

Figure S1. Quality control and preprocessing of scRNA-seq samples

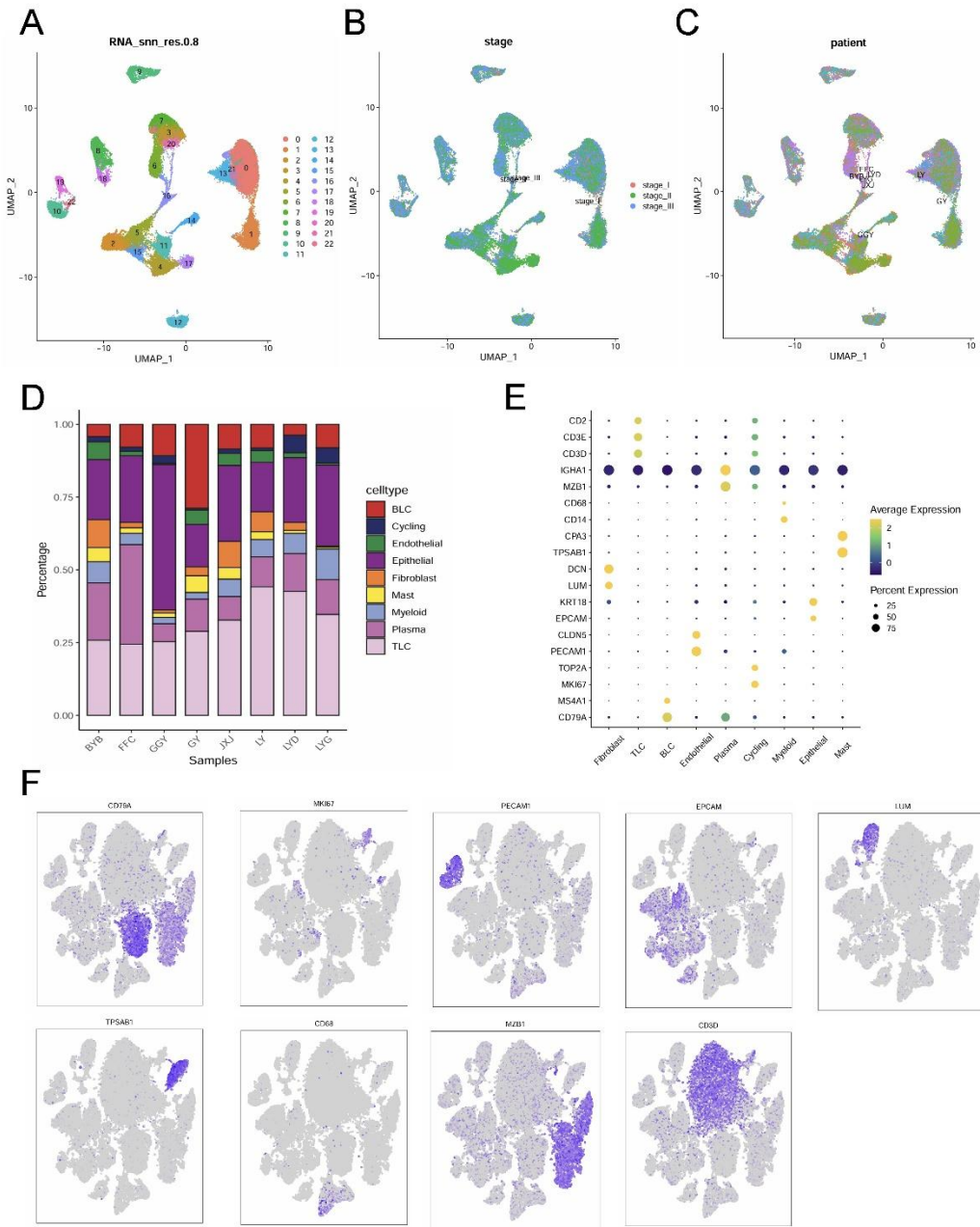


Figure S1. Quality control and preprocessing of scRNA-seq samples. (A) UMAP plot displaying 22 cell clusters identified in single-cell RNA sequencing (scRNA-seq) data based on SNN graph clustering (resolution = 0.8). (B) UMAP visualization of single cells colored by clinical tumor stages (stage I, stage II, and stage III), showing no obvious batch effects across stages. (C) UMAP plot of single cells categorized by individual patients (BYB, FFC, GGY, GY, JXJ, LY, LYD, and LYG). (D) Stacked bar plot showing the cell-type proportions of nine major cell types across different patient samples. (E) Dot plot showing the expression

