

Supplementary Materials

1'-O-methyl-averantin isolated from the endolichenic fungus *Jackrogersella* sp. EL001672 suppresses colorectal cancer stemness via sonic hedgehog and Notch signaling

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Supplementary Materials:

Supplementary Table 1.

ITS sequence of *Jackrogersella multiformis* EL001672

Supplementary Table 2.

Primer list for q-RT-PCR

Supplementary Figure 1-3.

Bioactivity-guided fractionation of a crude extract from EL001672

Supplementary Figure 4.

Viability of HEK293T cells treated with 1'-*O*-methyl-averantin

Supplementary Figure 5-9.

The NMR datas of 1'-*O*-methyl-averantin

Supplementary Figure 10-11.

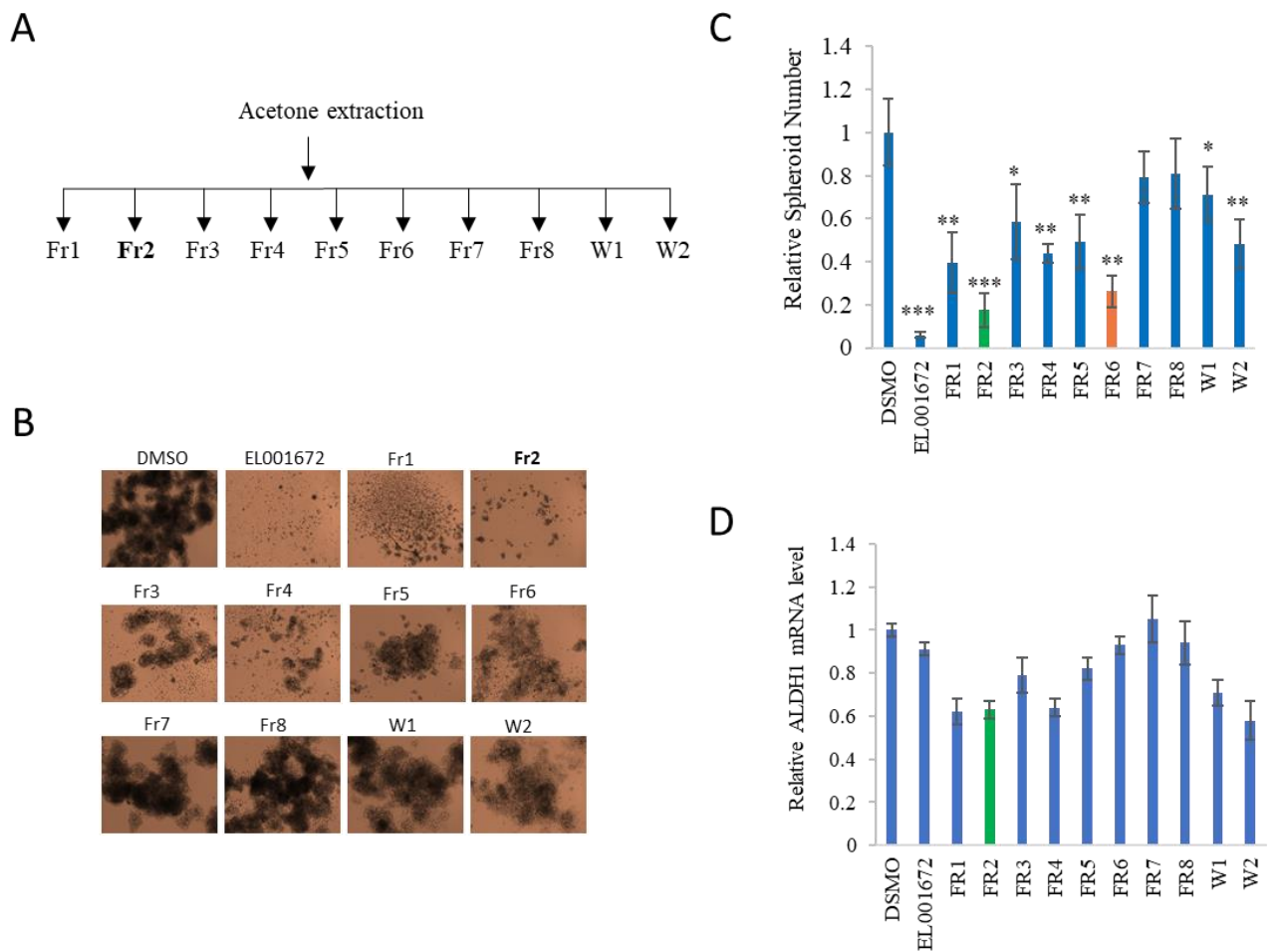
Full-length blots shown in Figure 3 and Figure 4.

