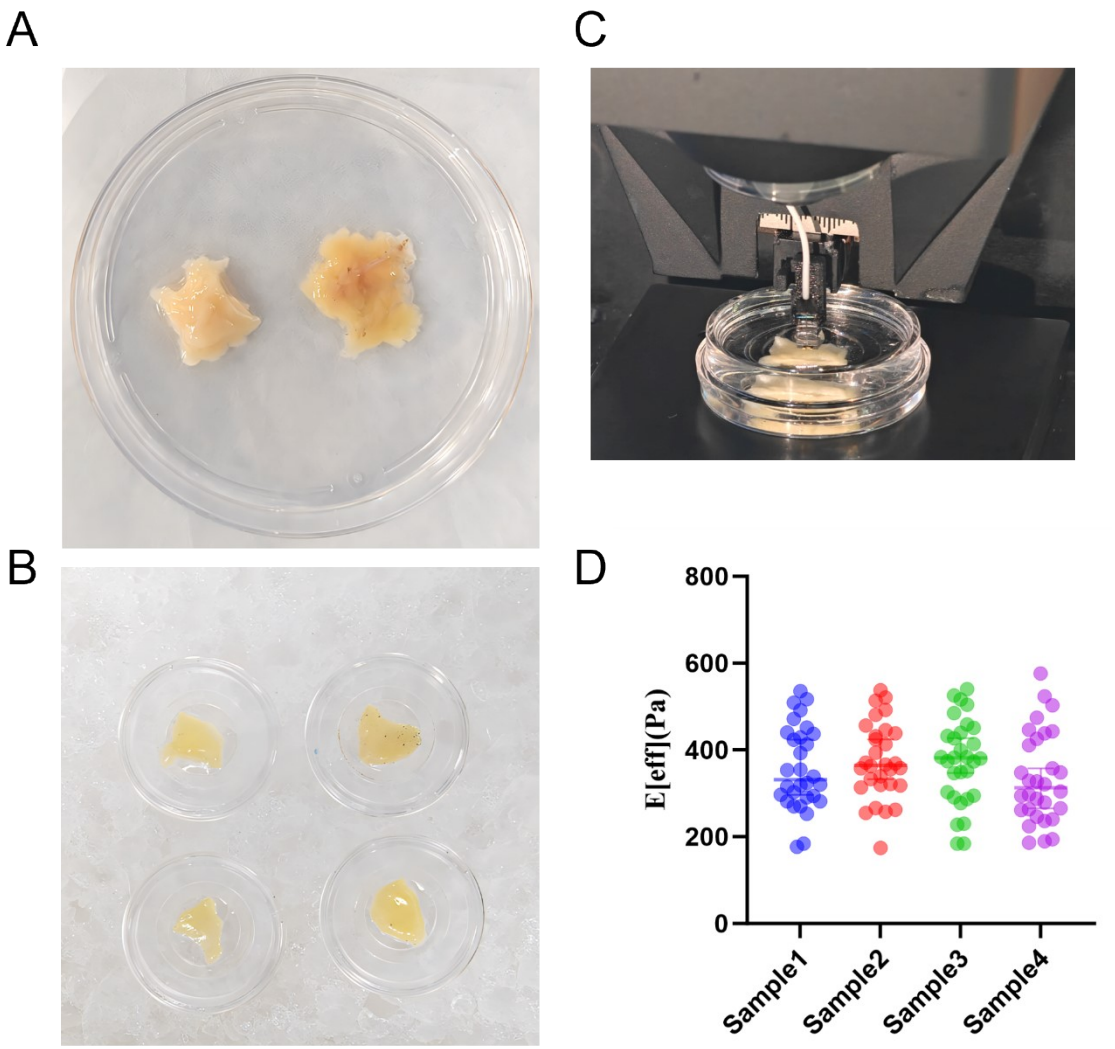
Supplementary Figure 1



Supplementary Figure 1. Mechanical characterization of the liposarcoma samples. (A) Gross view of the liposarcoma samples. (B) Liposarcoma samples prepared for Young's modulus measurement. (C) Measurement of the tissue's Young's modulus was performed using a nanoindenter. (D) The Young's modulus of liposarcoma samples (n=4).

Supplementary Materials, S1

Cell Lines and Culture Conditions

1. Quality Control

To ensure experimental reliability and reproducibility, all cell lines underwent short tandem repeat (STR) profiling upon receipt and prior to cryopreservation to verify identity and exclude cross-contamination. Additionally, cultures were routinely tested for mycoplasma contamination every 2–4 weeks using PCR-based detection kits, confirming all experiments were conducted under mycoplasma-free conditions.