levels and proportion of positive cells for key canonical marker genes across the nine major cell types. Dot size represents the percentage of expressing cells, and color intensity corresponds to average expression level. (F) Feature plots depicting the cell-type-specific spatial expression patterns of representative canonical marker genes (CD79A, MKI67, PECAM1, EPCAM, LUM, TPSAB1, CD68, MZB1, and CD3D) on UMAP embeddings.

Figure S2. Characterisation of Fibroblast subpopulations

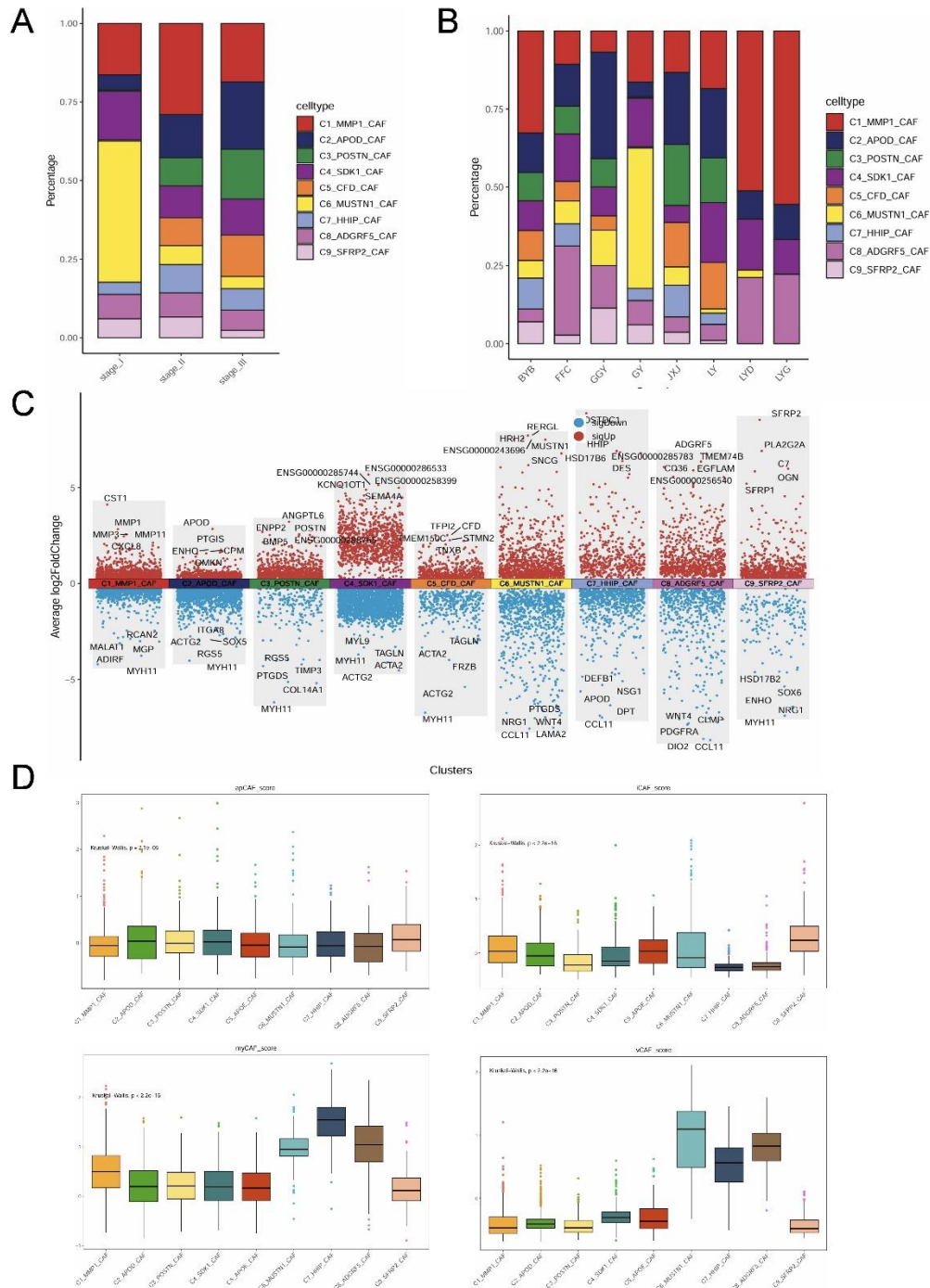


Figure S2. Characterisation of Fibroblast subpopulations. (A) Stacked bar plot showing the proportions of nine fibroblast subpopulations (C1_MMP1_CAF to C9_SFRP2_CAF) across different clinical tumor stages (stage I, stage II, and stage III). (B) Stacked bar plot illustrating the cell-type distribution of the nine