days. Scale bar indicate 200μm.

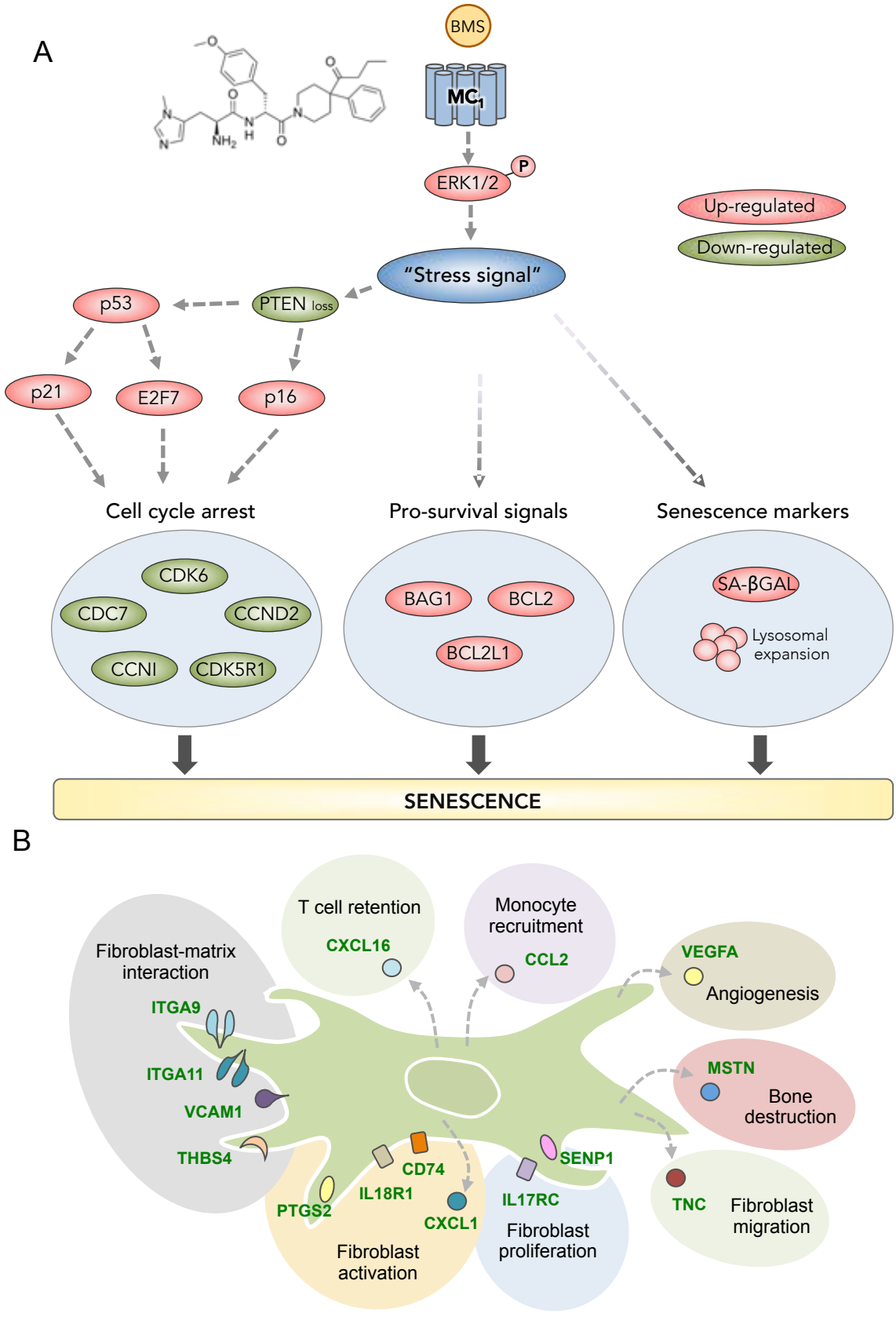
(H) Gene expression of tyrosinase (TYR) was analyzed on SF treated with 1μM BMS or 10μM αMSH. HPRT expression was used as control.

(I) Senescence was assessed by SA-βGal^{+} staining on BMS (1μM) treated skin fibroblasts. Scale bars indicate 200μm.

(J) Signaling via cAMP (15 min stimulation) and ERK-phosphorylation (5 min stimulation) induced by BMS was studied on HEK293 cells transiently transfected with *MC1R*, *MC3R*, *MC4R* or *MC5R*. Data represent mean values ±SE (n=2).