Supplementary Table 1. ITS sequence of *Jackrogersella multiformis* EL001672

Internal transcribed spacer sequence
TCAAAGGAACCAAGAATTTTTTTTGGATTTTTTATTTTGGTCTTACTTTTTTGGT GGTGGGGCCTTGTTTTCTTTTTTTTTTTGTTTCCTTTCCCGGTTTTGATATGCTT AAGTTCAGCGGGTATTCTACCTAATCCGAGGTCAACCACTAGAAATATAGGG GGTTTTACGGCAGGCAGCTAAGGCGACTGCAGAAGCGAGAAAAGATTTACTAC GCTCAGAGTACGCCATAACTCCGCCACTGACTTTGAGGAGCTACGCTATAACC GTAGGCTCCCAACGCTAAGCTACAGGGCTTAATGGTTCGAAATGACGCTCGAAT AGGCATGCCCACTAGAATACTAATGGGCGCAATGTGCGTTCAAAGATTCGATG ATTCATGAATTCTGCAATTCACATTACTTATCGCATTTTCGCTGCGTTCTTCATC GATGCCAGAACCAAGAGATCCGTTGTTGAAAGTTTTAACTTATTTTTGTTTGGGA GCATTCAGAAATACACTATTTTAAACAGAGTTTAGGGGTCCTCCGGCGGGCCG TGAGCCGGCTACAGGGTAGCCGCCGTTTTACAGGGCGGGCTACAGGGTAGCC GCAGCGAGCGCCGAGGCAACTGGAGGTAAGGTTACATAGGGTTTGGAGTTTA GTTTAACTCTGTAATGATCCCTCCGCTGGTTCACCAACGGAGACCTTGTTACGA CTTTTACTTCTCTAGGAAACCCAAAAAATTGTGGGGGTTAAAAGGAAGGCGA CGCCTACACC

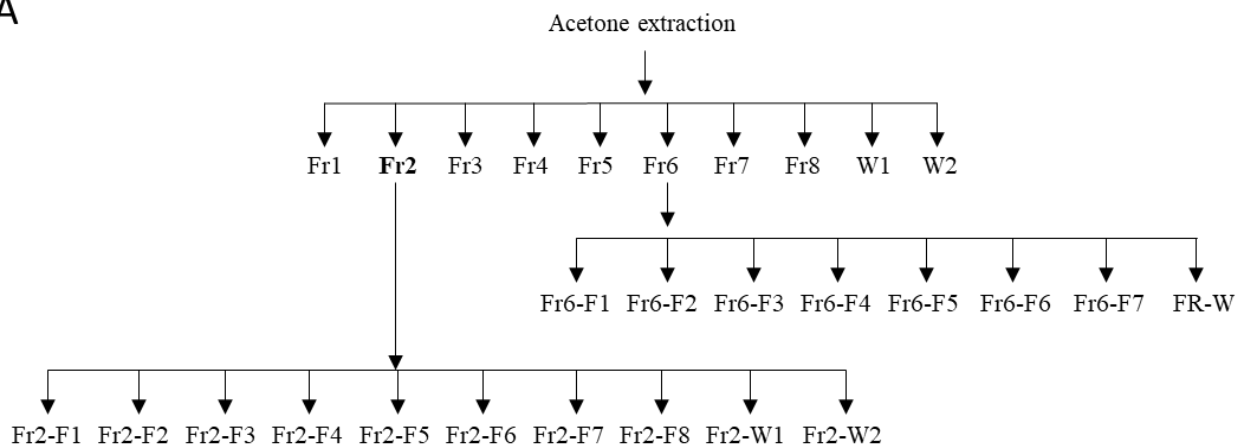
Supplementary Table 2. Primer list for q-RT-PCR

Gene Symbol	Primers sequences	
	For (5'-3')	Rev (5'-3')
GAPDH	ATCACCATCTTCCAGGAGCGA	AGTTGTCATGGATGACCTTGGC
ALDH1	TGTTAGCTCATGCCGACTTG	TTCTTAGCCCGCTCAACACT
CD44	TGCCGCTTTGCAGGTGTAT	GGCCTCCGTCCGAGAGA
CD133	GGACCCATTGGCATTCTC	CAGGACACAGCATAGAATAATC
Lgr-5	CTCTTCCTCAAACCGTCTGC	GATCGGAGGCTAAGCAACTG
Msi-1	ACCAAGAGATCCAGGGGTTT	TCGTTTCGAGTCACCATCTTG
Gli-1	CCATACATGTGTGAGCACGA	GGCACAGTCAGTCTGCTTT
Gli-2	CAACGCCTACTCTCCAGAC	GAGCCTTGATGTACTGTACCAC
SMO	CATCCCTGACTGTGAGATCA	CACCATCTTGGTGACATGCT
Hes-1	CTGAAGAAAGAT AGCTCGCG	ACTTCCCAGCACACTT
Bmi-1	CCA GGGCTTTTCAAAAATGA	CCGATCCAATCTGTTCTGGT
EphB-1	TGCAAGGAGACCTTCAACCT	CGGTGTTGATTTTCATGACG
Notch-1	TCAGCGGGATCCACTGTGAG	ACACAGGCAGGTGAACGAGTTG