fibroblast subpopulations across individual patient samples (BYB, FFC, GGY, GY, JXJ, LY, LYD, and LYG). (C) Jitter plot showing the differentially expressed genes for each fibroblast subpopulation, with red and blue dots indicating significantly up-regulated (sigUp) and down-regulated (sigDown) genes, respectively, and top characteristic marker genes labeled. (D) Box plots showing the gene signature scores for canonical CAF subtypes (apCAF, iCAF, myCAF, and vCAF) across the nine fibroblast subpopulations. Differences among clusters were evaluated using the Kruskal-Wallis test ($P < 0.0001$).

Figure S3. GO enrichment analysis of significantly upregulated marker genes in each fibroblast subtype

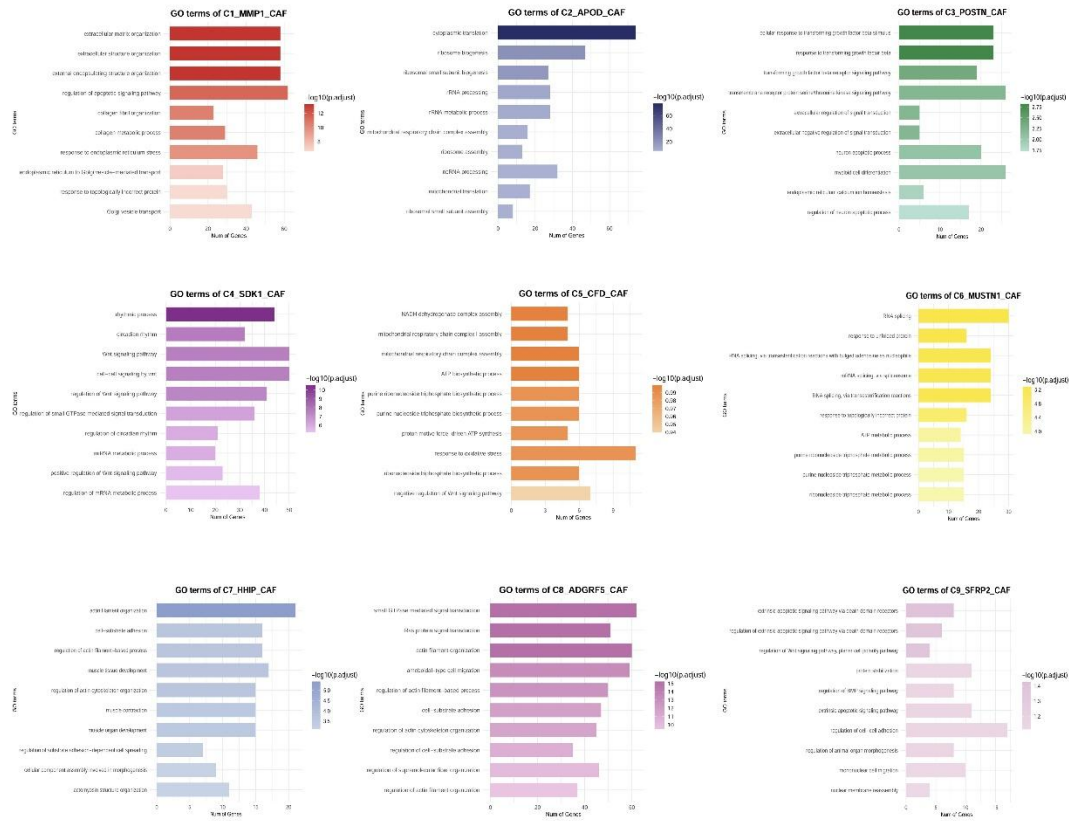


Figure S3. GO enrichment analysis of significantly upregulated marker genes in each fibroblast subtype.

