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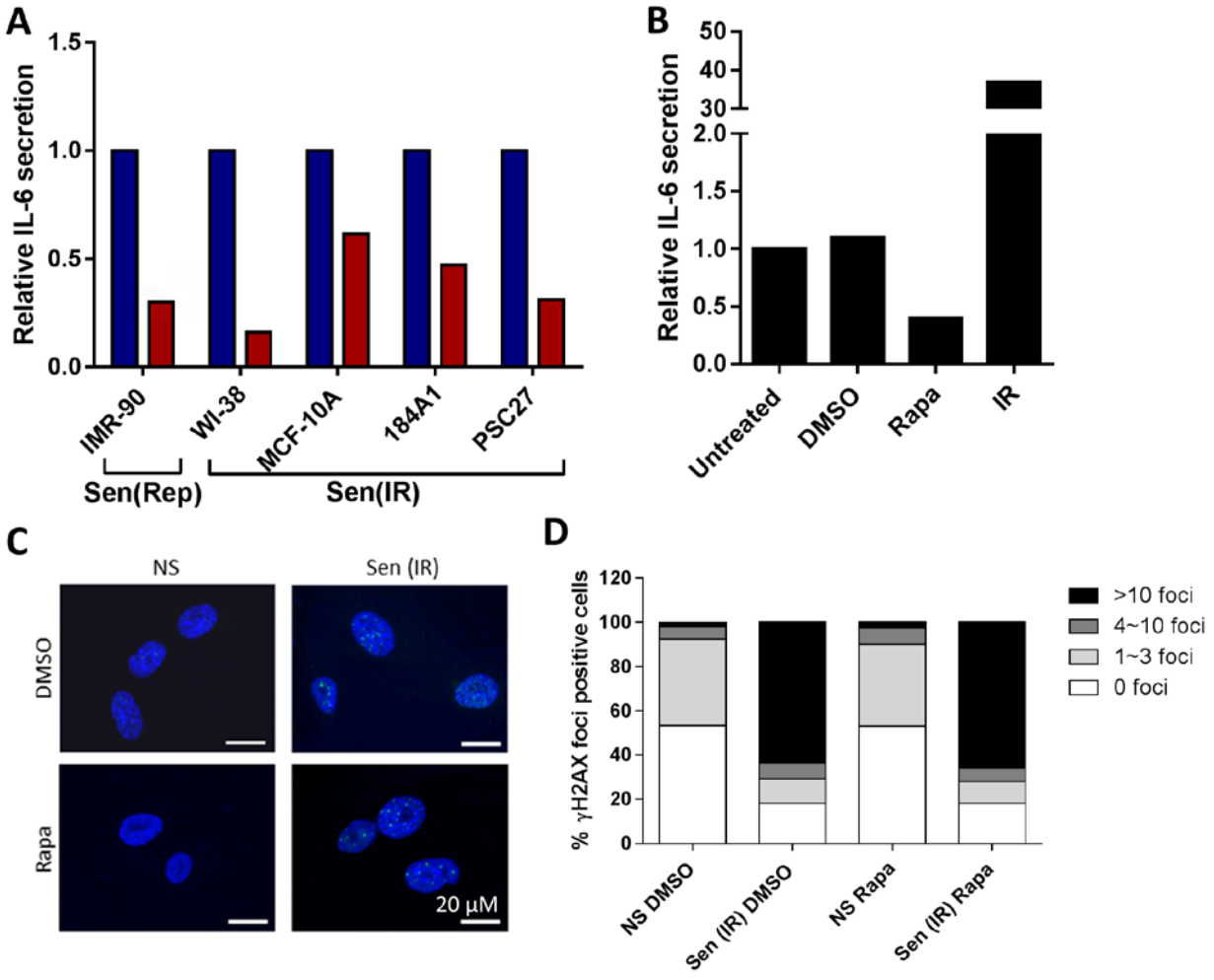
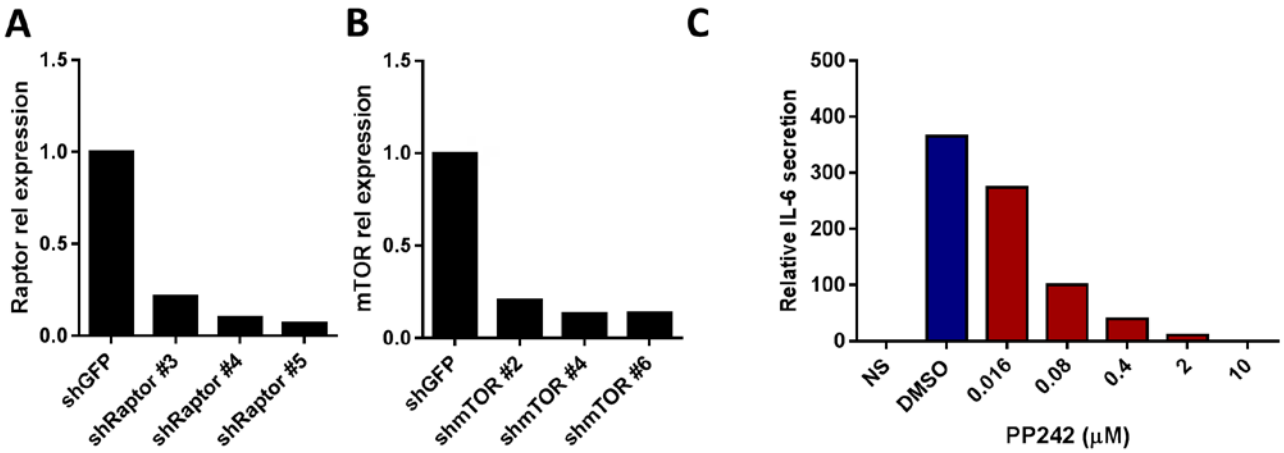
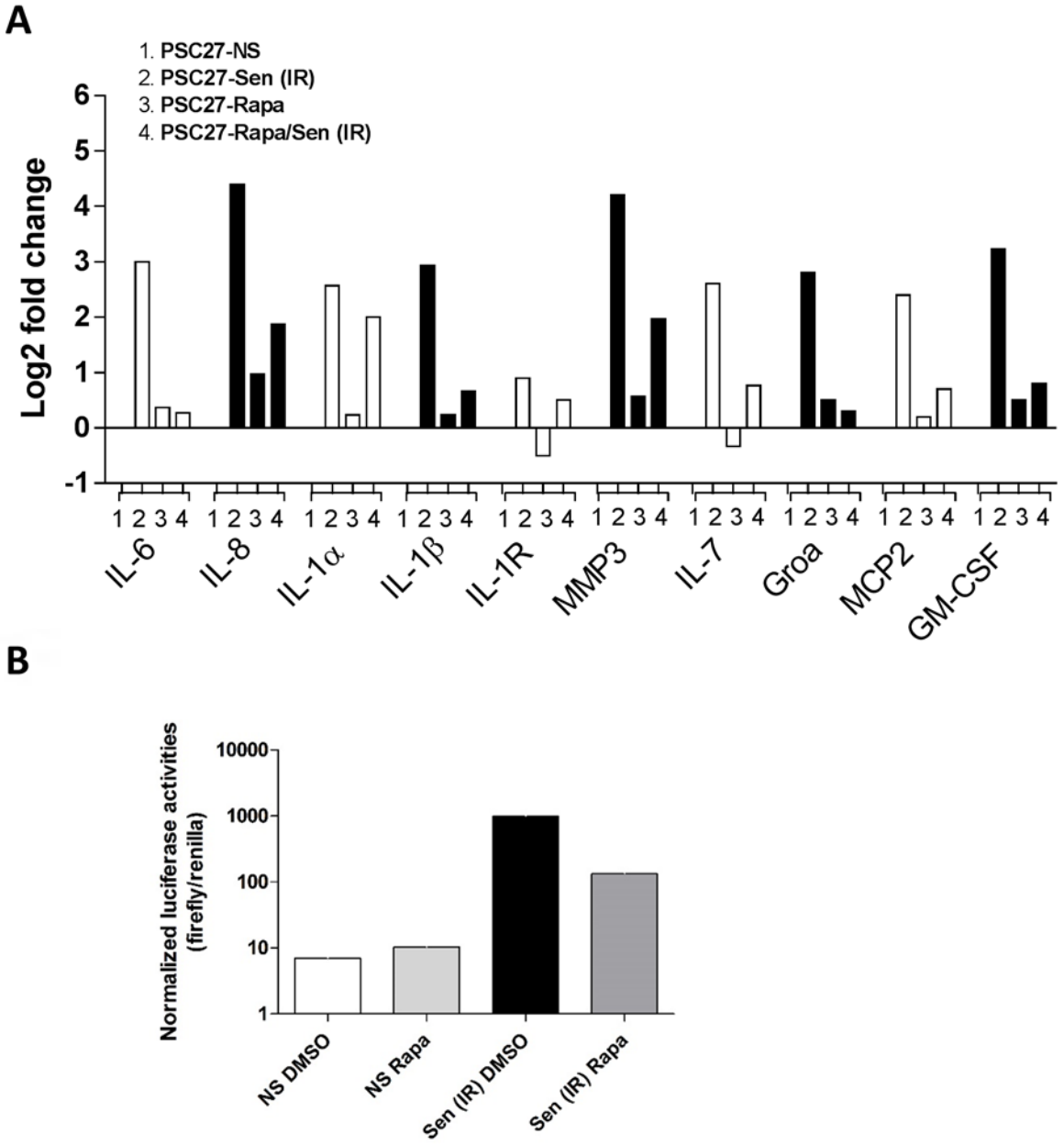
nature cell biology