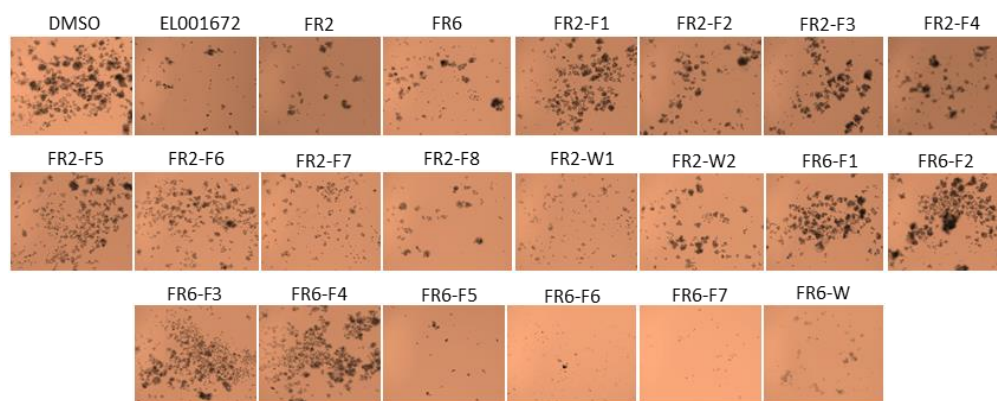


Supplementary Figure 1. (A) Flow chart of the fractionation. (B-C) Representative images of spheroid formation of CSC221 cells treated with fractions for 14 days and quantitative analysis of the number of spheroids following each treatment. (D) Quantitative analysis of mRNA expression levels of cancer stem markers aldehyde dehydrogenase-1 (ALDH1).

