Supplementary materials

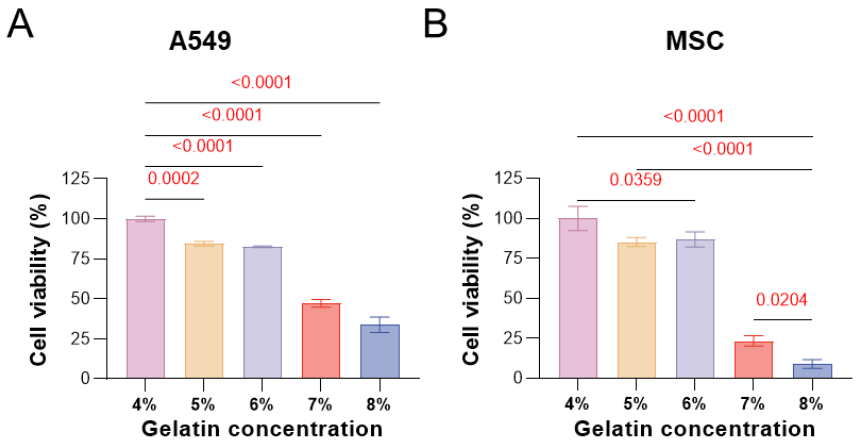

Fig. S1 Viability of A549 and mesenchymal stem cells cultured with various concentrations of gelatin hydrogels. **(A)** The cell viability of A549 cells was measured via CCK8 assay after incubation with different concentrations of gelatin hydrogels for 96 h. **(B)** The cell viability of mesenchymal stem cells was measured via CCK8 assay after incubation with different concentrations of gelatin hydrogels for 96 h. Data are plotted as mean ± standard deviation. n = 3.

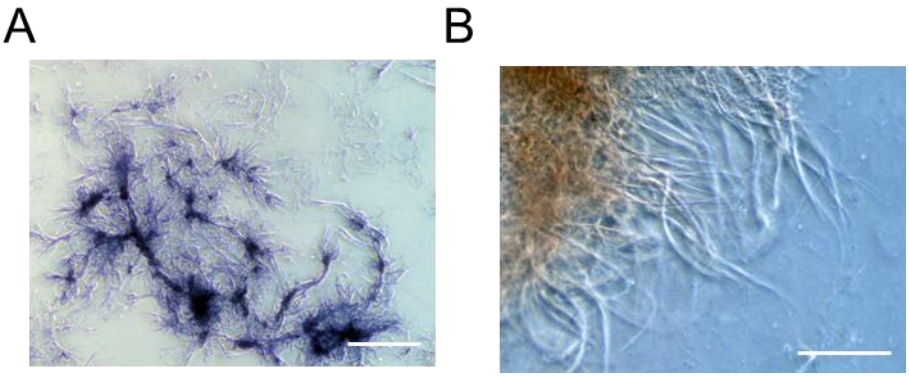

Fig. S2 Collagen bundles in tumor tissue. **(A)** The Masson staining image of the collagen bundles in tumor tissue. Scale bar, 100 μm. **(B)** The presence of collagen bundles in lung cancer organoid promoted tumor cell aggregation. Scale bar, 100 μm.