Figure S4. The evolutionary trajectories and transcriptional regulatory analysis of fibroblast subtypes

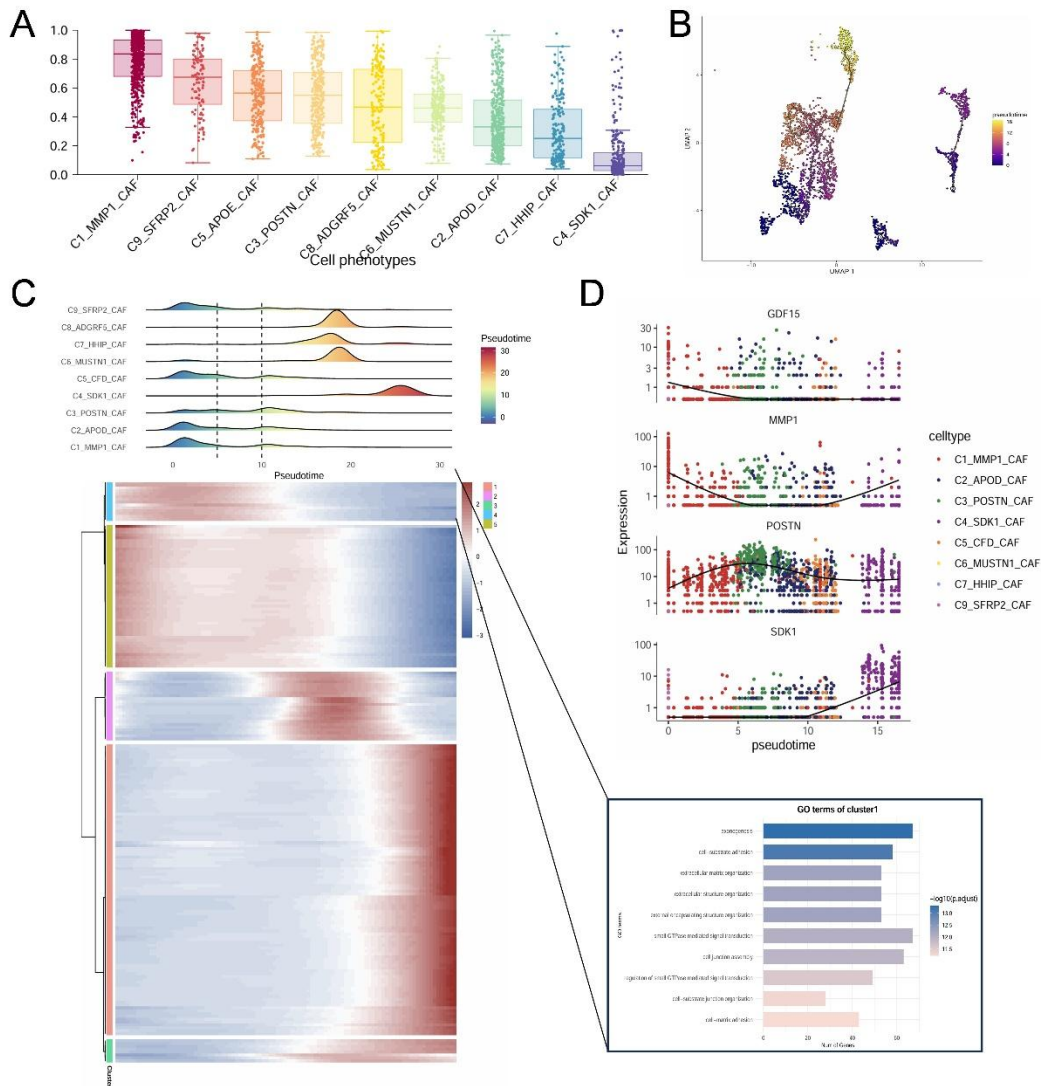


Figure S4. The evolutionary trajectories and transcriptional regulatory analysis of fibroblast subtypes. (A) Box plot with jittered points showing the distribution of developmental pseudotime scores across distinct fibroblast subpopulations (C1_MMP1_CAF to C9_SFRP2_CAF/C4_SDK1_CAF). (B) Monocle 2 pseudotime trajectory analysis of fibroblast subpopulations projected on UMAP space, with cells colored by pseudotime state progression from early (dark blue) to late (yellow).

(C) Ridge plot (top) displaying the density distribution of fibroblast subpopulations along the pseudotime trajectory, and heatmap (bottom) illustrating dynamic gene expression changes along pseudotime, clustered into distinct kinetic modules, alongside representative Gene Ontology (GO) biological process terms for gene cluster 1 (inset). (D) Scatter plots depicting the dynamic expression kinetics of key marker genes (GDF15, MMP1, POSTN, and SDK1) across pseudotime progression, with points colored by fibroblast subpopulation types.