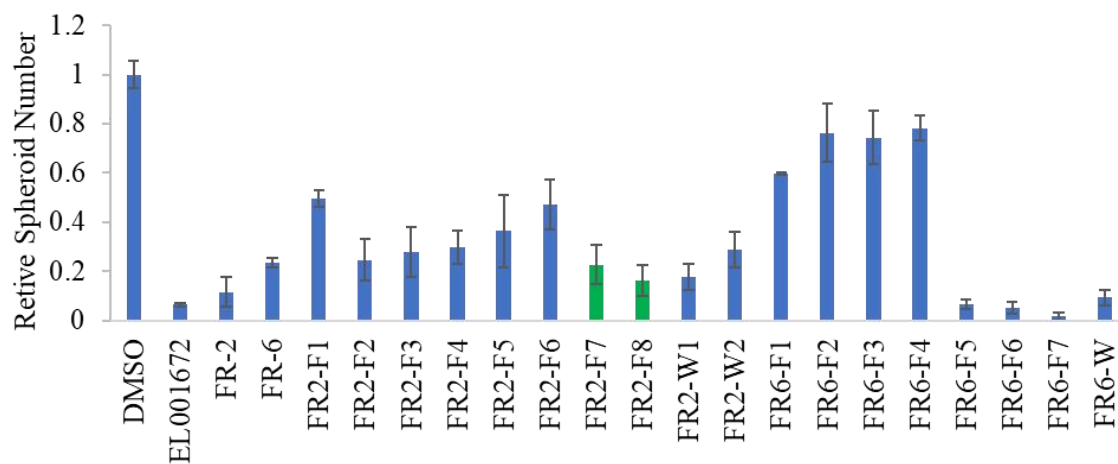
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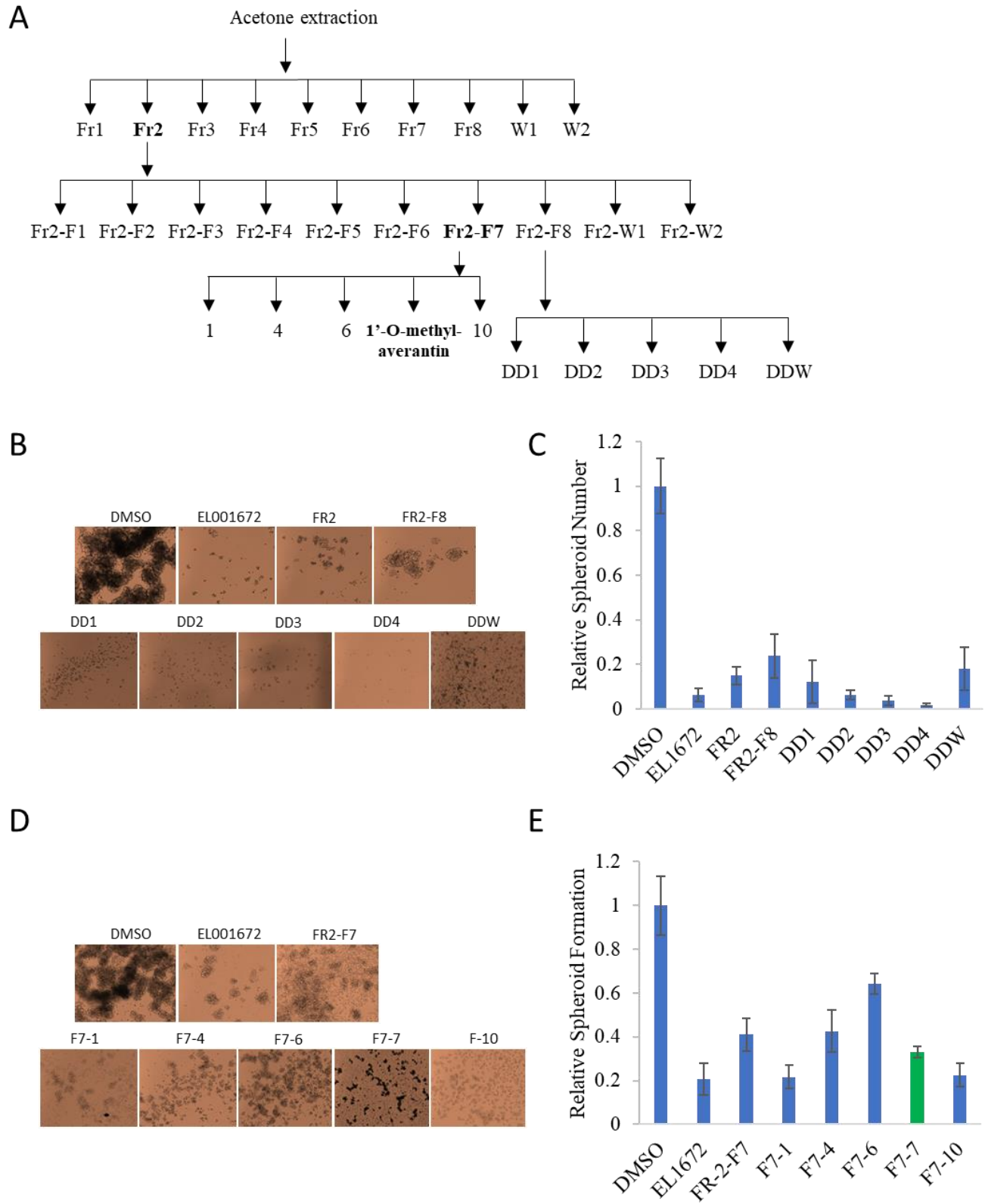
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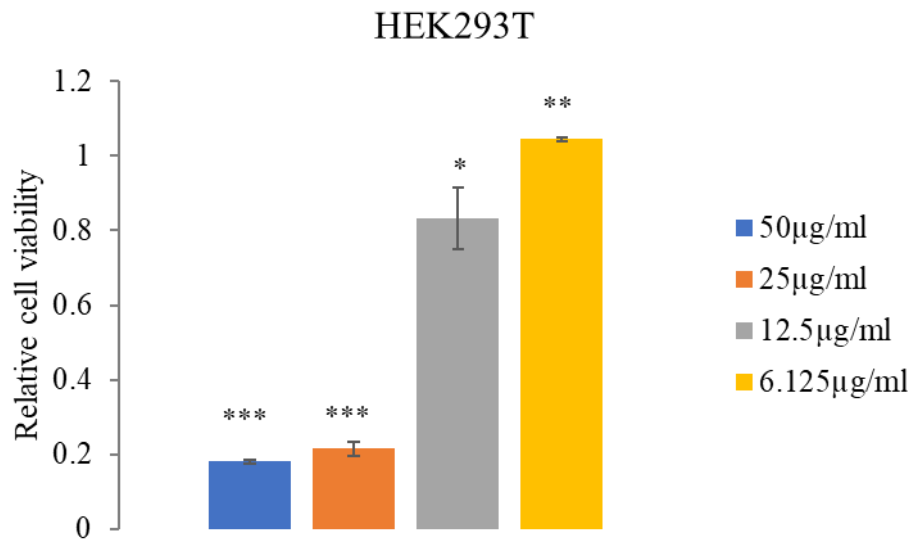
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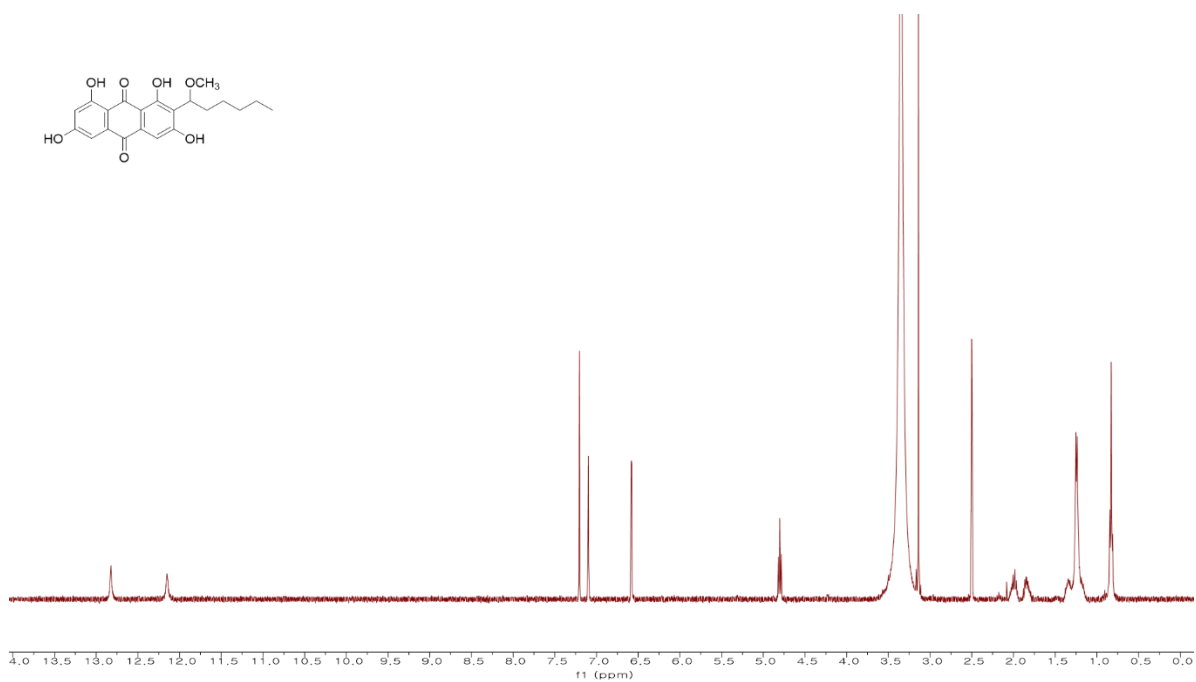
Supplementary Figure 2. (A) Flow chart of the fractionation. (B-C) Representative images of spheroid formation of CSC221 cells treated with fractions for 14 days and quantitative analysis of the number of spheroids following each treatment.