(K) Cytokines release was evaluated by ELISA on supernatants from senescent SF treated with 1μM BMS for 7 days. Data represent mean values ±SE (n=10, Student's t-test vs. control).

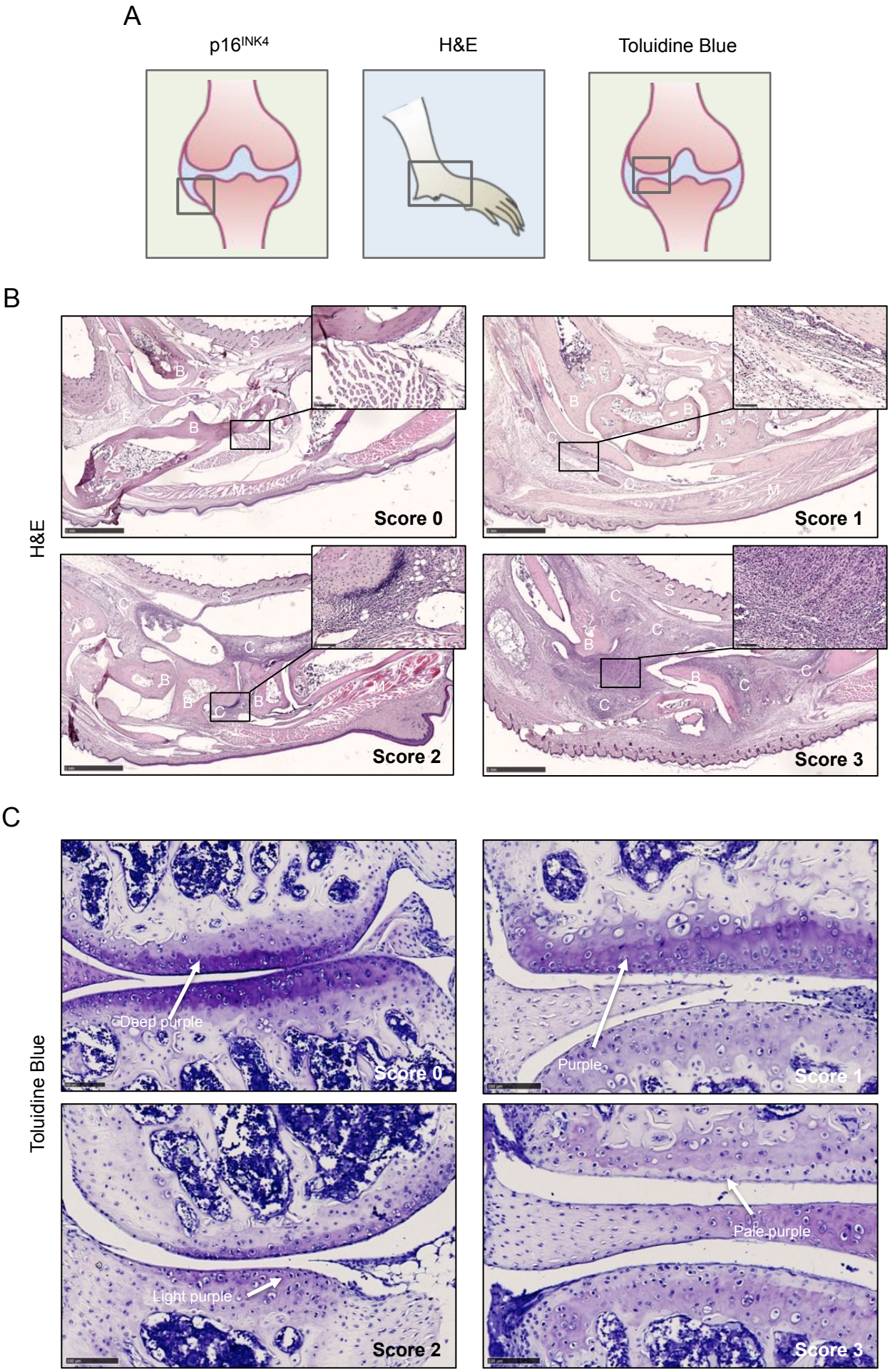
Source data are provided as Supplementary Source Data file.



Supplementary Figure 2. Genes identified by RNAseq associated with senescence and SF activation.

(A) Senescence-related genes reported on Figure 3C and other senescence markers identified, such as p53 (by western blot) and SA-βGal (by histochemistry). The chemical structure of BMS-470539 is shown.

