

Supplementary Materials: CD73-Positive Cell Spheroid Transplantation Attenuates Colonic Atrophy

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Supplementary Figures

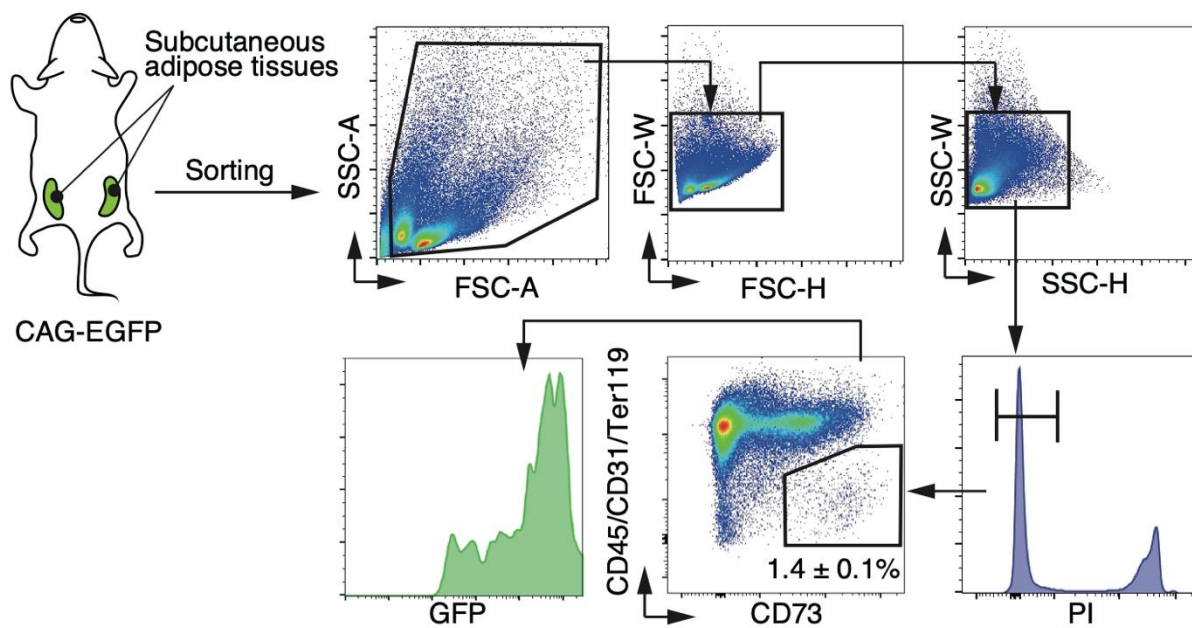


Figure S1. Representative FACS plots, representing the isolation of adipose-derived CD73⁺ cells from mouse subcutaneous fat ($n = 3$ mice).