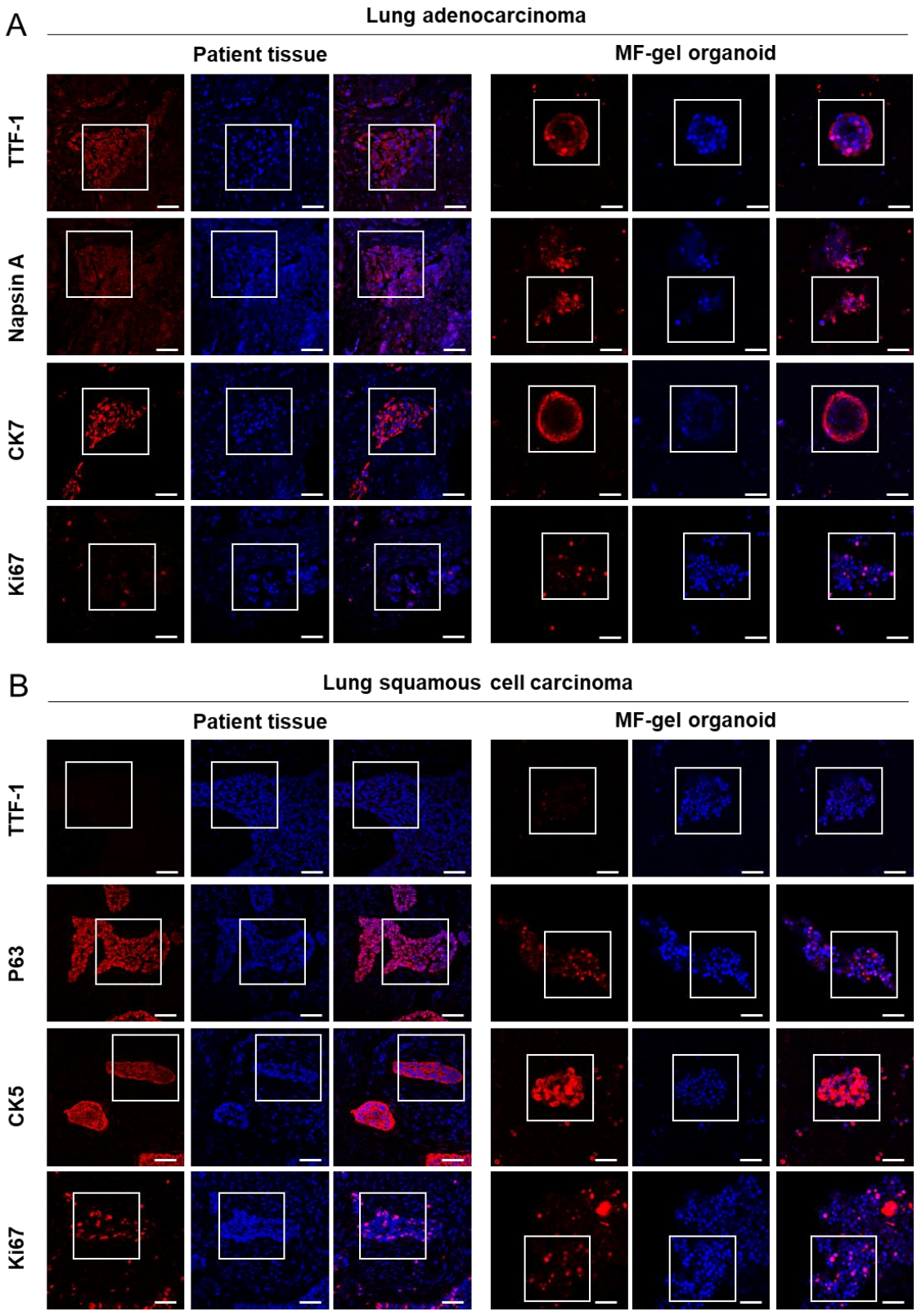

Fig. S3 Single-channel and merged immunofluorescence images corresponding to Fig. 4. **(A)** Individual

fluorescence channels and merged images for TTF-1, Napsin A, CK7, Ki67, and DAPI (nuclear counterstain) in lung adenocarcinoma tissue and matched MF-gel–cultured LCO. **(B)** Individual fluorescence channels and merged images for TTF-1, p63, CK5, Ki67, and DAPI in lung squamous cell carcinoma tissue and matched MF-gel–cultured LCO. The white-boxed regions were selected for presentation in Fig. 4.All images were acquired by confocal microscopy (Zeiss LSM 880); scale bars, 50 μm.