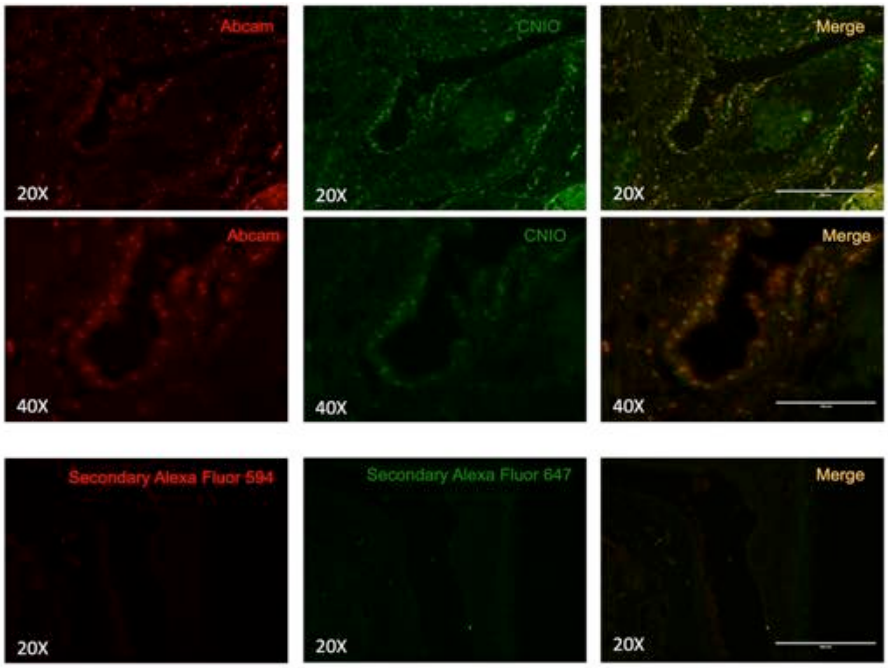
(B) Downregulation of genes related to SF activation and aggressiveness reported on Figure 3G.


A
p16INK4
H&E
Toluidine Blue
B
H&E
S
B
F
M
C
Score 0
Score 1
Score 2
Score 3
C
Toluidine Blue
Daep purple
Purple
Light purple
Pale purple
Score 0
Score 1
Score 2
Score 3

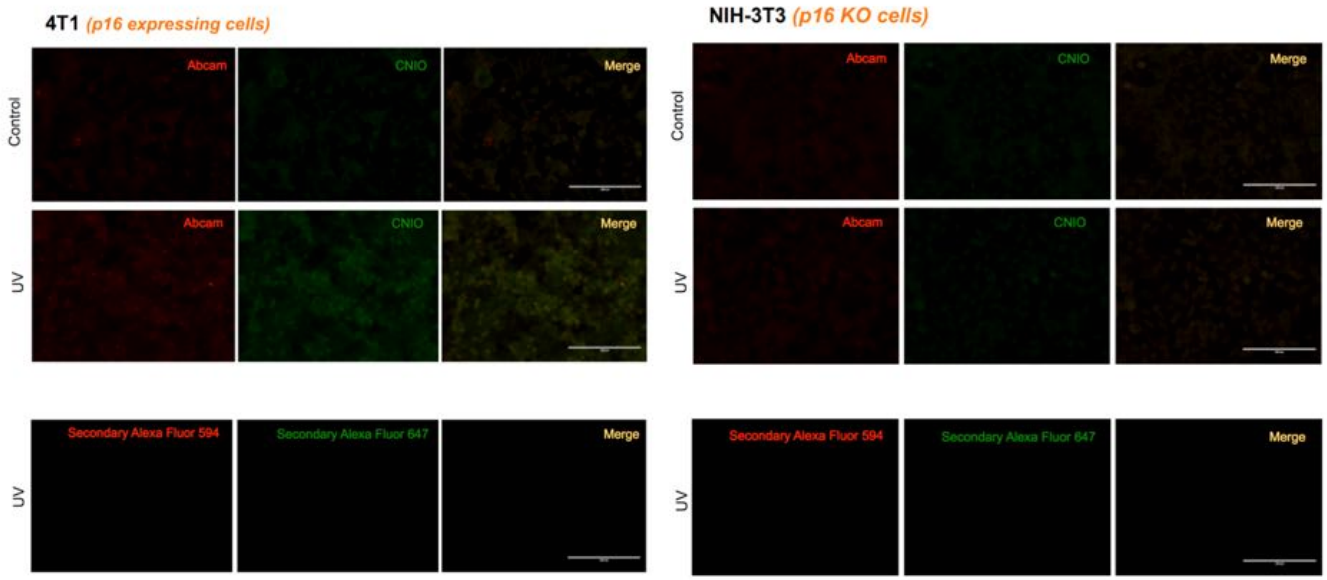
Supplementary Figure 3. Histological analysis on mouse joints.
(A) The location where images for IF and histological analysis on Figure 5 were taken are shown.
(B) Scale used for scoring degree of cell infiltration. Cell infiltration was analysed by H&E staining. Large images were taken at 2.5X magnification and scale bars indicate 1 mm. Small inserts images were taken at 20X magnification and scale bars indicate 100μm. B= bone, C= cell infiltration, F= fat, M= muscle, S= skin.
(C) Scale used for scoring degree of cartilage integrity. Cartilage integrity was analysed by toluidine blue staining. Deep purple colour indicates intact cartilage while gradual loss of colour accounts for loss of sulphated proteoglycans, indicative of cartilage damage. Images were taken at 20X magnification. Scale bars indicate 100μm.