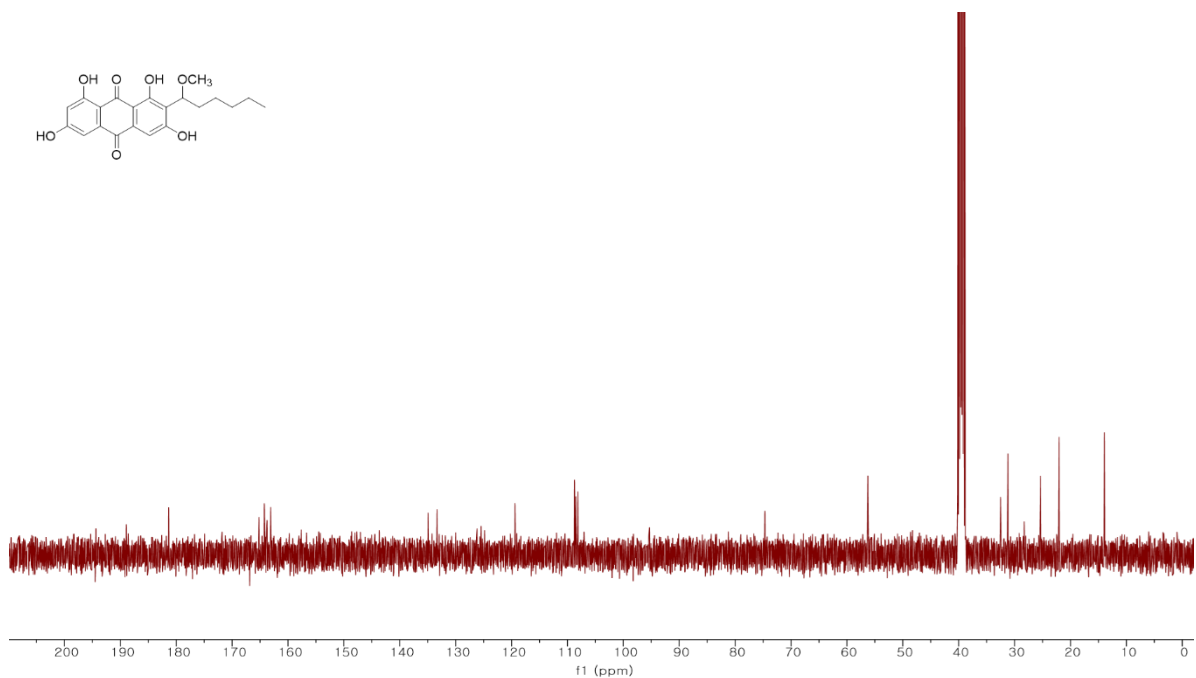
Supplementary Figure 3. (A) Flow chart of the fractionation. (B-C) Representative images of spheroid formation of CSC221 cells treated with fractions for 14 days and quantitative analysis of the number of spheroids following each treatment.



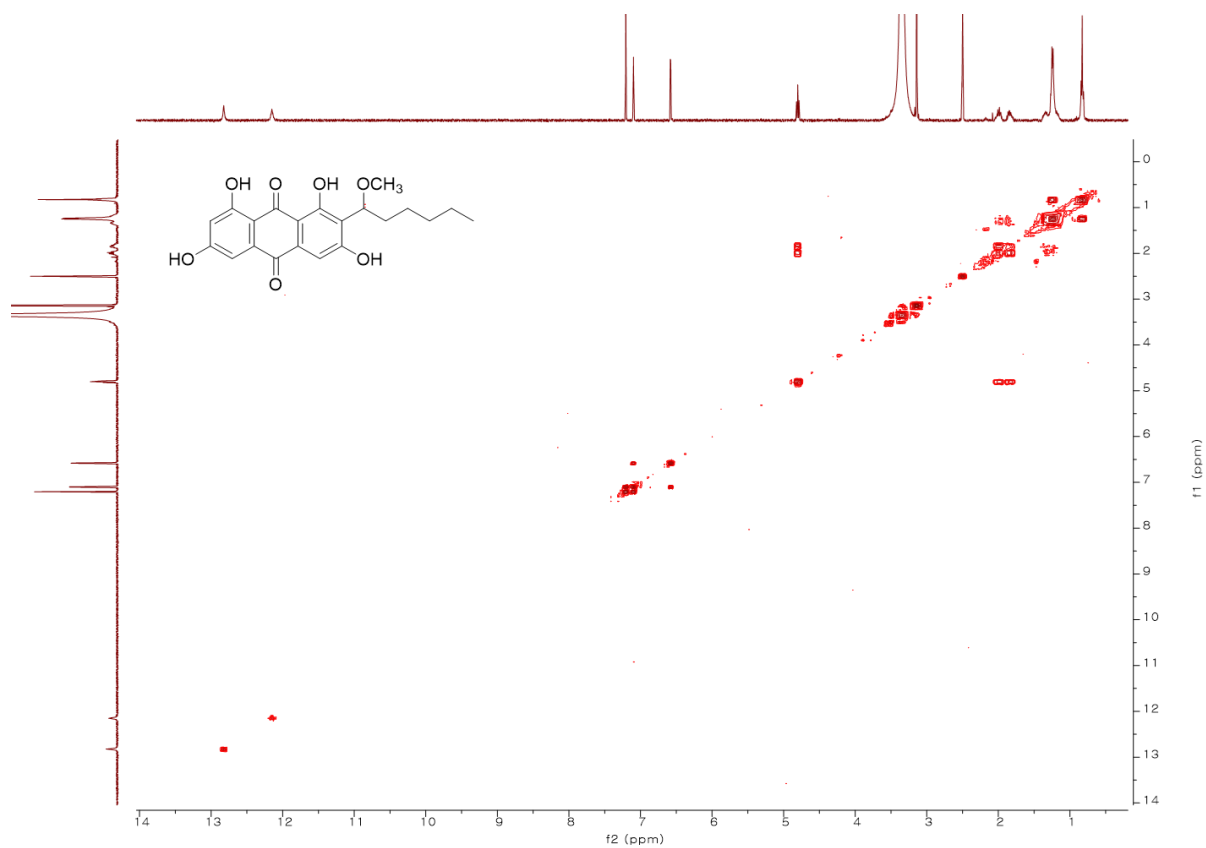
Supplementary Figure 4. Viability of HEK293T cells treated with 1'-*O*-methyl-averantin. DMSO used as a control. 50, 25, 12.5 and 6.125 µg/ml 1'-*O*-methyl averantin represent a concentration of 129.49, 64.74, 32.37 and 16.19 µM, respectively. Data represent the mean ± standard error of the mean, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (compared with DMSO treated cells).