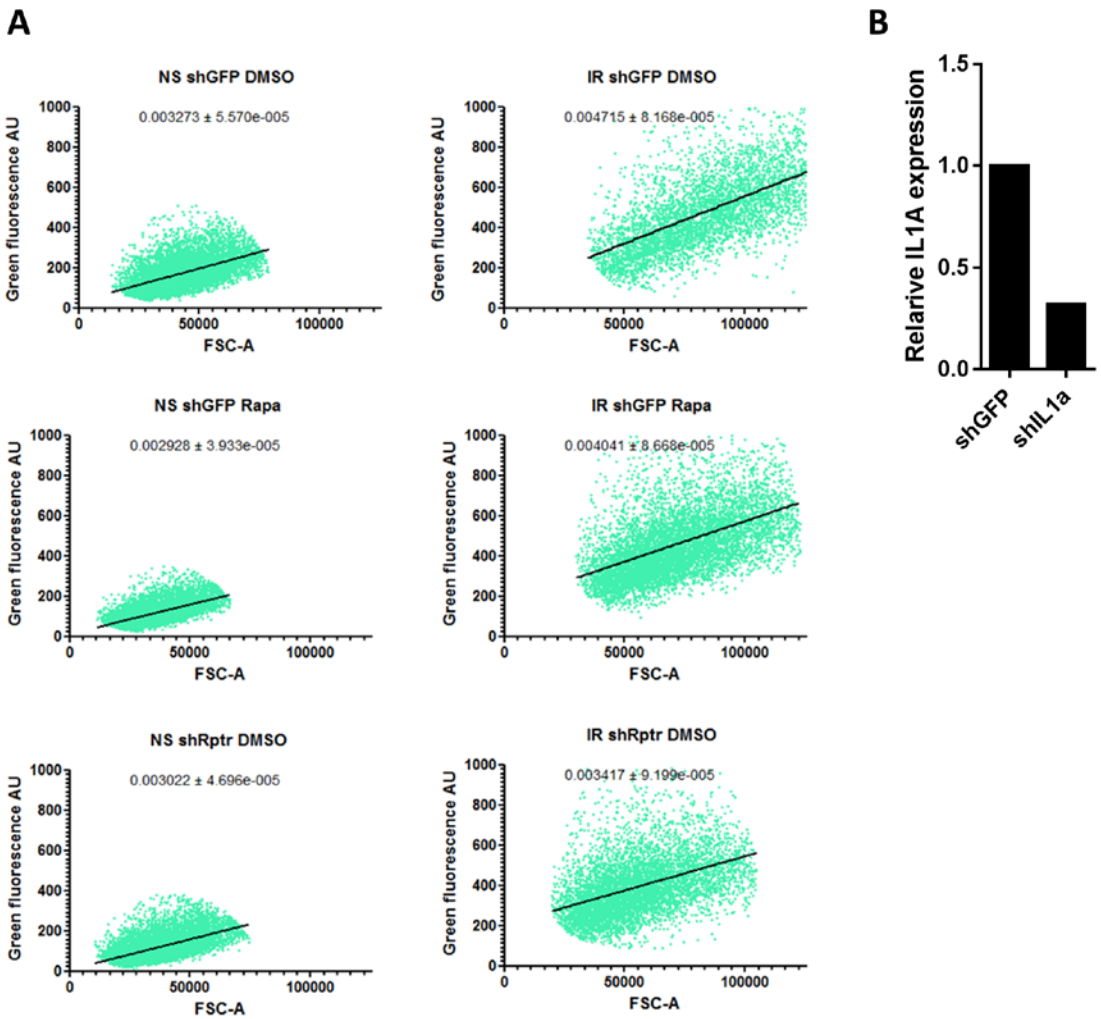
Supplementary Figure 1 Rapamycin suppresses the SASP in multiple cell strains and lines. (A) IL-6 secretion by IR-induced senescent cells, normalized to replicatively senescent IMR-90 normal fetal lung human fibroblasts. Shown are WI-38 normal fetal lung fibroblasts, MCF10-A and 184A1 immortal human breast epithelial cells and PSC27 normal adult prostate fibroblasts, treated with either DMSO (blue bars) or rapamycin (red bars). (B) We treated non-senescent (NS) HCA2 cells with rapamycin for 6 days, collected CM and analyzed IL-6 by ELISA. IL-6 secretion by irradiated senescent cells (Sen (IR)) is shown for comparison. (C) Immunofluorescence staining for 53BP1 was compared between NS and senescent (Sen IR) cells treated with DMSO or rapamycin (one representative image is shown). (D) We determined the number of foci in NS or senescent (Sen IR) cells treated with DMSO or rapamycin. With the exception of 184A1 experiment which was performed once, for panels A, B and D, shown is one representative of two or more independent experiments, each with triplicate samples. Raw data could be found in Supplementary Table 4.