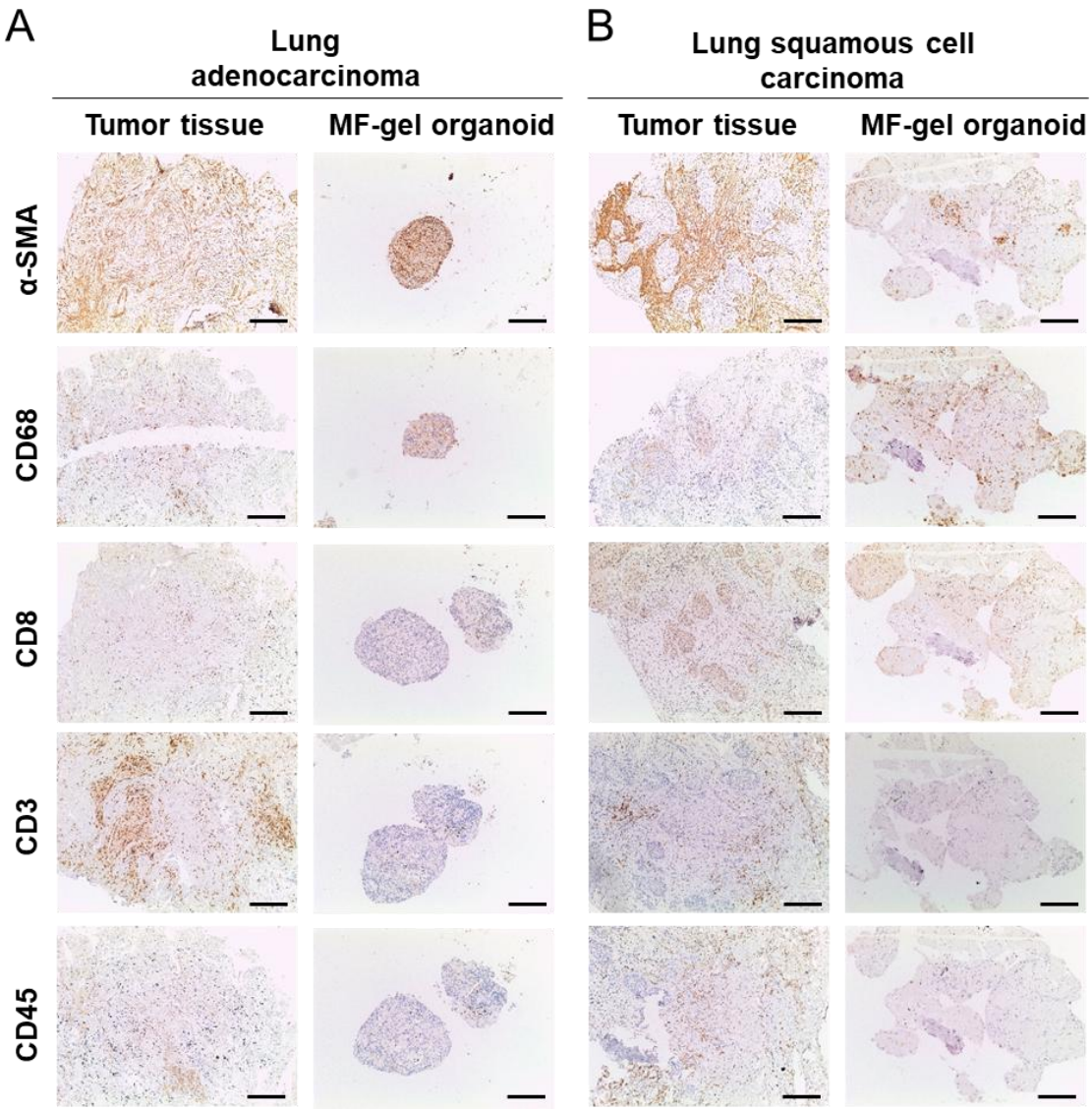

Fig. S4 Immunohistochemical (IHC) characterization of lung-cancer organoids (LCOs) cultured in MF-gel and their corresponding parental tumors. **(A)** Representative IHC images of parental lung adenocarcinoma tissue and matched LCOs grown in MF-gel, stained for α-SMA, CD68, CD8, CD3 and CD45. **(B)** Representative IHC images of parental lung squamous-cell carcinoma tissue and matched LCOs grown in MF-gel, stained for α-SMA, CD68, CD8, CD3 and CD45. Scale bar, 100 μm.