2. Culture of SW872 Liposarcoma Cells

SW872 cells were cultured in Leibovitz's L-15 medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (100 U/mL penicillin, 100 μg/mL streptomycin). Due to the medium's phosphate and free-base amino acid buffering system, which causes pH shifts upon CO_2 exposure leading to cellular toxicity, cells were maintained at 37°C in a humidified incubator with 100% air and no CO_2 supplementation.

3. Isolation and Culture of Primary Cancer-Associated Fibroblasts (CAFs)

Briefly, sterile fresh tumor tissue was washed in PBS, cleared of necrotic and adipose regions, and mechanically minced into 1–2 mm^3 fragments using a scalpel. Fragments were transferred to centrifuge tubes containing dissociation buffer with type I collagenase (1 mg/mL) and hyaluronidase (100 U/mL). The resulting cell suspension was filtered through a 70 μm cell strainer to remove debris, centrifuged, and resuspended in high-glucose DMEM supplemented with 10% FBS, 1% penicillin-streptomycin, and 2 mM L-glutamine before plating in tissue culture dishes. To enrich for CAFs, medium was replaced 2–4 h post-plating to remove non-adherent tumor cells, exploiting fibroblasts' faster attachment.

Isolated primary CAFs were used only at early passages to prevent phenotype drift and senescence from prolonged culture. CAF identity was confirmed via flow cytometry or immunofluorescence, showing high expression of α-smooth muscle actin (α-SMA) and fibroblast activation protein (FAP), with negligible expression of epithelial (EpCAM) and endothelial (CD31) markers, verifying an activated fibroblast phenotype.

4. Culture of Human Umbilical Vein Endothelial Cells (HUVECs)

HUVECs were cultured in Endothelial Cell Growth Medium-2 (EGM-2) BulletKit™, comprising Endothelial Basal Medium-2 (EBM-2) supplemented with vascular endothelial growth factor (VEGF), human fibroblast growth factor-B (hFGF-B), R3-insulin-like growth factor-1 (R3-IGF-1), human epidermal growth factor (hEGF), hydrocortisone, and FBS. This optimized medium supports HUVEC phenotype, proliferation, and angiogenic function. Culture flasks were precoated with 0.1% (w/v) gelatin for 30 min to enhance adhesion. Experiments employed low-passage (P3–P7) HUVECs to maintain robust proliferation and tubulogenesis.

Supplementary Materials, S2

RNA Sequencing and Data Preprocessing

1. RNA Sequencing and Data Preprocessing

To elucidate molecular mechanisms of TME-induced cellular reprogramming, FACS-sorted SW872 and CAF populations (TME co-culture vs. 3D monoculture controls) underwent high-throughput RNA sequencing. High-quality RNA (RIN > 8.0) was ensured. Strand-specific libraries were constructed using Illumina Stranded Total RNA Prep with Ribo-Zero Plus Kit, depleting rRNA to enrich mRNA and lncRNA while preserving strand information. Libraries were sequenced on Illumina NovaSeq, yielding 150 bp paired-end reads.

Raw reads underwent FastQC quality assessment, followed by Trimmomatic for adapter/low-quality trimming. Clean reads were aligned to the human reference genome (GRCh38/hg38) via STAR. Gene-level counts were generated using featureCounts, forming the expression matrix.

2. Differential Expression and Functional Enrichment

In R, DESeq2 normalized counts and identified differentially expressed genes (DEGs; adjusted $p < 0.05$, $|\log_2(\text{Fold Change})| > 1$). clusterProfiler performed Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment on up- and down-regulated DEGs separately to elucidate biological functions.

3. Gene Set Enrichment Analysis (GSEA)

GSEA (Broad Institute) detected coordinated gene set changes across all genes ranked by expression differences. The Molecular Signatures Database (MSigDB) "Hallmark" gene sets, highly curated and low-redundancy, identified key cancer processes like epithelial-mesenchymal transition (EMT), G2M checkpoint, and KRAS signaling.

4. Intercellular Communication Network Analysis

To infer signaling between SW872 cells and CAFs, iTALK R package analyzed transcriptomic data against its curated ligand-receptor database, identifying significant interactions. Comparing TME co-culture vs. monoculture networks highlighted TME-activated axes, such as TGF-β, integrin, and VEGF pathways.