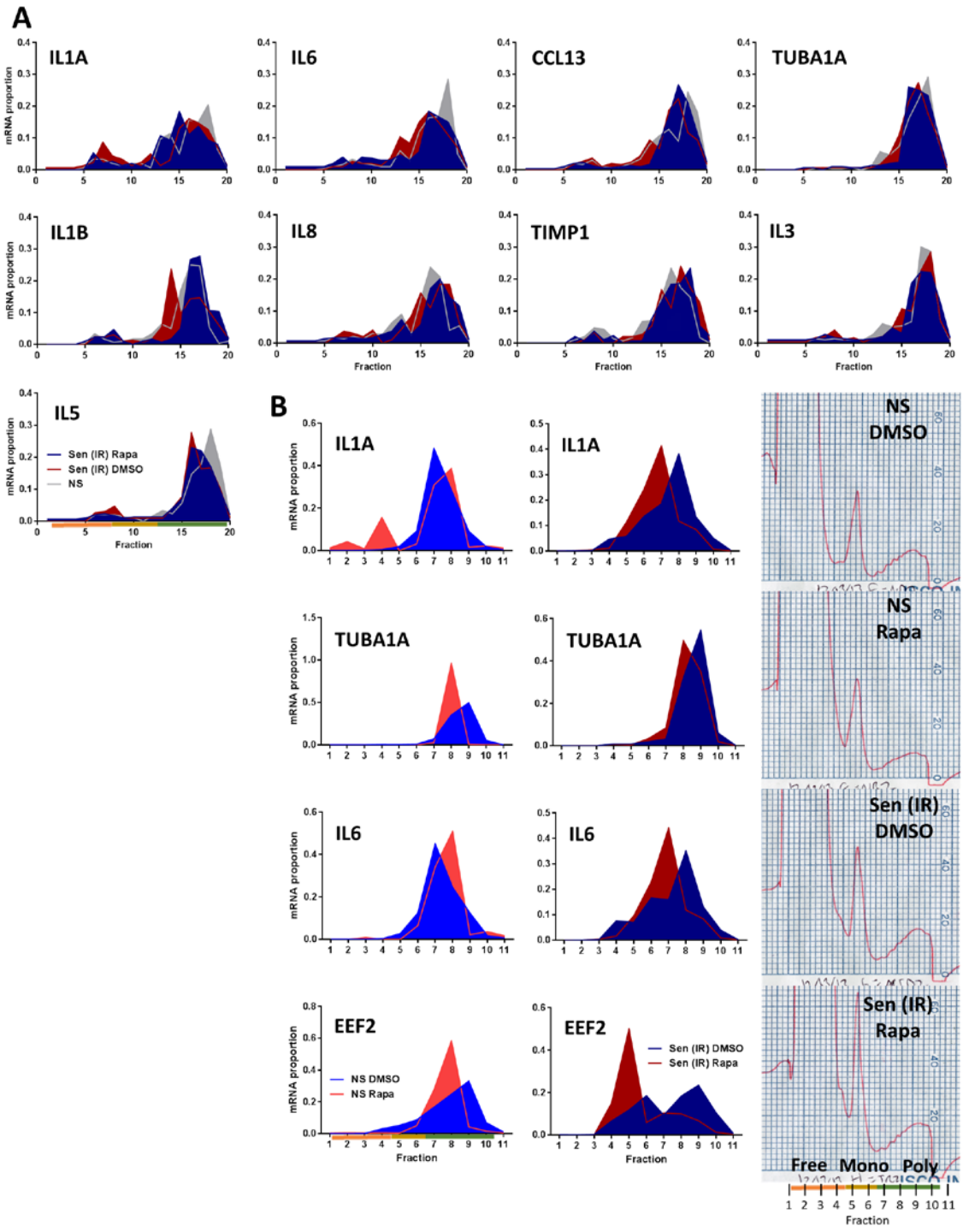
Supplementary Figure 2 The SASP is mTOR dependent. (A) HCA2 cells were infected with lentiviruses expressing shRNAs against GFP (shGFP; control) or one of three different shRNAs against raptor. Raptor transcript levels were measured and are shown relative the level in cells expressing shGFP. (B) HCA2 cells were infected with lentiviruses expressing shGFP (control) or one of three different shRNA against mTOR. The relative transcript level of mTOR was measured. (C) Normal HCA2 human foreskin fibroblasts, non-senescent (NS) or induced to senesce by IR, were treated with DMSO (control) or the indicated concentrations of the mTOR kinase inhibitor PP242. 7 days later, CM was collected and analyzed for IL-6 by ELISA. Values were normalized to the senescent cell level. For all panels, shown is one representative of two independent experiments, each with triplicate samples. Raw data could be found in Supplementary Table 4.



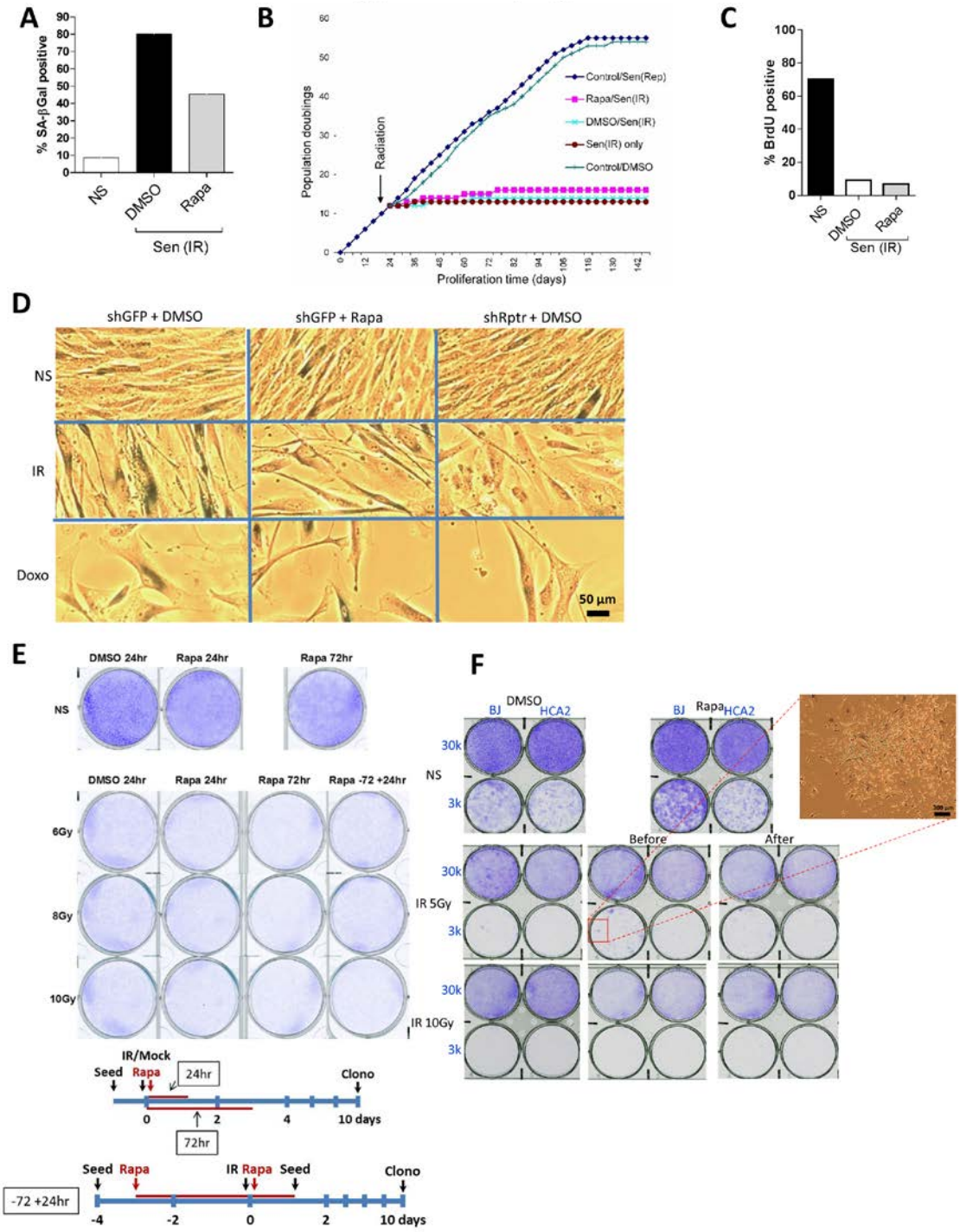
Supplementary Figure 3 Rapamycin reduces SASP transcript levels and NF-kB activity in senescent prostate fibroblasts. (A) Transcript levels of indicated SASP factors were quantified by qRT-PCR in NS and Sen (IR) PSC27 fibroblasts with or without treatment with rapamycin for 7 days. (B) NF-κB activity was measured after treatment with rapamycin for 7 days using a reporter assay. For all panels, shown is one representative of three independent experiments, each with triplicate samples. Raw data could be found in Supplementary Table 4.