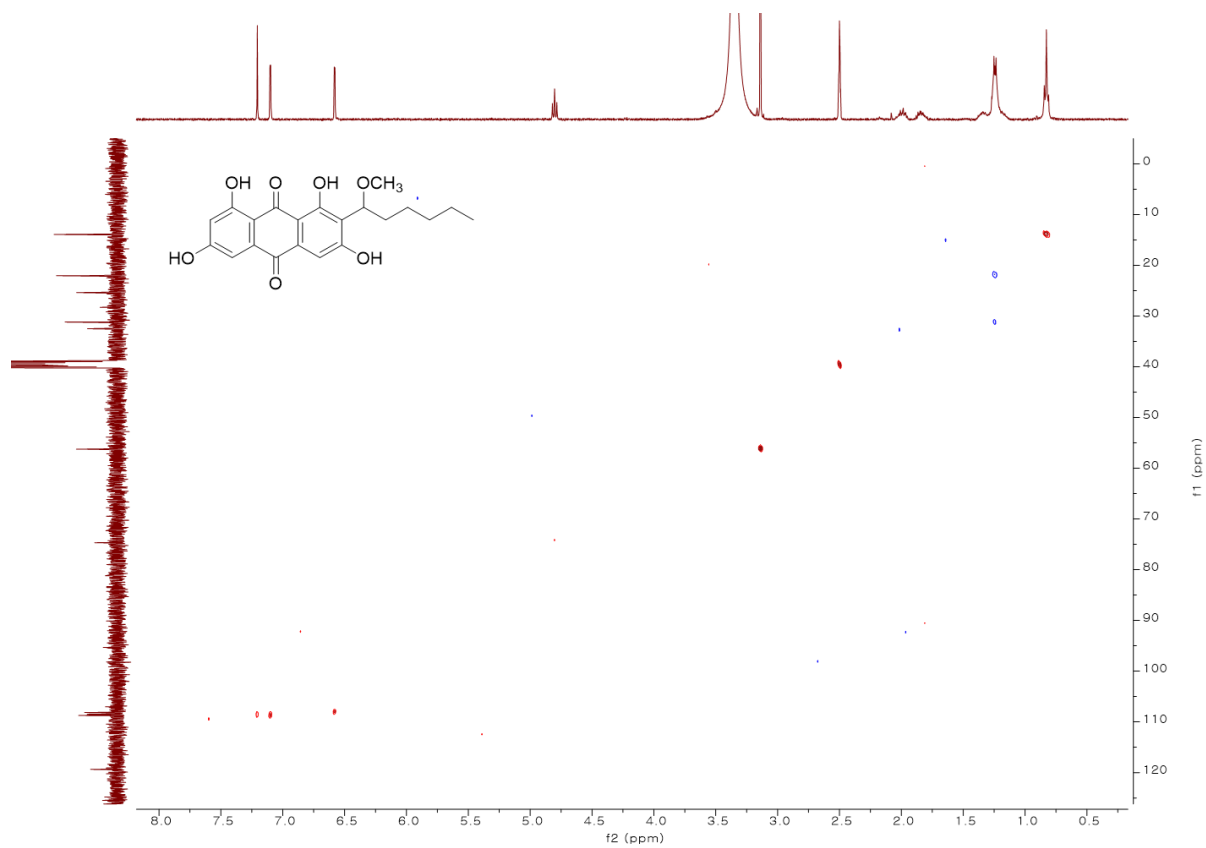
Supplementary Figure 5. ¹H NMR spectrum of 1'-*O*-methyl-averantin in DMSO-*d*₆