Supplementary Figure 4. Anti-mouse p16^{INK4} antibody validation.
(A) p16^{INK4} expression was assessed by immunofluorescence on knee joints on mice treated with BMS. The Abcam ab54210 (clone 2D9A12) -in red-, was compared to the knock-out validated antibody from the CNIO (clone PABLO33B) -in green-. Scale bars indicate 200μm (20X) and 100μm (40X).
(B) p16^{INK4} expression was assessed by immunofluorescence on 4T1 and NIH 3T3 cells three days after treatment with UV light. Scale bars indicate 200μm.

A



B

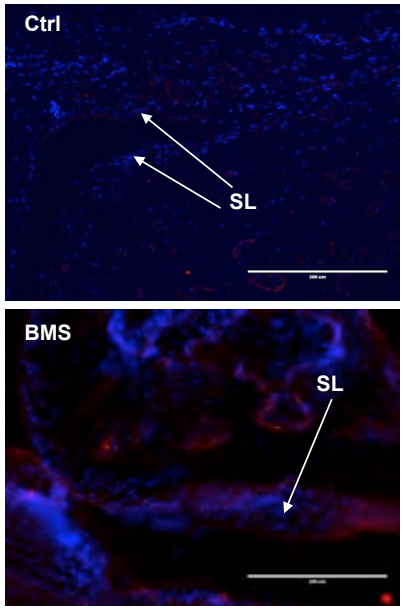


DAPI

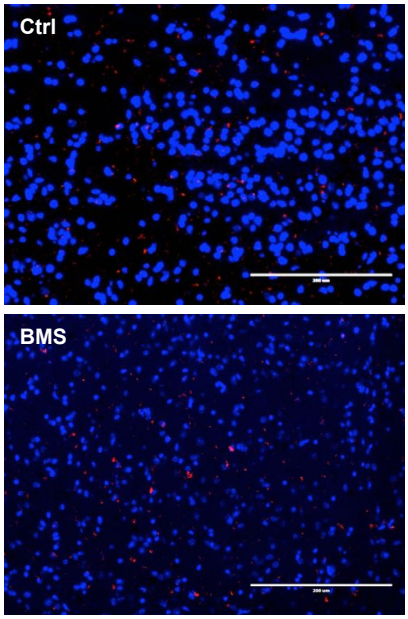
p16^{INK4}

200μm

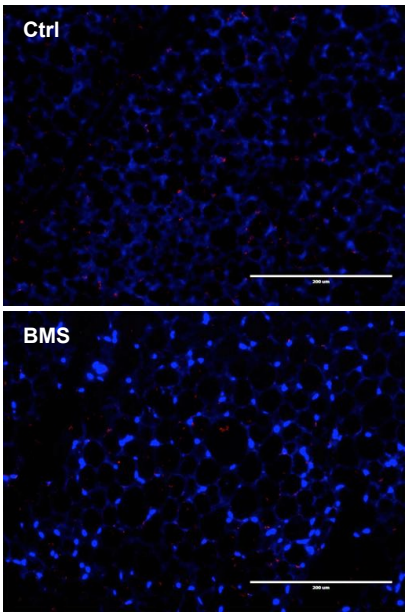
Articular joint