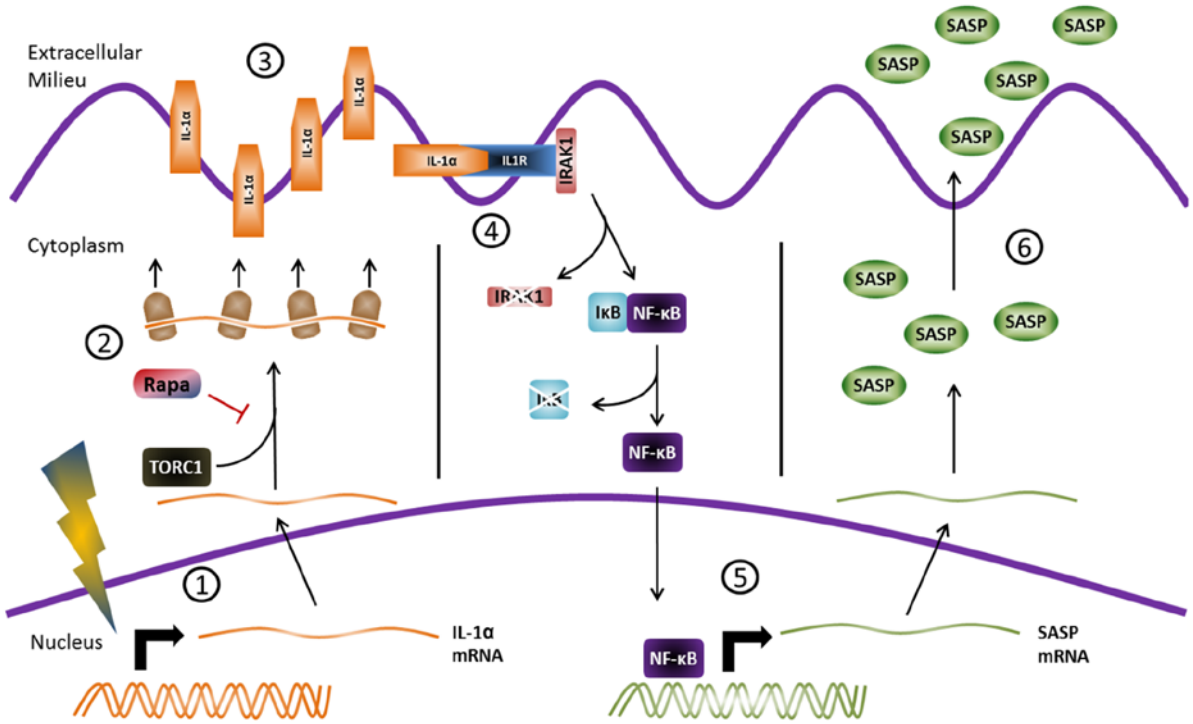
Supplementary Figure 4 FACS analysis of cell surface IL-1α. (A) Flow cytometry for cell surface IL-1α was performed using NS or senescent (IR) HCA2 cells expressing shRNAs against GFP or raptor and treated with DMSO or rapamycin and a FITC-tagged antibody. Slope of a trend-line of fluorescence over forward scattering (FSC) was determined to discriminate fluorescence intensity from the effect of cell size (shown is the result of one of two independent experiments, 10,000 cells were counter before gating). (B) HCA2 cells were infected with a lentivirus expressing shRNA against IL-1α and the relative IL-1α mRNA level was measured. Shown is one representative of two independent experiments, each with triplicate samples. Raw data could be found in Supplementary Table 4.



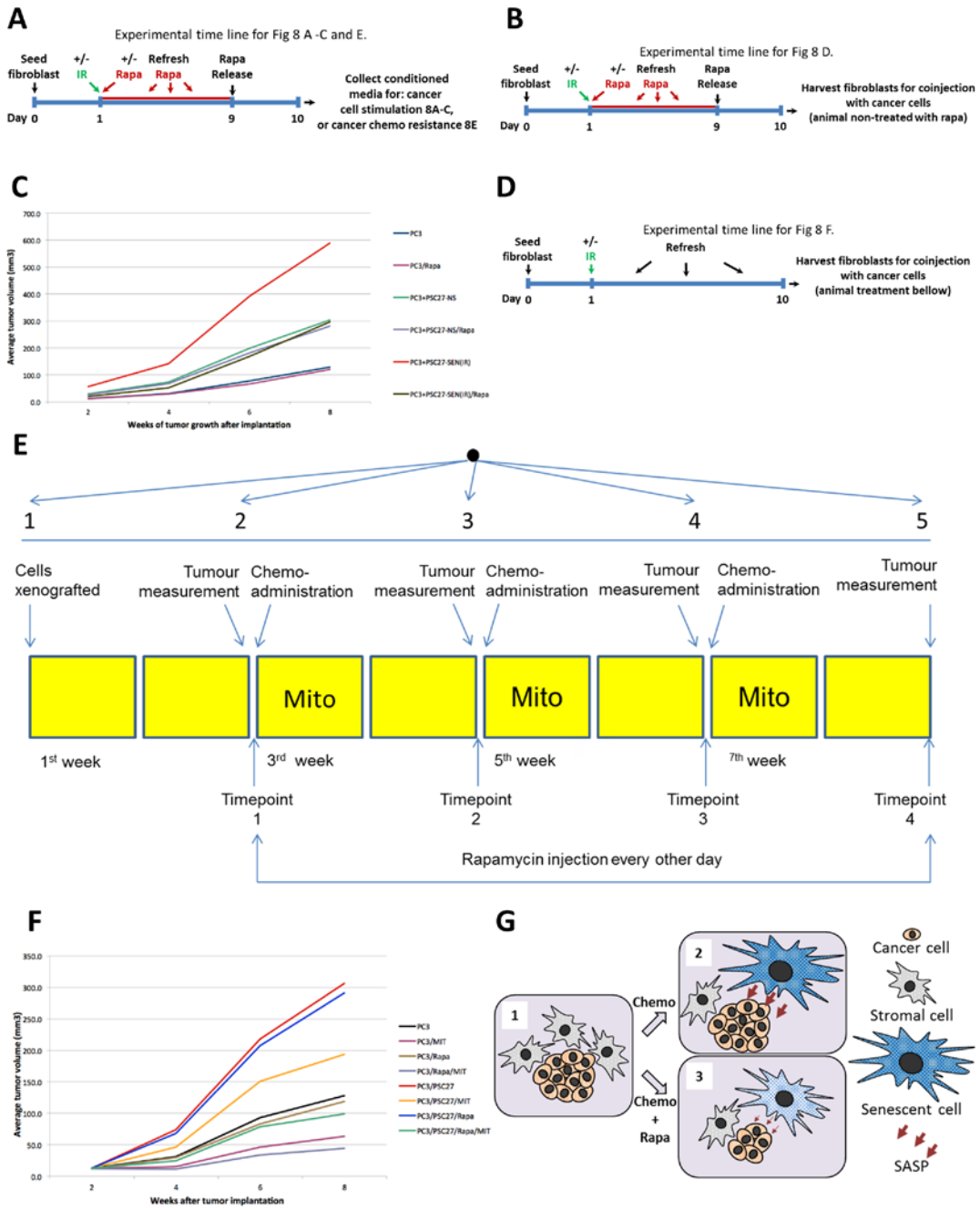
Supplementary Figure 5 Translation status of various transcripts after rapamycin treatment. (A) After IR, senescent (Sen (IR)) HCA2 cells were treated for 1 day with rapamycin or DMSO followed by 1 day in serum-free media, after which cells were harvested and mRNA collected for polysome profiling. qPCR was performed on each fraction for IL1A, IL1B, IL6, IL8, IL3, IL5, TIMP1, CCL13 and TUBA1A mRNA (one representative experiment is shown). Fractions 1-7: Free RNA; 8-12: 40-60S; 13-20: polysomes. (B) NS or senescent (Sen (IR)) HCA2 cells were cultured for 7 days and then treated with DMSO or rapamycin for 4 hours, after which cells were harvested and mRNA collected for polysome profiling. qPCR was performed on each fraction for IL1A, TUBA1A, IL6, and EEF2 mRNA (one representative experiment is shown). Fractions 1-4: Free RNA; 5-6: 40-60S; 7-11: polysomes. Right panel: polysome profiles used to determine the translated fractions. All polysome profile data are based on at least two independent replicates; representative polysome traces are shown. Raw data could be found in Supplementary Table 4.