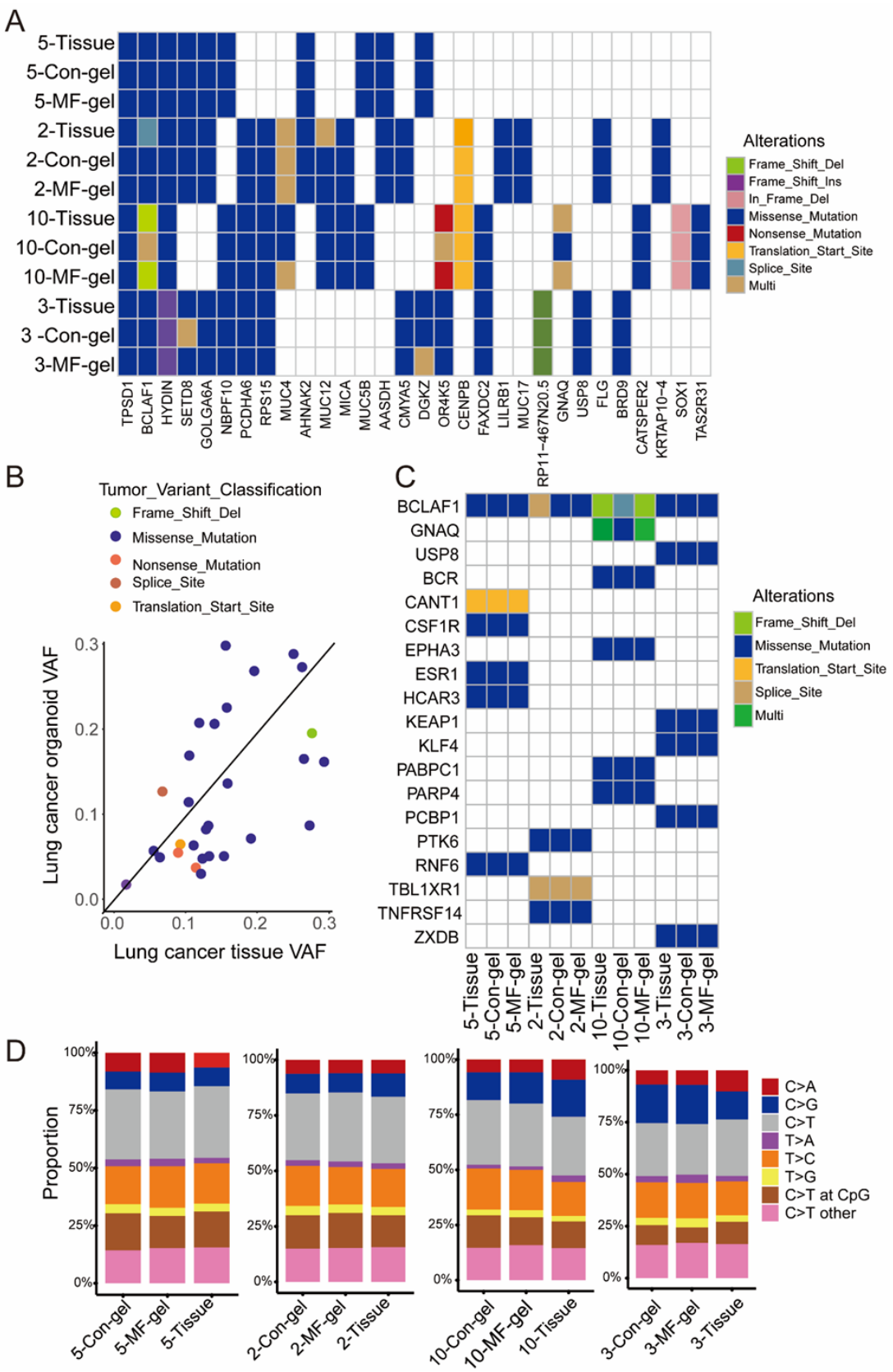

Fig. S5 Lung cancer tissues' genetic characteristics are preserved in MF-gel culture. (**A**) The heat-map

analysis involved the examination of the most significant mutations found in lung cancer samples from patients and lung cancer organoids in two gels. (**B**) The comparison was made between the VAF of genetic alterations in lung cancer organoids within MF-gel and lung cancer samples. (**C**) A visual representation illustrating the distribution of genetic alterations in cancer driver genes within LC samples and their corresponding lung cancer organoids cultured in two gels. (**D**) Comparison of mutation ratios between lung cancer samples from patients and lung cancer organoids in two gels. The corresponding indications of specific base substitutions are provided. VAF represents the fraction of variant alleles.

