Supplementary Information

Senescent Cells in 3D Culture Show Suppressed Senescence Signatures

Parul Yadav¹, Kaushik Chatterjee^{1,2*} and Deepak Kumar Saini^{1,3*}

¹Centre for BioSystems Science and Engineering, Indian Institute of Science, C.V Raman Avenue, Bangalore, India 560012

²Department of Materials Engineering, Indian Institute of Science, C.V Raman Avenue, Bangalore, India 560012

³Department of Molecular Reproduction, Development and Genetics, C.V Raman Avenue, Indian Institute of Science, Bangalore, India 560012



Supplementary figure S1: (A) Viability assessment using live stained by Calcein-AM (green) and dead stained by EthD-1 (red) staining of senescent and non-senescent cells in the scaffold. Scale bars represent 10 μ m. (B) Live cell imaging videos of non-senescent and senescent GFP- HeLa cells in scaffold. Scale bars represent 10 μ m. (C) PCNA gene expression analysis for senescent and non-senescent cells in 3D scaffolds. Unpaired t test was performed. ** indicates p<0.01.

Table S1: Details of antibody

S. No.	Antibody	Company	Dilution
1	Alexa Fluor 488 Phalloidin	Invitrogen A12379	1: 400
2	Alexa Fluor 546 Phalloidin	Invitrogen A22283	1: 400
3	Vimentin (D21H3) Rabbit mAb	CST 5741	1: 100
4	Goat anti-Rabbit IgG Secondary Antibody, Alexa Fluor 488	Invitrogen A-11034	1:500

Table S2: Details of primers

S. No.	Gene	Sequence
1	P21 FW	5'-GGAAGACCATGTGGACCTGT -3'
2	P21 RW	5'-TAGGGCTTCCTCTTGGAGAA -3'
3	HIF1a FW	5'- GAAGTGGCAACTGATGAGCA -3'
4	HIF1a RW	5'-GCGCGAACGACAAGAAA -3'
5	IL6 FW	5'- AGACAGCCACTCACCTCTTCAG -3'
6	IL6 RW	5'- TTCTGCCAGTGCCTCTTTGCTG -3'
7	IL8 FW	5'- GAGAGTGATTGAGAGTGGACCAC -3'
8	IL8 RW	5'- CACAACCCTCTGCACCCAGTTT -3'
9	NFκB FW	5'- GCAGCACTACTTCTTGACCACC -3'
10	NFκB RW	5'- TCTGCTCCTGAGCATTGACGTC -3'
11	PCNA FW	5'-AGGAGGAAGCTGTTACCATAGAG -3'
12	PCNA RW	5'-AAGTGTCCCATATCCGCAAT -3'
13	COL1A1 FW	5'-GATTCCCTGGACCTAAAGGTGC-3'
14	COL1A1 RW	5'-AGCCTCTCCATCTTTGCCAGCA-3'
15	FN1 FW	5'-ACAACACCGAGGTGACTGAGAC-3'
16	FN1 RW	5'-GGACACAACGATGCTTCCTGAG-3'
17	GAPDH FW	5'- GAGTCAACGGATTTGGTCGT -3'
18	GAPDH RW	5'- GACAAGCTTCCCGTTCTCAG -3'

Video files: Live-cell imaging videos of non-senescent (video V1) and senescent (video V2) GFP-HeLa cells in the scaffold. Imaging was performed for 24 h after three days of seeding. Scale bars represent $10 \mu m$.