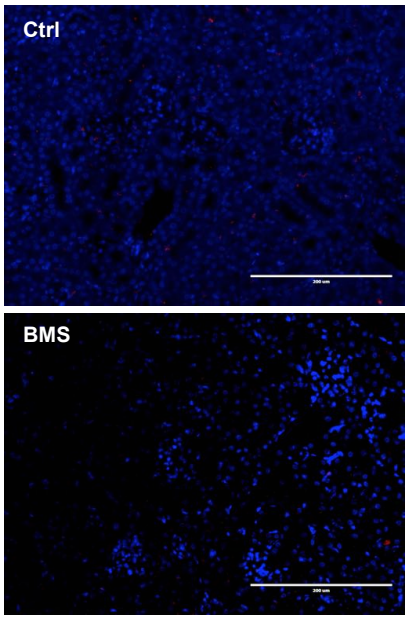
Brain



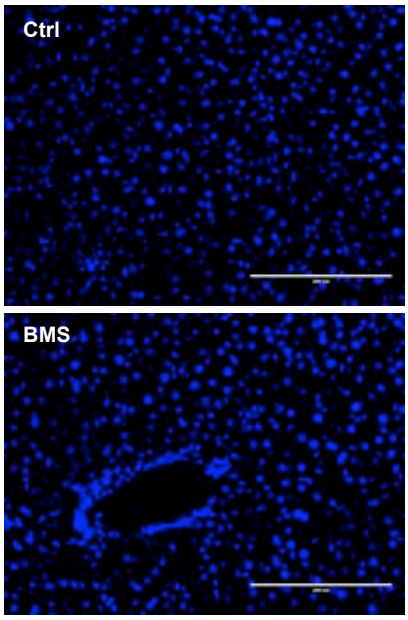
Fat



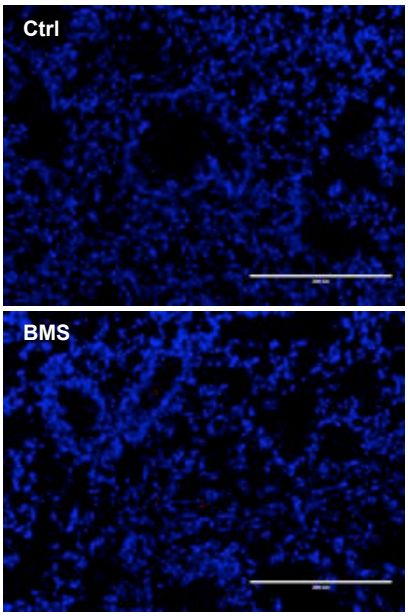
Kidney



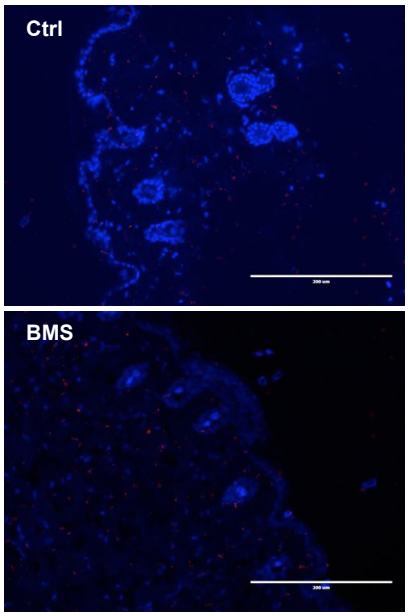
Liver



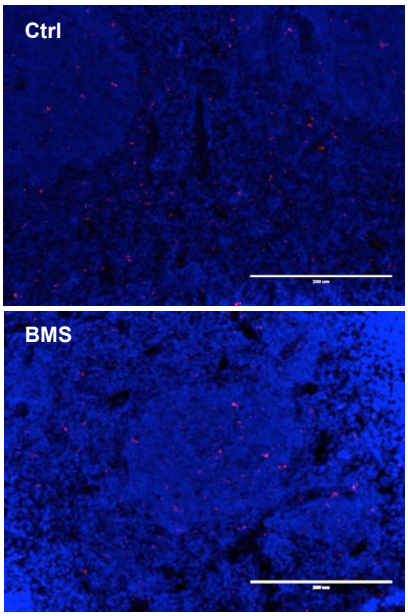
Lung



Skin



Spleen



Supplementary Figure 5. Expression of $p16^{INK4}$ on mouse tissues.
To account for off-target effects, $p16^{INK4}$ expression was assessed by immunofluorescence on brain, fat, kidney, liver, lung, skin and spleen on mice treated with 18mg/kg BMS intraperitoneally for 6 days, compared with control (vehicle treated) mice. Tissues were formalin-fixed and embedded in paraffin. Scale bars indicate 200μm.