Table S1. Key commercial reagents, kits, consumables, and antibodies.

Materials	Supplier (city, country)	Catalog number
Human liposarcoma cell line SW872	Nobelbio (Hangzhou, China)	nobcell0426
Human umbilical vein endothelial cells (HUVEC)	Lonza (Basel, Switzerland)	C2517A
Leibovitz's L-15 medium	ATCC (Manassas, VA, USA)	30-2008
Endothelial Cell Growth Medium-2 (EGM-2) BulletKit™	Lonza (Basel, Switzerland)	CC-3162
High-glucose DMEM medium	Gibco (Grand Island, NY, USA)	C11995500BT
Fetal bovine serum (FBS)	Gibco (Grand Island, NY, USA)	10270106
Penicillin–streptomycin solution (100×)	Gibco (Grand Island, NY, USA)	15140122
L-glutamine	Gibco (Grand Island, NY, USA)	25030081
Accutase® cell dissociation reagent	Cytion (Heidelberg, Germany)	810100a
0.25% trypsin–EDTA	Gibco (Grand Island, NY, USA)	25200072
Type I collagen, rat tail, 10 mg/mL	ibidi (Gräfelfing, Germany)	50206
Phosphate-buffered saline (PBS), 10×	Gibco (Grand Island, NY, USA)	70011044
Sodium hydroxide (NaOH)	Sigma-Aldrich (St. Louis, MO, USA)	S8045
Lentiviral expression vector (pLenti-CMV-Puro)	Addgene (Watertown, MA, USA)	#17448
Polybrene (hexadimethrine bromide)	Sigma-Aldrich (St. Louis, MO, USA)	H9268
Puromycin	Sigma-Aldrich (St. Louis, MO, USA)	P8833
Doxorubicin	Sigma-Aldrich (St. Louis, MO, USA)	D1515
LIVE/DEAD™ Viability/Cytotoxicity Kit	Invitrogen (Carlsbad, CA, USA)	L3224
CellTiter-Glo® 3D Cell Viability Assay Kit	Promega (Madison, WI, USA)	G9681
Annexin V–FITC/PI Apoptosis Detection Kit	Vazyme (Nanjing, China)	A211-02
Bright-Glo™ Luciferase Assay System	Promega (Madison, USA)	E2610

TRIzol™ reagent	Invitrogen (Carlsbad, CA, USA)	15596026
HiScript® III RT SuperMix for qPCR (+gDNA wiper)	Vazyme (Nanjing, China)	R323-01
ChamQ Universal SYBR qPCR Master Mix	Vazyme (Nanjing, China)	Q711-02
Transwell® permeable support inserts (0.4 μm)	Corning (Corning, NY, USA)	CLS3413
BIO X™ 3D bioprinter	CELLINK (Gothenburg, Sweden)	-
LSRFortessa™ flow cytometer	BD Biosciences (San Jose, CA, USA)	-
FACSAria™ III cell sorter	BD Biosciences (San Jose, CA, USA)	-
LSM 880 confocal laser scanning microscope	ZEISS (Oberkochen, Germany)	-
Piuma nanoindenter	Optics11 Life (Amsterdam, Netherlands)	-
Rotational rheometer	Anton Paar (Graz, AUSTRIA)	-

Table S2. Primers Used for RT-qPCR

Gene Symbol	Forward Primer (5'→3')	Reverse Primer (5'→3')
MKI67	GGGCCAATCCTGTCGCTTAAT	GTTATGCGCTTGCGAACCT
CXCL12	ATTCTCAACACTCCAAACTGTGC	CTTTAGCTTCGGGTCAATGC
COL1A1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
COL1A2	GCCCCACCCAGAACTCATAC	GGTCTTGATGTCACCCTTTGC
CCN1	AATGCCTGCACCAGACCAAC	GCGAGCTGCATTCGAAACTC
CD34	CTACAACACCTAGTACCCTTGGA	GGTGAACACTGTGCTGATTACA
VEGFA	TTGCCTTGCTGCTCTACCTCCA	GATGGCAGTAGCTGCGCTGATA
MMP9	GCCACTACTGTGCCTTTGAGTC	CCCTCAGAGAATCGCCAGTACT
PCNA	CCTGCTGGGATATTAGCTCCA	CAGCGGTAGGTGTCGAAGC
GAPDH	TGTGGGCATCAATGGATTTGG	ACACCATGTATTCCGGGTCAAT