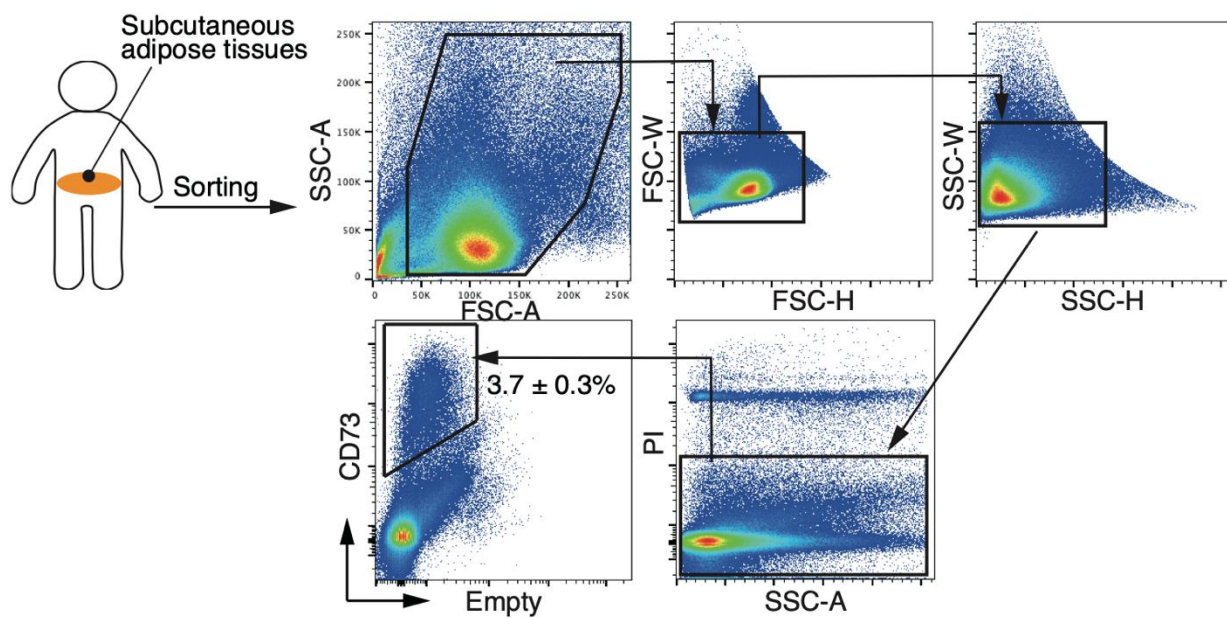


Figure S2. Representative FACS plots, representing the isolation of CD73⁺ cells from human adipose tissue ($n = 3$ specimens).

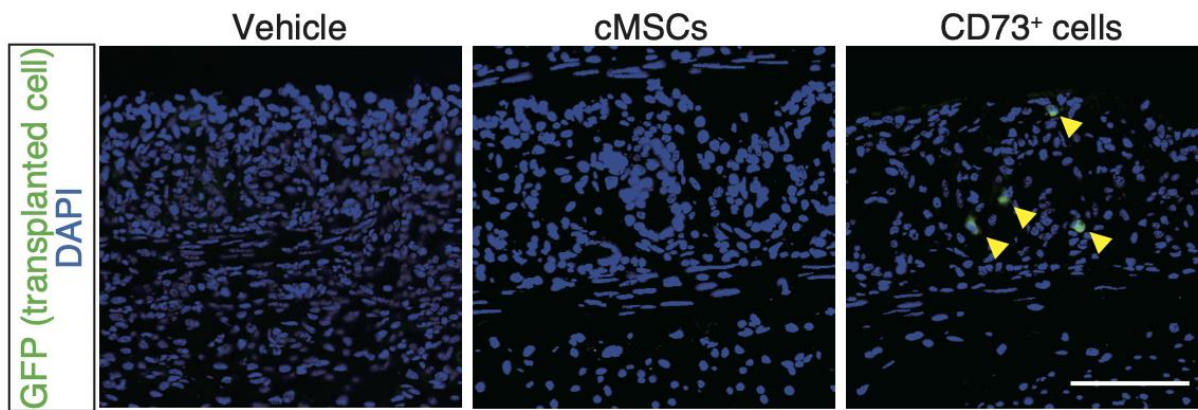


Figure S3. Transplanted cell engraftment by intravenous injection. Representative immunohistochemistry images of GFP⁺-transplanted cells after intravenous injection. Arrowheads indicate transplanted cells. No cell engraftment was observed in the PBS-treated and cMSC-transplanted groups. Vehicle: PBS-treated group; cMSCs: cMSC-transplanted group; CD73⁺ cells: CD73⁺ cell-transplanted group. Scale bar: 100 μ m.