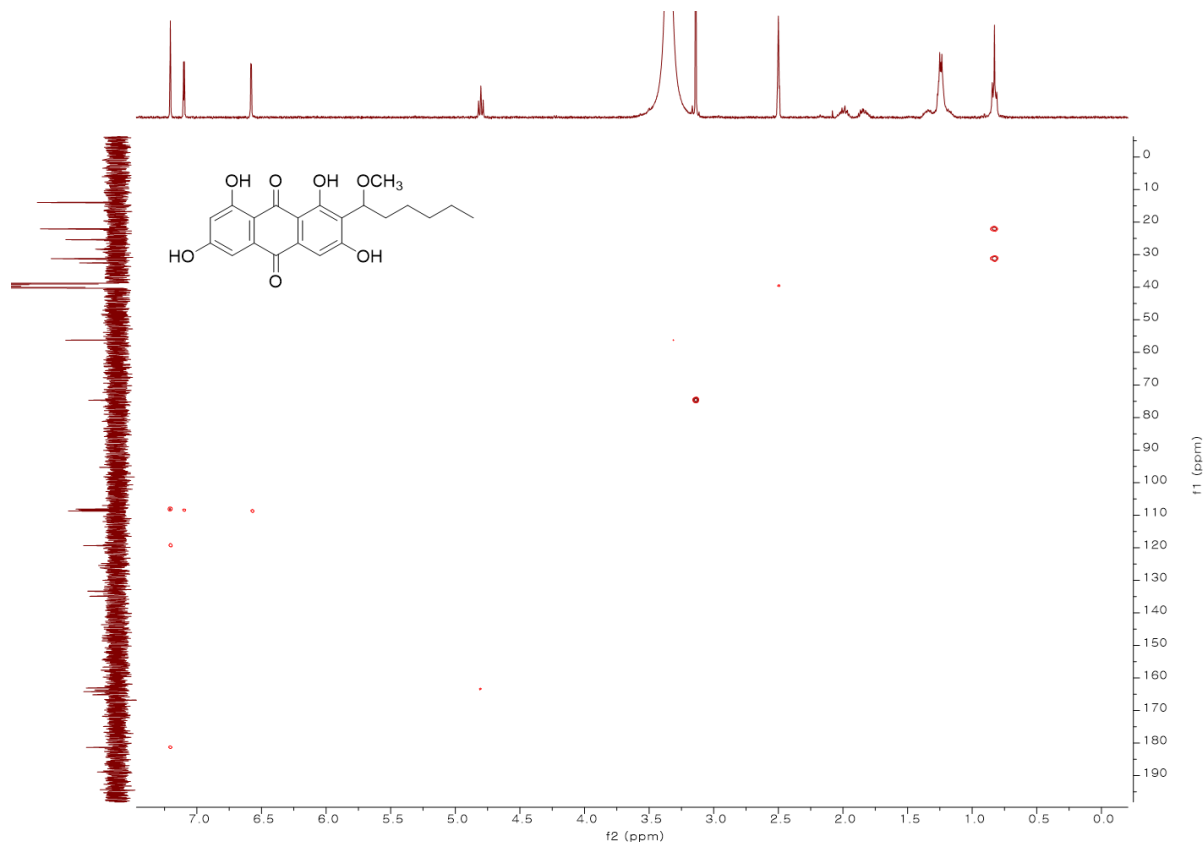
Supplementary Figure 6. ¹³C NMR spectrum of 1'-*O*-methyl-averantin in DMSO-*d*₆



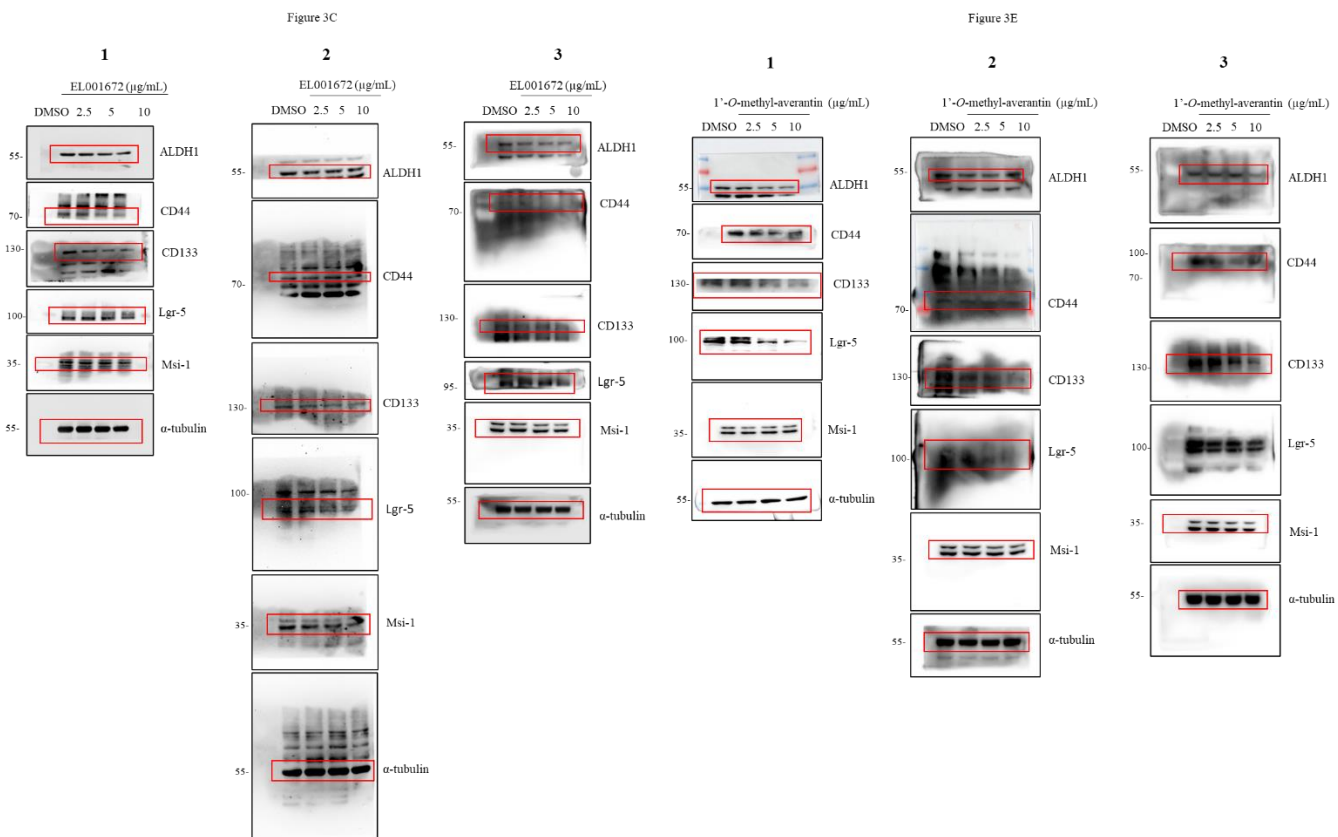
Supplementary Figure 7. COSY spectrum of 1'-O-methyl-laverantin in DMSO-*d*₆