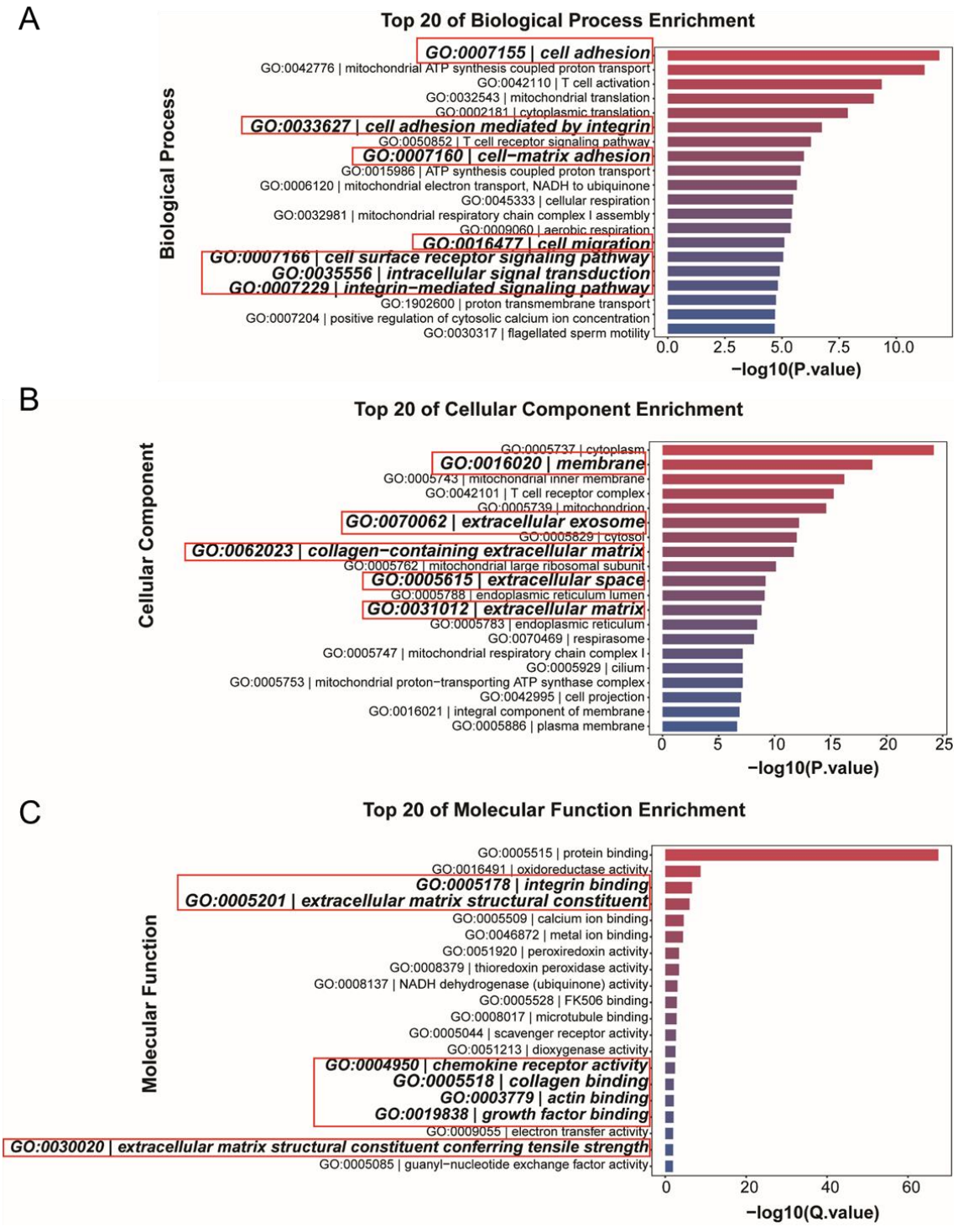

Fig. S6 Gene Ontology (GO) analysis of MF-gel-cultivated lung cancer organoids (LCOs) compared to Con-gel-cultivated LCOs. (A) GO analysis of top 20 Biological Process Enrichment on MF-gel-cultivated LCOs compared to Con-gel-cultivated LCOs. (B) GO analysis of top 20 of Cellular

Component Enrichment on MF-gel-cultivated LCOs compared to Con-gel-cultivated LCOs. (C) GO analysis of top 20 of Molecular Function Enrichment on MF-gel-cultivated LCOs compared to Con-gel-cultivated LCOs