Supplementary Figure 6 mTORC1 inhibition does not reverse the senescence growth arrest. (A) Effect of rapamycin on the number of senescent cells with detectable senescence-associated β-gal (SA-β-gal) activity. (B) Proliferative potential of PSC27 fibroblasts was measured under the indicated culture conditions. (C) PSC27 cells, NS or made senescent by IR and treated with DMSO or rapamycin for 6 days, were pulsed with BrdU for 24 hours and the fraction that incorporated BrdU was determined by fluorescence microscopy. (D) SA-β-gal expression was determined for the cell populations described in Figure I (one representative experiment is shown). (E) Clonogenic assays were performed on HCA2 cells to compare the effects of rapamycin and DMSO on NS cells or cells irradiated at 6, 8 or 10 Gy (one representative experiment is shown). (F) BJ and HCA2 human fibroblasts were irradiated at 5 or 10 Gy and clonogenic assays performed in the presence of DMSO or rapamycin (one representative experiment is shown). For panels A, B and C, shown is one representative of three independent experiments, each with triplicate cell culture samples. For panel D, E and F, shown is one clonogenic assay experiment replicated once. Raw data could be found in Supplementary Table 4.

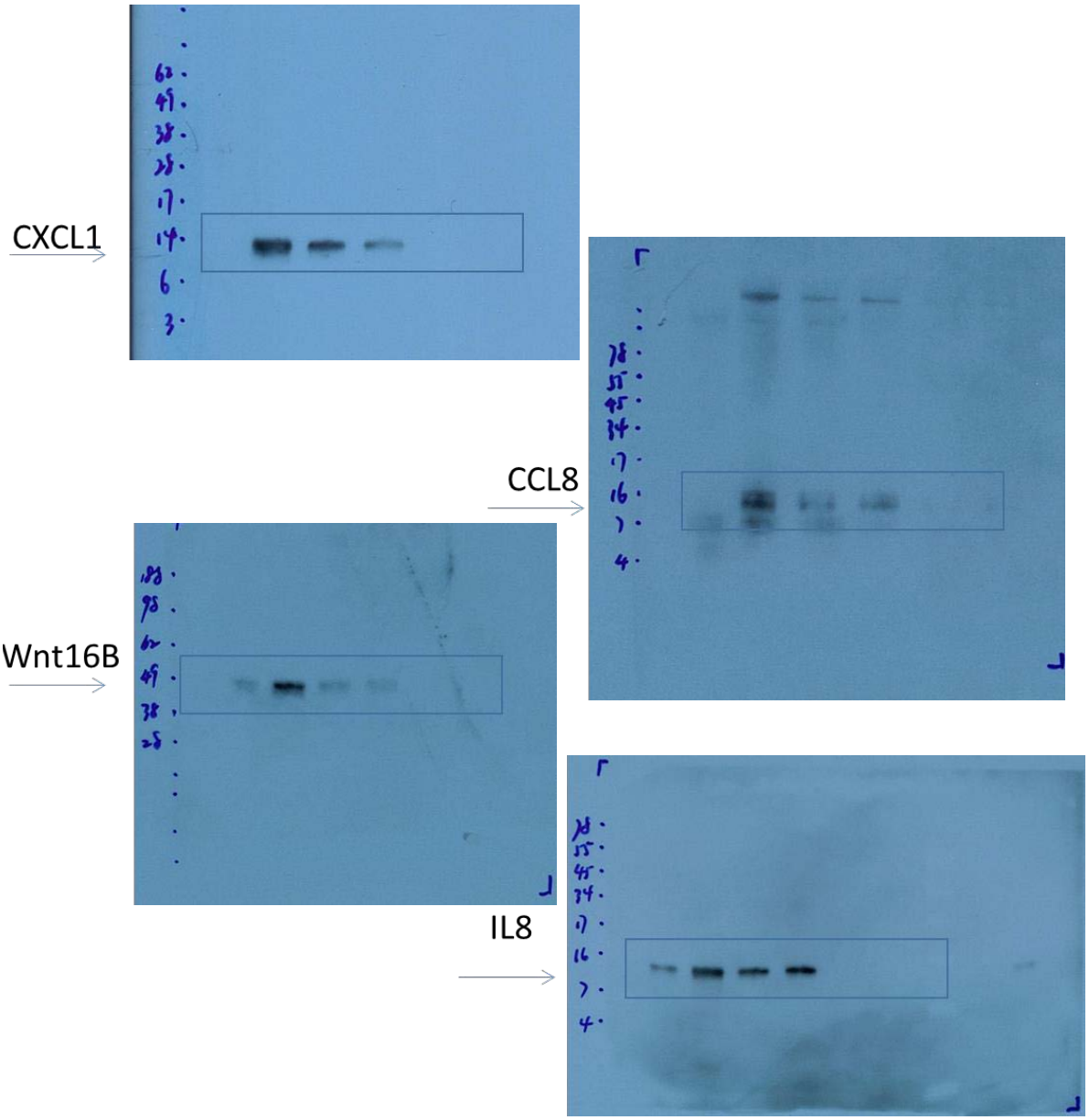


Supplementary Figure 7 Model for SASP suppression by rapamycin. 1) Senescence signals activate IL-1α transcription; 2) IL-1α translation is mTOR-dependent and sensitive to rapamycin; 3) IL-1α at the plasma membrane binds the IL1R; 4) IL1R occupancy activates IL1R signaling; 5) IL1R signaling releases IκB, allowing NF-κB translocation to the nucleus, where it activates the transcription of genes encoding SASP factors; 6) SASP factors are transcribed, translated and secreted.