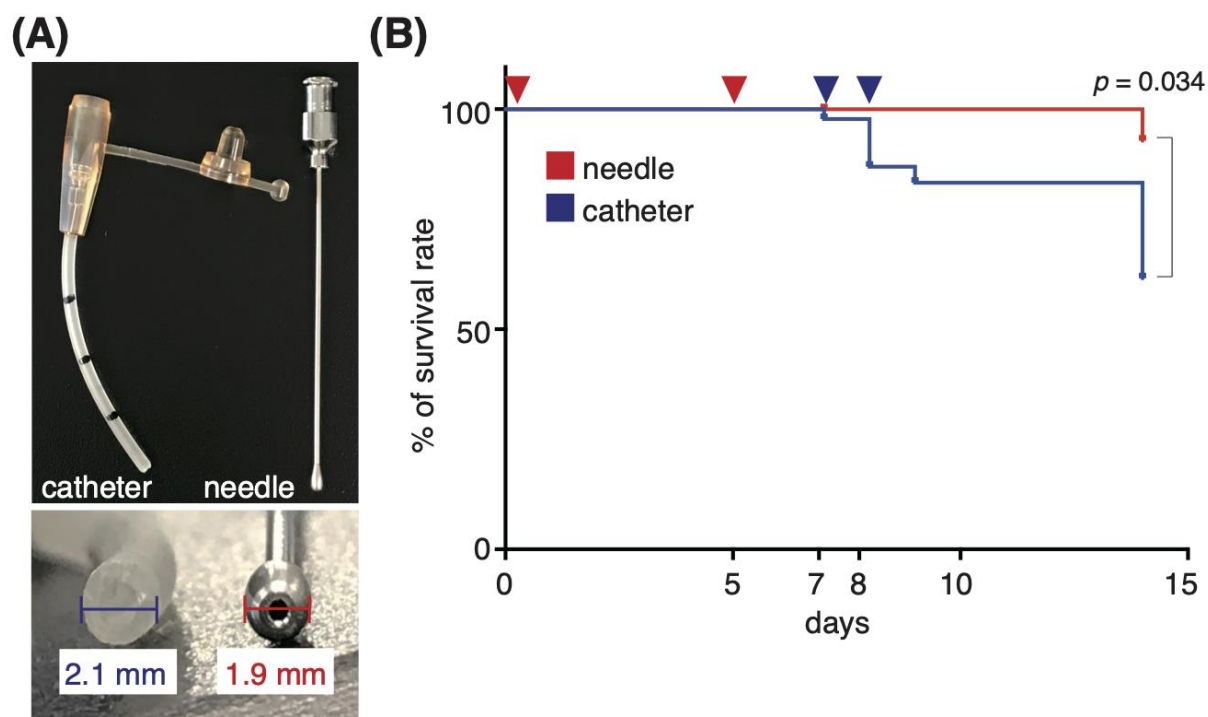


Figure S4. Improvement of trans-anal transplantation method. (A) Comparison of a thin, flexible catheter and a stainless steel needle used for cell injection. (B) Survival rate for each method. $n = 5-6$ mice. Arrowheads indicate transplantation day. Statistical significances were determined using Welch's t-test.