Figure S5. The correlation between MMP1⁺ CAF and Immature ECs

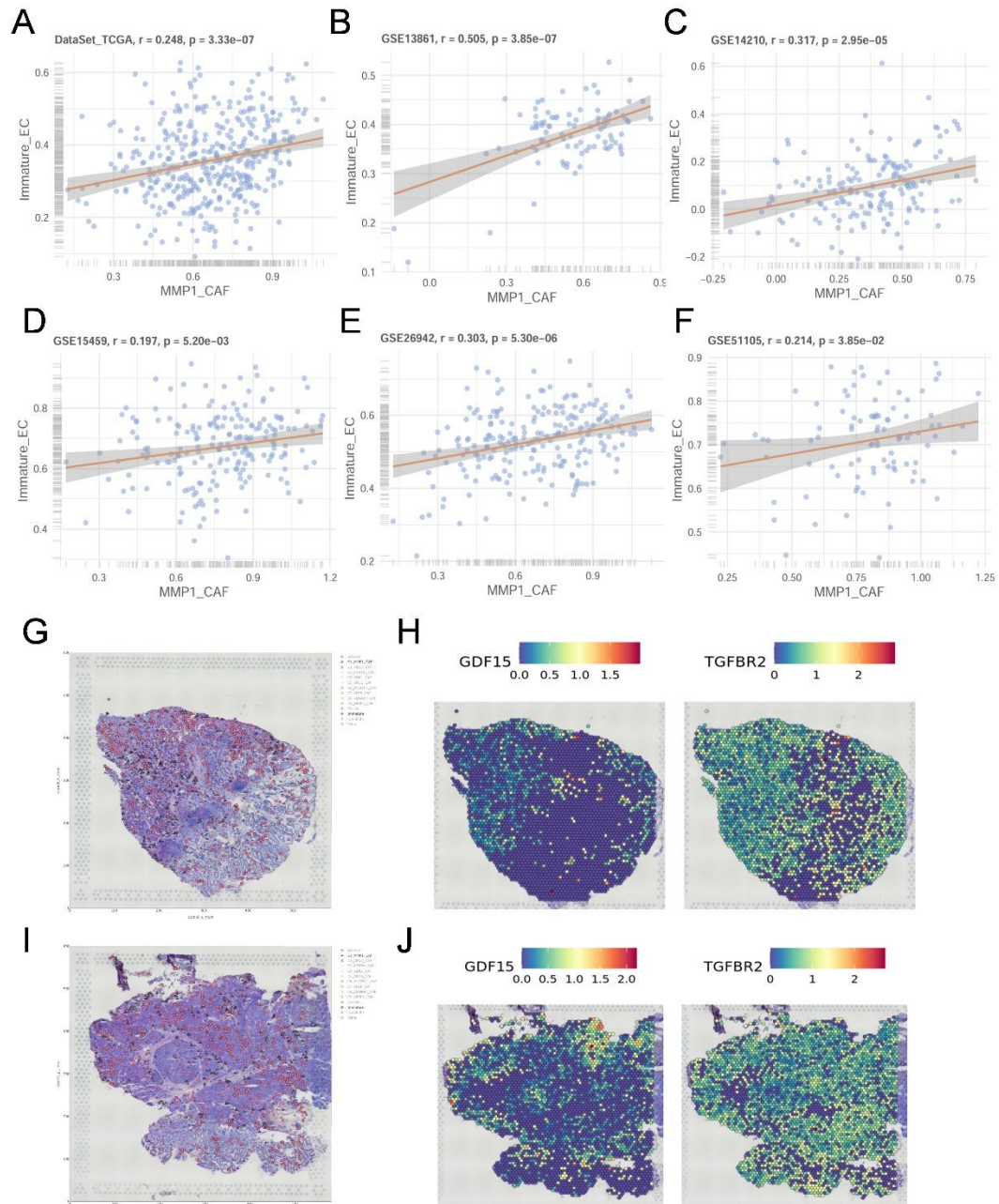


Figure S5. The correlation between MMP1+ CAF and Immature ECs. (A – F) Scatter plots depicting the positive Pearson correlation between MMP1+CAF infiltration scores and Immature EC scores across public datasets, including the TCGA cohort (A), GSE13861 (B), GSE14210 (C), GSE15459 (D), GSE26942 (E), and GSE51105 (F). Linear regression lines are shown with 95% confidence intervals (gray shading). Pearson correlation coefficients (r) and P values are indicated above each plot. (G, I) Spatial transcriptomic mapping displaying the spatial co-localization patterns of cell subpopulations across two representative tissue sections (Section 1 in G, Section 2 in I). (H, J) Spatial expression profiles of GDF15 and TGFBR2 across spatial transcriptomic spots corresponding to Section 1 (H) and Section 2 (J). Color intensity corresponds to gene expression levels, ranging from blue (low) to red (high).