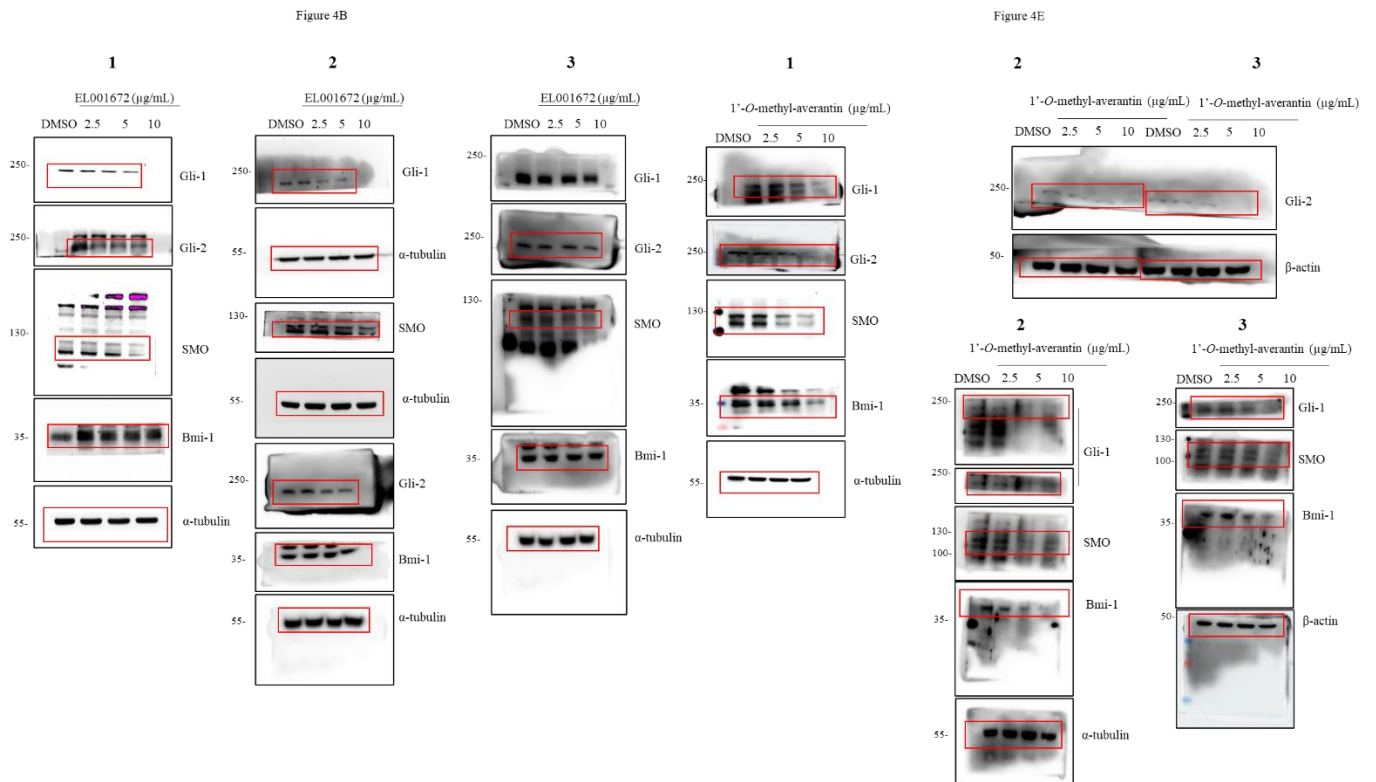
Supplementary Figure 8. HSQC spectrum of 1'-O-methyl-averantin in DMSO-*d*₆



Supplementary Figure 9. HMBC spectrum of 1'-O-methyl-averantin in DMSO-*d*₆



Supplementary Figure 10. Western blots in Figure 3 in the manuscript



Supplementary Figure 11. Western blots in Figure 4 in the manuscript