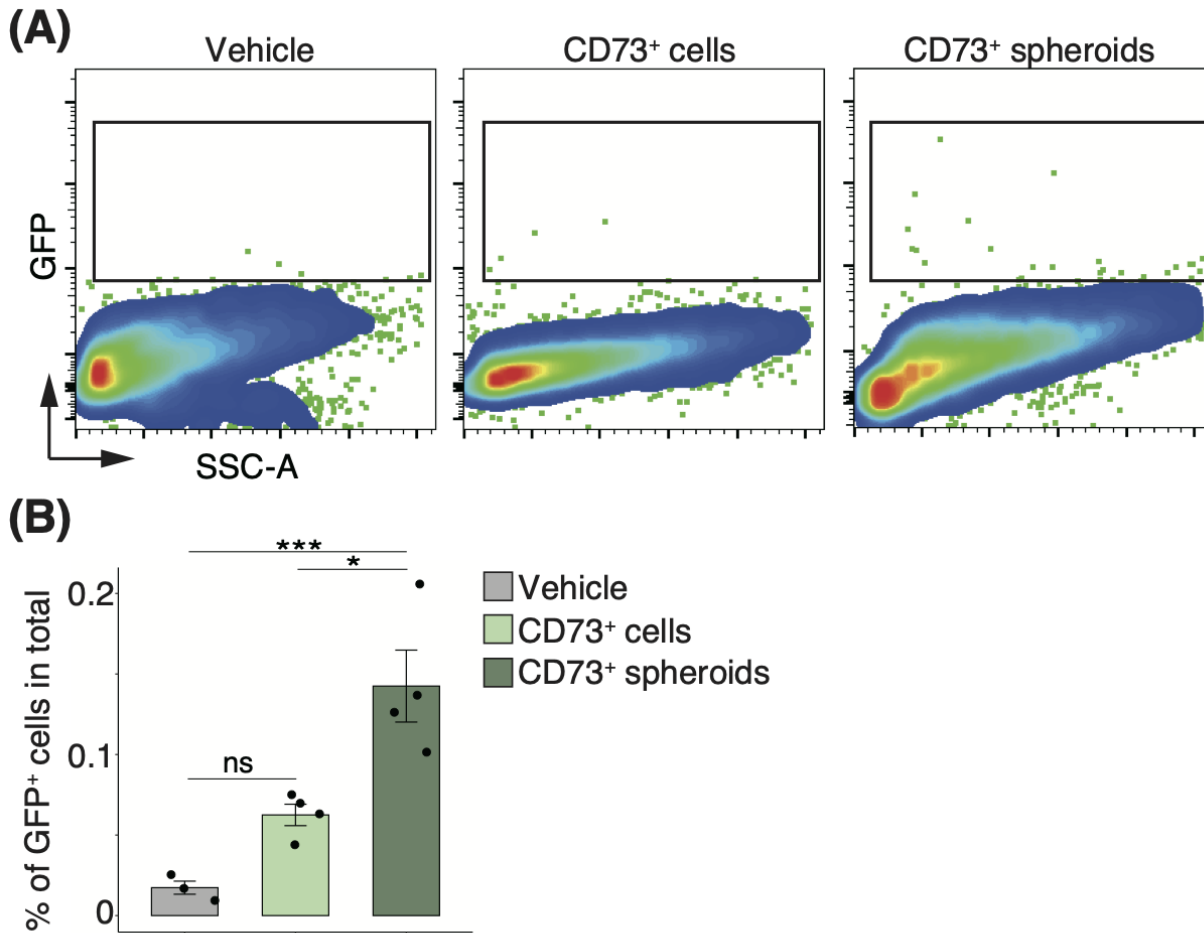


Figure S5. 3D culture improves the engraftment of CD73⁺ cells into colonic tissue after DSS treatment. (A) Representative FACS plots of GFP⁺-transplanted cells after trans-anal transplantation, using a stainless steel needle. (B) Graph showing the percentage of GFP⁺-transplanted cells. Vehicle: PBS-treated group; CD73⁺ cells: CD73⁺ 2D cell-transplanted group; CD73⁺ spheroids: CD73⁺ cell spheroid-transplanted group. $n = 3-4$ mice. Statistical significances were determined using the Tukey's test. Data are shown as mean \pm SEM. * $p < 0.05$; *** $p < 0.005$; ns, not significant.