Figure S6. Transcription Factors with high activity in immatured ECs

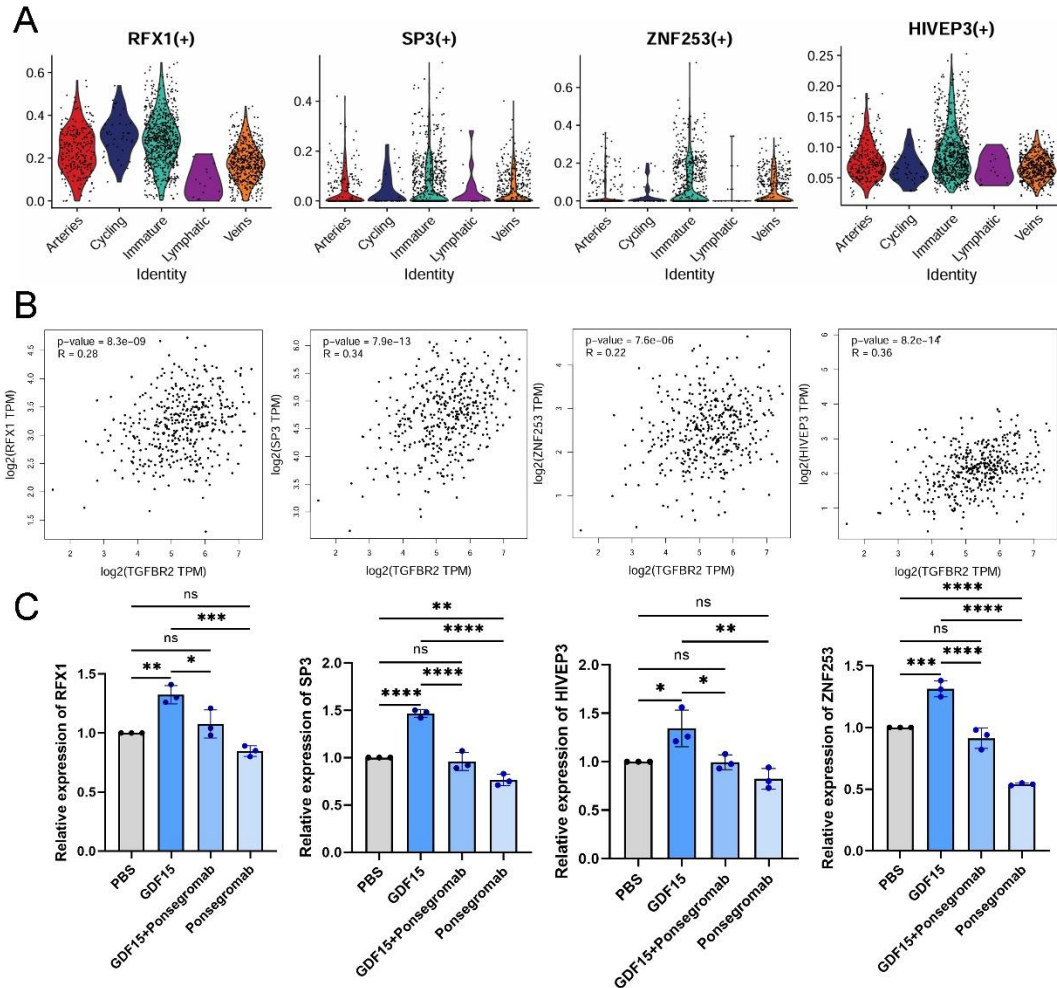


Figure S6. Transcription Factors with high activity in immatured ECs. (A) Violin plots with overlaid individual cell data points showing the regulon activity scores of representative transcription factors (RFX1, SP3, ZNF253, and HIVEP3) across EC subtypes (Arteries, Cycling, Immature, Lymphatic, and Veins). (B) Scatter plots depicting the Pearson correlation between TGFB2 expression and the mRNA expression levels of RFX1, SP3, ZNF253, and HIVEP3. Correlation coefficients (R) and P

values are indicated above each panel. (C) Bar plots showing the relative mRNA expression levels of RFX1, SP3, ZNF253, and HIVEP3 across different treatment groups (PBS, recombinant GDF15, GDF15 combined with Pongsegromab, and Pongsegromab alone). Data are shown as mean $\pm$ SD (n = 3 independent biological replicates); ns, not significant, *P < 0.01, ***P < 0.001, ****P < 0.0001.