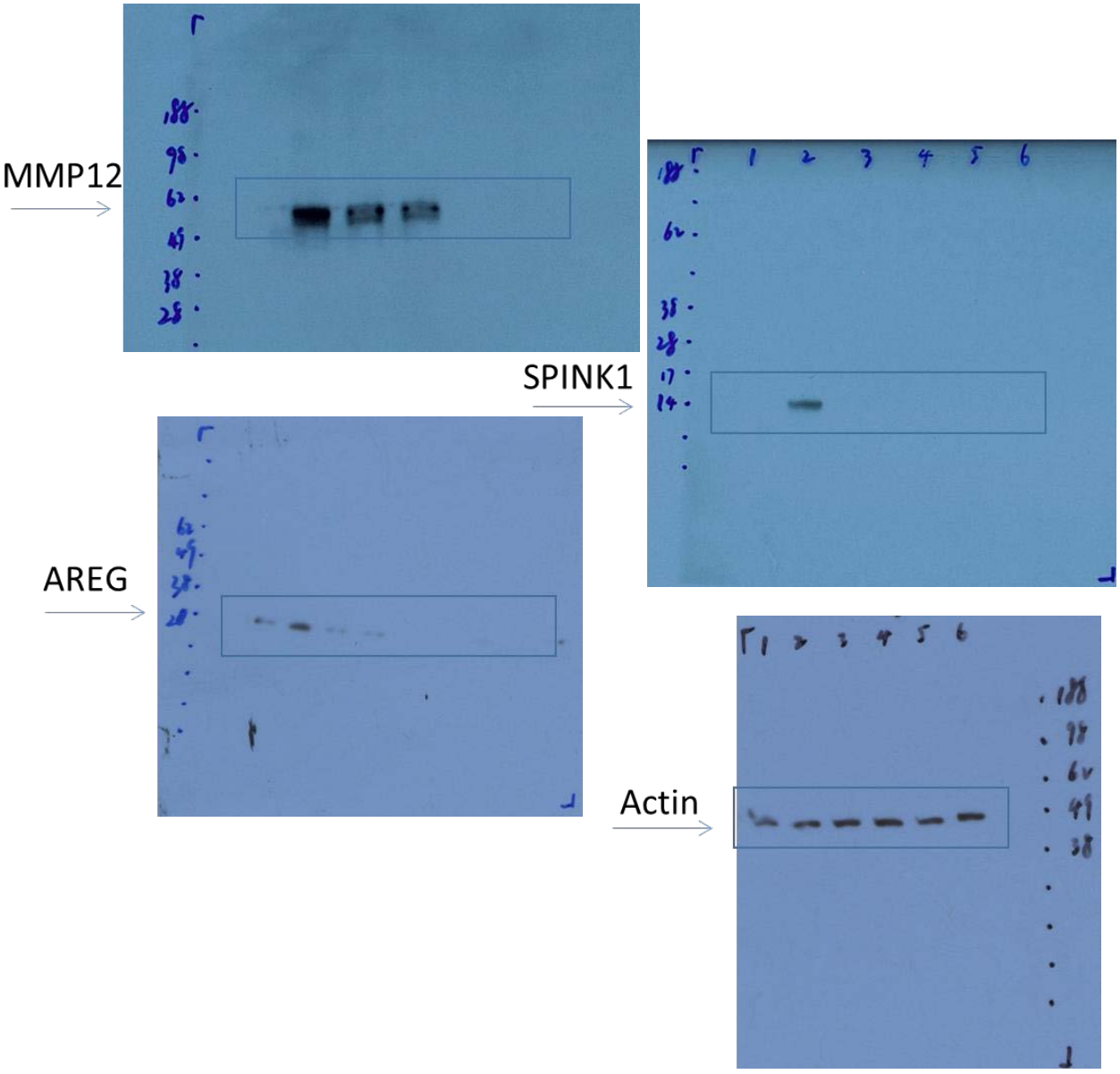
Supplementary Figure 8 Rapamycin suppresses tumour cell growth. (A) Experimental timeline corresponding to Fig. 8A-C and E. (B) Experimental timeline corresponding to Fig. 8D. (C) PC3 prostate tumour cells were implanted subcutaneously with or without PSC27 prostate fibroblasts, and tumour sizes were measured every 2 weeks. PC3 cells were either exposed to DMSO or rapamycin *ex vivo* prior to implantation. PSC27 cells were exposed to ionizing radiation (IR), rapamycin (Rapa) or both *ex vivo* prior to implantation (n=8 per type of treatment). (D) Timeline corresponding to the cell culture experiment presented in Fig. 8F. (E) Timeline corresponding to the *in vivo* experiment presented in Fig. 8F. Scheduled timing of mitoxantrone given as 3 doses 2 weeks apart, and rapamycin given every 2 days, to SCID mice over the course of an 8 week regimen. The mice were engrafted with PC3 cells alone, or combined with either PSC27-NS (control fibroblasts, without pre-treatment) or PSC27-Sen (IR) (fibroblasts pretreated with IR in culture). At the end of the treatment period, tumours were excised and volumes were determined, with 8-10 mice used per treatment arm. (F) PC3 prostate tumour cells were implanted subcutaneously with or without PSC27 prostate fibroblasts. After 2 weeks of tumour growth, mice were treated with vehicle (control), rapamycin (Rapa) and/or mitoxantrone (MIT). Tumour sizes were measured every 2 weeks (n=10 per type of treatment). (G) 1) Tumour cells (orange) are surrounded by stromal cells (grey); 2) Treatment with DNA-damaging chemotherapy induces senescence in the stroma (blue cell). The SASP (red arrows) from these senescent cells fuels the proliferation of the remaining tumour cells; 3) Rapamycin reduces the intensity of the SASPs induced by chemotherapy, and tumour cell proliferation is decreased.

Uncropped westerns for Fig 2D

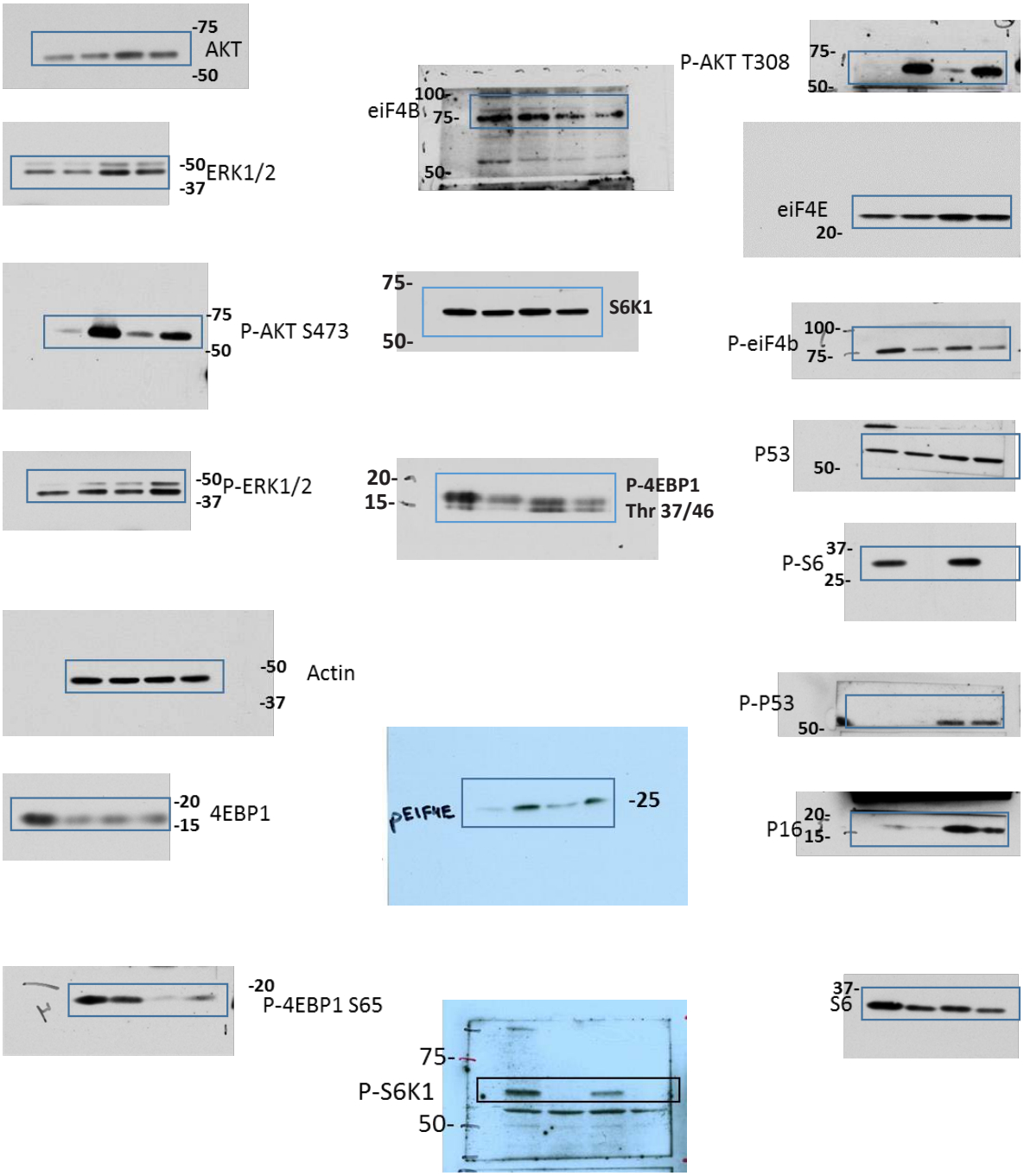


Supplementary Figure 9 Unprocessed western blots from experiments displayed in Figures 2D, 2E, 4C and 7C.