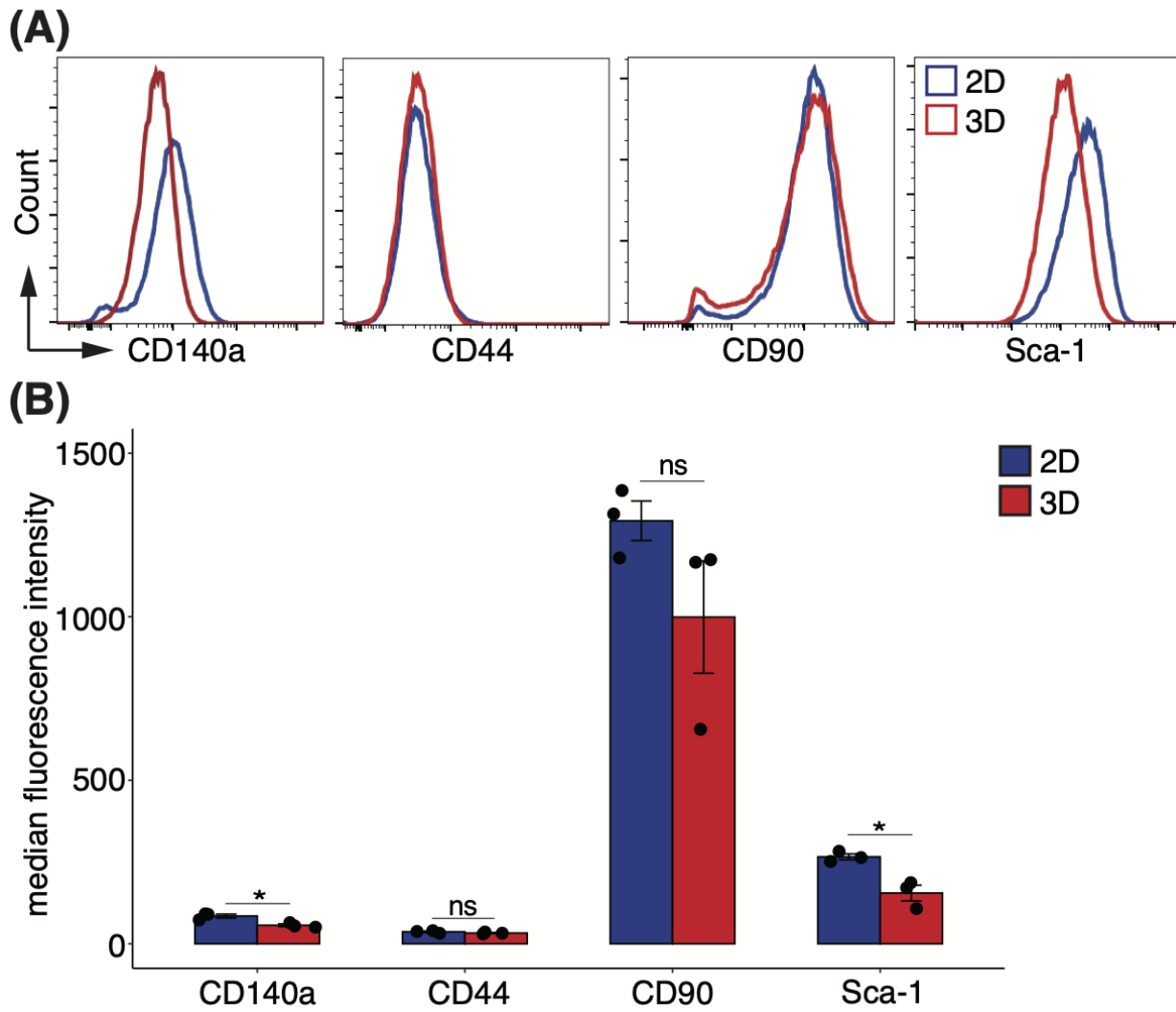


Figure S6. Protein expression of MSC markers under 3D culture, determined using FACS analysis. (A) Representative histograms of each MSC marker in CD73⁺ 2D cells and 3D spheroids. (B) Graph showing the mean fluorescent intensity of MSC markers. $n = 4$. Statistical significances were determined using Welch's t-test. Data shown as mean \pm SEM. * $p < 0.05$. ns, not significant.

Table S1. Mouse qPCR primers sequences used in the present study.