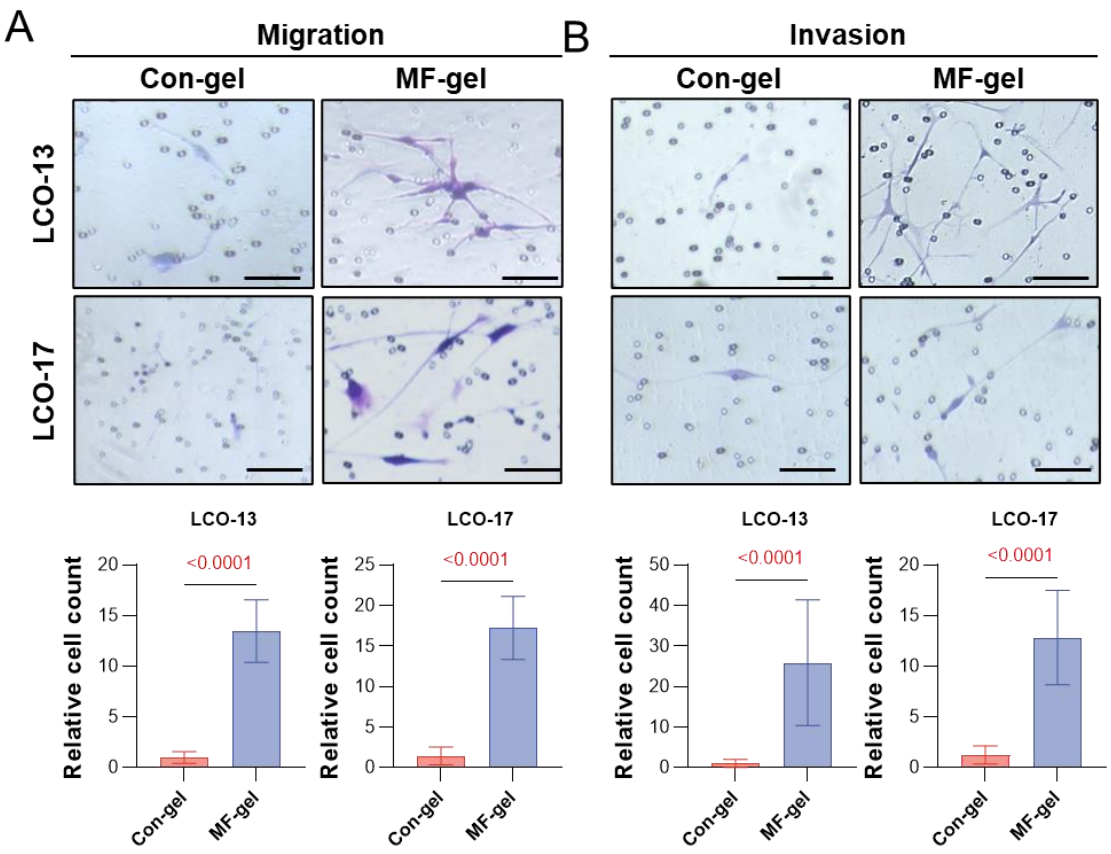

Fig. S7. MF-gel increases the ability of migration and invasion of lung cancer organoids (LCOs). **(A)** Migration assays of LCOs cultivated in Con-gels and MF-gels. Representative images (upper) and quantitative data (bottom) are shown. **(B)** Invasion assays of LCOs cultivated in Con-gels and MF-gels. Representative images (upper) and quantitative data (bottom) are shown. Data are plotted as mean ± standard deviation. Scale bar, 100 μm.