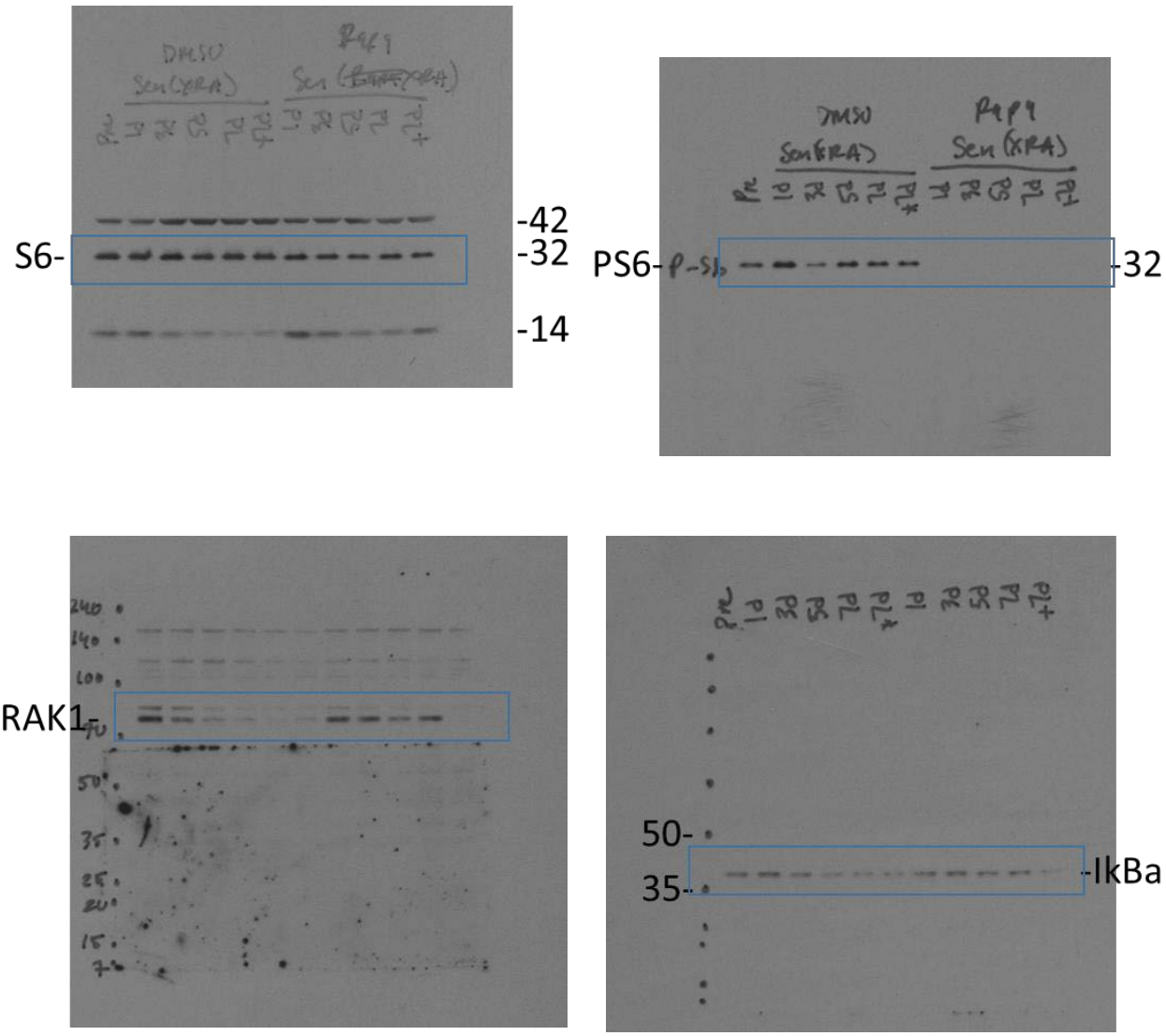
Uncropped westerns for Fig 2D



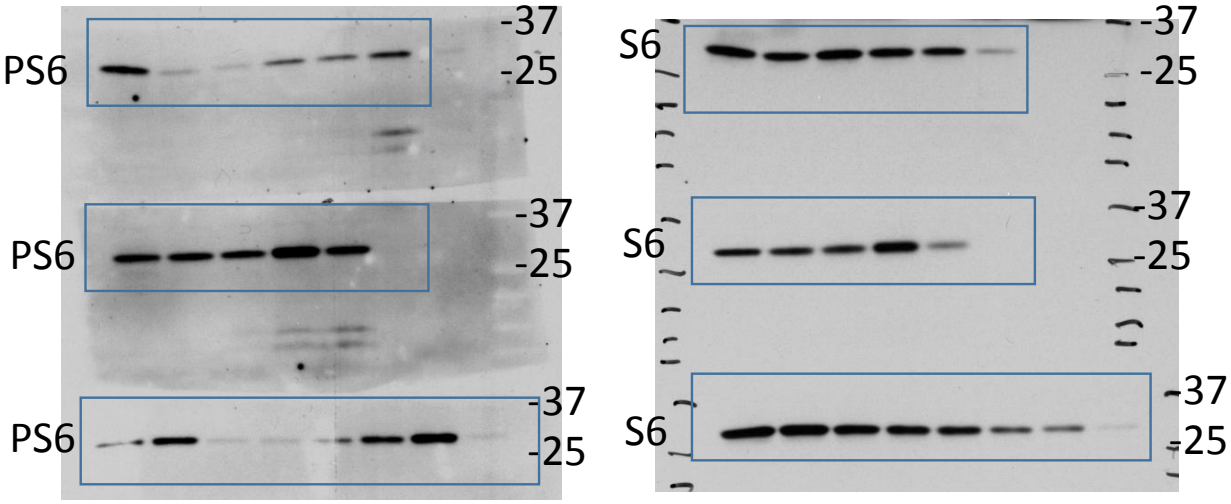
Uncropped westerns for Fig 2E



Uncropped westerns for figure 4C



Uncropped westerns for figure 7C



Target	Figure	Source	Cat#	Clone #	Dilution
IκBα	Figure 4 C	Cell Signaling	9242	Polyclonal	1:250
p70 S6 Kinase	Figure 2 E	Cell Signaling	2708	Polyclonal	1:500
Phospho-p70 S6 Kinase (Thr389)	Figure 2 E	Cell Signaling	9234	Polyclonal	1:1000
Phospho-Akt (Thr308)	Figure 2 E	Cell Signaling	2965	Polyclonal	1:1000
Phospho-Akt (Ser473)	Figure 2 E	Cell Signaling	4060	Polyclonal	1:1000
Akt (pan)	Figure 2 E	Cell Signaling	4691	Polyclonal	1:1000
Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204)	Figure 2 E	Cell Signaling	9101	Polyclonal	1:1000
p44/42 MAPK (Erk1/2)	Figure 2 E	Cell Signaling	4695	Polyclonal	1:1000
Phospho-eIF4B (Ser422)	Figure 2 E	Cell Signaling	3591	Polyclonal	1:500
eIF4B	Figure 2 E	Cell Signaling	3592	Polyclonal	1:500
Phospho-eIF4E (Ser209)	Figure 2 E	Cell Signaling	9741	Polyclonal	1:500
eIF4E	Figure 2 E	Cell Signaling	9742	Polyclonal	1:500
Phospho-S6 Ribosomal Protein (Ser235/236)	Figure 2E, 4C and 7C	Cell Signaling	4858	Polyclonal	1:1000
S6 Ribosomal Protein	Figure 2E, 4C and 7C	Cell Signaling	2217	Polyclonal	1:1000
Phospho-4E-BP1 (Thr37/46)	Figure 2 E	Cell Signaling	2855	Polyclonal	1:500
Phospho-4E-BP1 (Ser65)	Figure 2 E	Cell Signaling	9451	Polyclonal	1:500
4E-BP1	Figure 2 E	Cell Signaling	9644	Polyclonal	1:500
Phospho-p53 (Ser15)	Figure 2 E	Cell Signaling	9284	Polyclonal	1:500
p53	Figure 2 E	Oncogene	OP29	Ab-3	1:1000
p16	Figure 2 E	Santa Cruz Biotechnology	56330	JC8	1:500
β-Actin	Figure 2 E	Sigma-Aldrich	A2228	AC-74	1:5000
IRAK-1	Figure 4 C	Santa Cruz Biotechnology	5288	F4	1:500
CXCL1	Figure 2 D	R & D Systems	MAB275	Clone 20326	1:500
CCL8	Figure 2 D	R & D Systems	AF-281-NA	Polyclonal	1:400
WNT16B	Figure 2 D	BD Pharmingen	552595	clone F4-1582	1:1000
IL8	Figure 2 D	Epitomics	1856-1	Clone EP117Y	1:300
MMP-12	Figure 2 D	Santa Cruz Biotechnology	sc-30072	polyclonal	1:500
SPINK1	Figure 2 D	Abnova	H00006690-M01	clone 4D4	1:250
AREG	Figure 2 D	Abcam	ab33558	polyclonal	1:500
Actin	Figure 2 D	Santa Cruz Biotechnology	SC-1616	polyclonal	1:1000

Supplementary Table 1 shRNA list.

Product	id	Figure	Source and cat#	Antisense Sequence 5'-3'
shGFP	NA	various figures	Open Biosystems #RHS4459	NA
shRaptor	3	Figure 2A and S2A	Open Biosystems #TRCN0000332954	TACACGGAGATGTAGTAGTCG
shRaptor	4	Figure 2A and S2A	Open Biosystems #TRCN0000221531	ATACGACCTCATAATCCTTTC
shRaptor	5	Figure 2A, 4A, 6F, 6G, S2A, S6D, S6E, S6F, S6G	Open Biosystems #TRCN0000332886	ATTGTAGTGAAAGAGGACTCG