Gene	Forward	Reverse
Ago2	TCCCAGGATATGCCTTCAAA	TAAAGTGCTGGACCATGTGC
Axin2	AGAAGCGACCCAGTCAATCC	TTACTCCCCATGCGGTAAGG
Bmp-2	TAGATCTGTACCGCAGGCACTC	CTGCAGATGTGAGAACTCGTC
Col1a1	ATGTTTCAGCTTTGTGGACCT	CAGCTGACTTCAGGGATGT
Col4a1	ATTGTGGTGGCTCTGGCTGT	GCCAGGAAGTCCAGGTTCTC
Ctgf	CCACCCGAGTTACCAATGAC	GCTTGGCGATTITAGGTGTC
Cxcl1	CAGCCACCCGCTCGCTTCTC	AGGGAGCTTCAGGGTCAAGG
CyclinD1	TGGTGAACAAGCTCAAGTGG	TGGAAGAAAGTGCGTTGTG
Egf	CCCGAATTCTCAAACAGAGG	CCATCAGGAAGCAGAACAAATC
Fbxo22	GACAGTTCTCTACATGGCAGATT	ACAATTCCCGGGGTCAACA
Fgf-1	AAAAGCCCCAACTGCTCTACTG	GTATAAAAGCCCTTCGGTGTCC
Fgf-2	AAGCGGCTCTACTGCAAGAAC	CATAGCAAGGTACCGGTTGG
Fn1	GCTTTGACAAATACACTGGGAAC	CATTTCCAGACACAGACACTC
Gadd45a	GCAGAGCAGAAGACCGAAAG	GCAGGCACAGTACCACGTTA
Gapdh	AAAGGGTCATCATCTCCGCC	CTCGTGGTTCACACCCATCA
Hgf	AGTGTGCCAACAGGTGTATCAG	GTCCCTTTATAGCTGCCTCCTT
Icam-1	GAGAGTGGACCCAACTGGAA	GGGTGAGGTCTTGCTACT
Igf-1	CAGCTGGACCAGAGACCCCTT	ATCACAGCTCCGGAAGCAAC
Il-6	GGATACCACTCCCAACAGACC	TCCAGTTTGGTAGCATCCATC
Il-8	TCAAGAGCTACGATGTCTGTGT	GGCCAACAGTAGCCTTCACC
Itgb1	CCTTCAATTGCTCACCTTGTTT	CGATGATTAGCTGGATCACATTAC
Itgb3	CCACACGAGGCGTGAAGTC	CTTCAGGTTAC ATCGGGGTGA
Itgb5	GAAGTGCCACCTCGTGTGAA	GGACCGTGGATTGCCAAAGT
Itgb7	ACCTGAGCTACTCAATGAAGGA	CACCGTTTTGTCCACGAAGG
Itgb8	CTGAAGAAATA CCCCCTGGA	ATGGGGAGGCATACAGTCT
Lef1	GTCAGACAAGCCCCGTCCT	GCTGTTTCATATTGGGCATCATT
Mgp	GCTCCCTCTGGCCATCCT	GCTTTAGCTCGCCACCTCT
Mmp2	ACCAGAACACCATCGAGACC	TACTTTTAAGGCCCGAGCAA
N-myc	TCCTGGGAAGTGGGTTGGAG	GACCGCCGAAGTAGAAGTCA
Nox4	CTTTTCATTGGGCGTCCTC	GGGTCCACAGCAGAAAACTC
Paxillin	ACTACTGCAACGGACCCATC	TAGTGGACCTCACAGTACGG
Pdgfr	CTCCATCCGCTCCTTTGATG	GATCGATGAGGTTCCGAGAGA
Postn	CTCAGCACTACTCCGATGTCTC	TTAACCATGTGGCTGTGTAAGG
Rankl	AAACGCAGATTTGCAGGACT	ACATCCAACCATGAGCCTTC
Runx2	GCGTCAAACAGCCTCTTCAG	GTTGTTGCTGTTGCTGTTGT
S100a13	TGGTCTCTACTTTCTTCACCTTTG	TCACATCCAAGGTCTTCATCTTT
Slc3a2	CAGCTCCTACCTGTCAAATTCC	TGGTAGAGTCGGAGAAGATGGT
Smad4	AGGACATTGATTCAAACCATC	TTTCAAAGTAAGCAATGGAGCA
Sox9	AAGAACAAGCCACACGTCAA	CGTTCTTCACCGACTTCCTC
Tcf7	ATCCTTGATGCTGGGATCTG	CTTCTCTGCCTTGGGTTCTG
Tgf-β1	GTCAGACATTCGGAAGCAGT	GGTCAGCAGCCGTTACCAAG
Tsg-6	CTGGCAGATACAAGCTCACCTAC	GGGTATCCGACTCTACCCTTG
Vegfa	CCATGAACCTTCTGCTCTCTTG	GTAGCTTCGCTGGTAGACATCC
Vinculin	GCCAAGCAGTGCACAGATAA	TTCTTTCTGGTGTGTGAAGC
Wnt11	CTGCACCTCTGGCGACCT	CAGAAGTCAGGGGAGCTCTGT
Wnt3	AGCGTAGCAGAAGGTGTGAAG	GTGGCCCCCTTATGATGTGAGT
Wnt5a	GTCTTTTGAGATGGGTGGTATC	ACCTCTGGGTTAGGGAGTGTCT
Wnt5b	CCCCAGGCCAGAGAAAGC	CCTCCCCGATGTAGGACAT