Table S1. Summary table of clinical information of patient donors

Line	Age	Sex	Pathology type	Differentiation	(y)pStage	Tumor size (longest diameter/cm)	Neoadjuvant therapy	Location
LCO-1	30	Male	Lymphoepithelial carcinoma	Poor	pT2bN1M0	4.2	None	Left lower lobe
LCO-2	68	Male	Squamous cell carcinoma	Moderate	pT1cN1M0	3	None	Left upper lobe
LCO-3	57	Male	Adenocarcinoma and neuroendocrine tumor	Poorly	ypT3N2M0	7	Yes	Left upper lobe
LCO-4	74	Male	Adenocarcinomas	Moderate-poor	ypT2bN1M0	4.1	Yes	Left upper lobe
LCO-5	78	Male	Adenocarcinoma	Poor	pT4N0M0	7.1	None	Right lower lobe
LCO-6	72	Male	Squamous cellcarcinoma	Moderate	pT2aN0M0	3.7	None	Left upper lobe
LCO-7	76	Male	Adenocarcinoma	Poor	pT3N2M0	5.5	None	Right upper lobe
LCO-8	54	Male	Squamous cellcarcinoma	Moderate	ypT2N1M0	5	Yes	Right median lobe
LCO-9	69	Male	Adenocarcinoma	Poor	ypT1bN0M0	3.3	Yes	Right lower lobe
LCO-10	69	Male	Squamous cell carcinoma	Well-moderate	ypT4N0M0	7.8	Yes	Left upper lobe
LCO-11	55	Female	Adenocarcinoma	Moderate-poor	ypT2bN2M0	4.7	Yes	Left lower lobe
LCO-12	61	Male	Squamous cell carcinoma	Moderate	ypT0N0M0	2.4	Yes	Right upper lobe
LCO-13	71	Male	Adenocarcinoma	Poor	ypT1cN0M0	2.8	Yes	Right upper lobe
LCO-14	67	Male	Squamous cell carcinoma	Moderate-poor	pT2bN0M0	4.8	None	Left upper lobe
LCO-15	58	Female	Adenocarcinoma	Poor	pT3N2M0	5.8	None	Right upper lobe

Table S1. (continued)

Line	Age	Sex	Pathology type	Differentiation	(y)pStage	Tumor size (longest diameter/cm)	Neoadjuvant therapy	Location
LCO-16	64	Male	Squamous cell carcinoma	Moderate	pT2aN0M0	3.8	None	Right lower lobe
LCO-17	71	Male	Squamous cell carcinoma	Well	ypT0N0M0	4.5	Yes	Right upper lobe
LCO-18	58	Male	Adenocarcinoma	Moderate	pT2bN0M0	4.5	None	Right lower lobe
LCO-19	73	Female	Adenocarcinoma	Moderate	pT1cN0M0	4.2	None	Left lower lobe
LCO-20	48	Male	Adenocarcinoma	Poor	ypT3N0M0	5.4	Yes	Right upper lobe
LCO-21	79	Female	Adenocarcinoma	Moderate	pT2N2M0	4.7	None	Right median and lower lobe
LCO-22	48	Male	Adenocarcinoma	Poor	ypT3N0M0	3.9	Yes	Right lower lobe
LCO-23	78	Male	Squamous cell carcinoma	Moderate	ypT1aN0M0	3.7	Yes	Left lower lobe
LCO-24	73	Female	Adenocarcinoma	Moderate-poor	pT2aN0M0	3.8	None	Left lower lobe
LCO-25	85	Male	Squamous cell carcinoma	Well-moderate	ypT1bN0M1	1.5	Yes	Right lower lobe

Table S2. Primer sequences for RT-qPCR analysis of inflammatory markers in THP-1 cells

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
β-actin	AGGCACCAGGGCGTGAT	GCCCACATAGGAATCCTTCTGAC
CD86	CCATCAGCTTGTCTGTTTCATTCC	GCTGTAATCCAAGGAATGTGGTC
IL-6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
IL-12A	CCTTGCACTTCTGAAGAGATTGA	ACAGGGCCATCATAAAAGAGGT
TNF	CCTCTCTCTAATCAGCCCTCTG	GAGGACCTGGGAGTAGATGAG
CCL1	CTCATTTGCGGAGCAAGAGAT	GCCTCTGAACCCATCCAACTG
CD206	AGCCAACACCAGCTCCTCAAGA	CAAAACGCTCGCGCATTGTCCA
IL-10	TCAAGGCGCATGTGAACTCC	GATGTCAAACTCACTCATGGCT

Table S3. Inflammatory marker expression in THP-1 cells after 72 h exposure to CMF, Con-gel, or MF-gel extracts.

Pro-inflammatory macrophage (M1) markers		Anti-inflammatory macrophage (M2) markers	
CD86	Undetectable	*CCL1*	Undetectable
IL-6	Undetectable	*CD206*	Undetectable
IL-12A	Undetectable	*IL-10*	Undetectable
TNF	Undetectable		