shmTOR	2	Figure 2A and S2B	Open Biosystems #TRCN0000038675	AATTCTCCTATTGTTGCCAGG
shmTOR	4	Figure 2A and S2B	Open Biosystems #TRCN0000038677	AAAGAATGAATAGATTCTGGC
shmTOR	6	Figure 2A and S2B	Open Biosystems #TRCN0000221542	AATATATTCTTCAACAGCAGC
siScrmbl	NA	Figure 2 C, D	Open Biosystems / NA	ACTACCGTTGTATAGGTGTT
siS6K	NA	Figure 2 C, D	Cell Signaling #6568	CTCAGTGAGAGTGCCAACCAA
shIL1A	NA	Figure 4B, 6F, S4A, S4B, S6D	Open Biosystems #TRCN0000059208	GCCCTCAATCAAAGTATAATT
shIL-6	1	Figure 6F, 6G, S6D	NA	TGATCCAGTTCCTGCAGAA
shIL-6	2	Figure 6F, 6G, S6D	NA	GAACTTATGTTGTTCTCTA
shRelA	NA	Figure 6F, 6G, S6D	Open Biosystems #TRCN0000014687	TAGGCGAGTTATAGCCTCAGG

Supplementary Table 2 Antibodies used for western blotting.

target	Figure	UPL Probe#	Forward primer 5'-3'	Reverse primer 5'-3'
IL6	Figure 3A, 3B, 5A, S5A, S5B, S6D	45	GCCCAGCTATGAACTCCTTCT	GAAGGCAGCAGGCAACAC
IL8	Figure 3A, 3B, 5A, S5A	72	AGACAGCAGAGCACACAAGC	ATGGTTCCTTCCGGTGGT
IL1A	Figure 3A, 3B, 5A, S5A, S5B	6	GGTTGAGTTTAAGCCAATCCA	TGCTGACCTAGGCTTGATGA
IL1B	Figure 3A, 3B, 5A, S5A	78	CTGTCCTGCGTGTTGAAAGA	TTGGGTAATTTTTGGGATCTACA
CCL13	Figure 3A, 3B, 5A, S5A	40	ACCTTCAACATGAAAGTCTCTGC	GGACGTTGAGTGCATCTGG
TIMP1	Figure 3A, 3B, 5A, S5A	76	GGGCTTCACCAAGACCTACA	TGCAGGGGATGGATAAACA
IL3	Figure 3A, 3B, 5A, S5A	60	TTGCCTTTGCTGGACTTCA	CTGTTGAATGCCTCCAGGTT
IL5	Figure 3A, 3B, 5A, S5A	25	GGTTTGTTGCAGCCAAAGAT	TCTTGGCCCTCATTCTCACT
CXCL1	Figure 3A	52	TCCTGCATCCCCCATAGTTA	CTTCAGGAACAGCCACCAGT
CXCL2	Figure 3A	69	CCCATGGTTAAGAAAATCATCG	CTTCAGGAACAGCCACCAAT
CCL2	Figure 3A	40	AGTTCTTGCCGCCCTTCT	GTGACTGGGGCATTGATTG
TUBA1A	Figure 3A, 3B, 5A, S5A, S5B	58	CTTCGTCTCCGCCATCAG	TTGCCAATCTGGACACCA
mTOR	Figure S2 B	53	TCATCAAACAAGCGACATCC	GGGCCTCCAGTTACCAGAA
Raptor	Figure S2 A	45	AGACACACCTGGCCCTCA	TGTCCTGCCTTGTCACGTC
EEF2	Figure S5B	25	CTGGAGATCTGCCTGAAGGA	GAGACGACCGGGTCAGATT
IL6	Figure S3A	NA	TACCCCCAGGAGAAGATTCC	TTTTCTGCCAGTGCCTCTTT
IL8	Figure S3A	NA	GTGCAGTTTTGCCAAGGAGT	CTCTGCACCCAGTTTTCCTT
IL1A	Figure S3A	NA	AATGACGCCCTCAATCAAAG	TGGGTATCTCAGGCATCTCC
IL1B	Figure S3A	NA	GGGCCTCAAGGAAAAGAATC	TTCTGCTTGAGAGGTGCTGA
IL-1R1	Figure S3A	NA	ATTGATGTTCGTCCCTGTCC	CCTCCACCTTAGCAGGAACA
MMP3	Figure S3A	NA	GCAGTTTGCTCAGCCTATCC	GAGTGTCGGAGTCCAGCTTC
IL7	Figure S3A	NA	CGCAAGTTGAGGCAATTTCT	CTCTTTGTTGGTTGGGCTTC
CXCL1	Figure S3A	NA	AGGGAATTCACCCCAAGAAC	TGGATTTGTCACTGTTCAGCA
CCL8	Figure S3A	NA	TCACCTGCTGCTTTAACGTG	ATCCCTGACCCATCTCTCCT
GM-CSF	Figure S3A	NA	ATGTGAATGCCATCCAGGAG	AGGGCAGTGCTGCTTGTAGT
RPL13A	Figure S3A	NA	GTACGCTGTGAAGGCATCAA	CGCTTTTTCTTGTCGTAGGG

Supplementary Table 3 Primers and probes list.

Supplementary Table 4 Statistics source data.