Figure S7. Cell-cell communication between MMP1+ CAFs and Epithelial

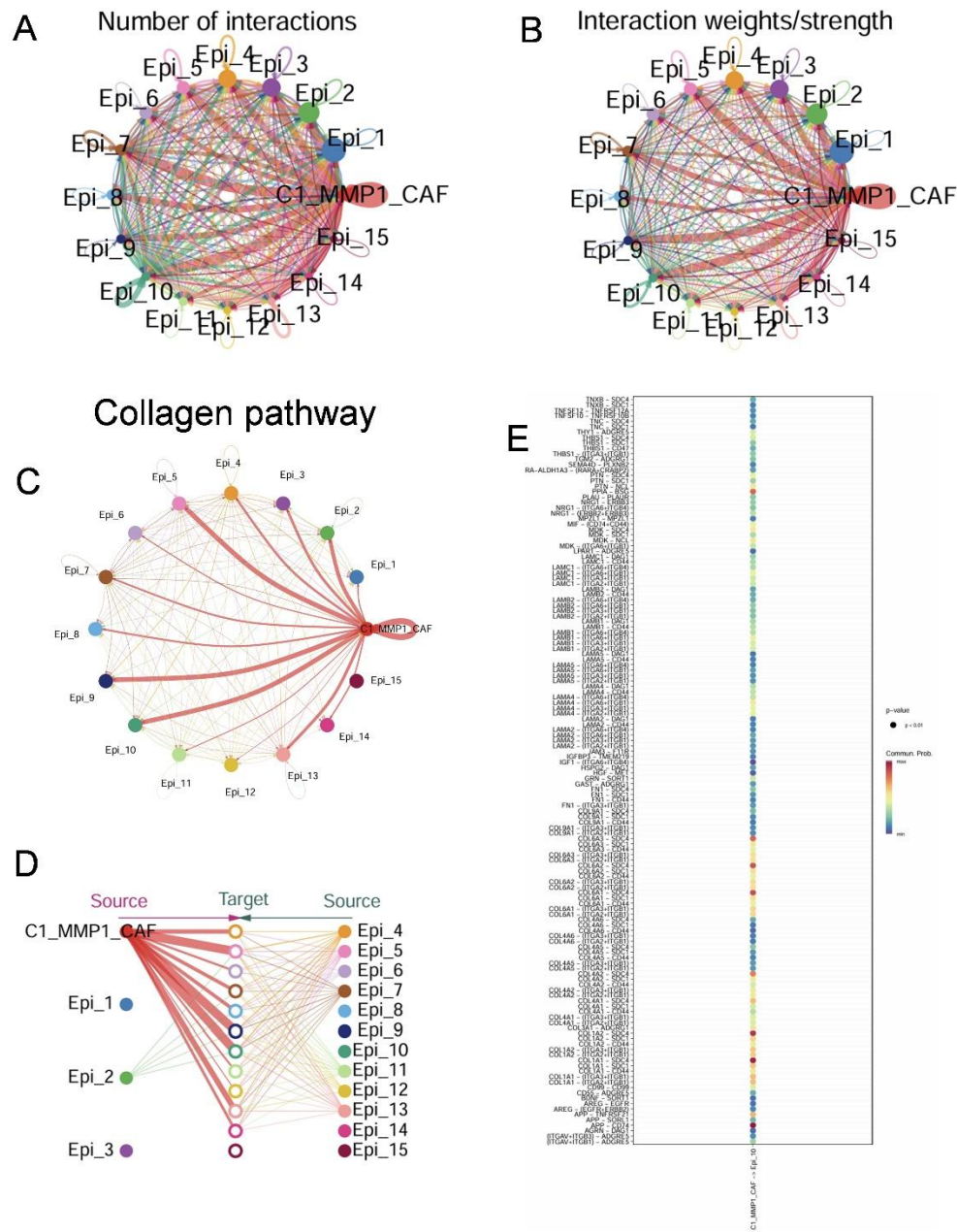


Figure S7. Cell-cell communication between MMP1+ CAFs and Epithelial. (A, B) Circle plots showing the total number of ligand-receptor interactions (A) and interaction weights/strengths (B) among C1_MMP1_CAF and epithelial cell clusters (Epi_1 to Epi_15). Edge thickness is proportional to the number or strength of interactions. (C) Circle plot highlighting the inferred cellular crosstalk network specifically mediated by the Collagen signaling pathway between C1_MMP1_CAF and various epithelial cell clusters. (D) Hierarchical plot depicting the outgoing and incoming signaling flows of Collagen signaling from sender cells (Source: C1_MMP1_CAF or epithelial clusters) to receiver cells (Target). (E) Dot plot showing the specific ligand-receptor pairs involved in the cell-cell communication from C1_MMP1_CAF to the Epi_10 subpopulation. Dot size reflects the P value ($P < 0.01$), and color intensity represents communication probability (Commun. Prob.).

scaled expression levels (Z-score) of top marker genes across five identified macrophage clusters (C1_IL4I1_Macro, C2_IGLC2_Macro, C3_FOLR2_Macro, C4_JUNB_Macro, and C5_MKI67_Macro). (D, E) Stacked bar plots depicting the relative proportions of the five macrophage clusters across clinical tumor stages (stage I, stage II, and stage III in D) and individual patient samples (BYB, FFC, GGY, GY, JXJ, LY, LYD, and LYG in E). (F) Scatter plots showing Pearson correlation analyses between MMP1+CAF infiltration levels and the signatures of distinct macrophage subpopulations in the TCGA cohort. Pearson correlation coefficients (r) and P values are provided above each plot.

Supplementary Table 1. Clinical information of patients in house cohort

PatientID	TNM stage	Age	Gender	Tissue	Lauren	MSI/MSS	Tumor status
GY	IA	68	F	Tumor	Diffuse	pMMR	Primary
BYB	IIA	69	M	Tumor	Intestinal	pMMR	Primary
FFC	IIA	62	M	Tumor	Diffuse	pMMR	Primary
GGY	IIA	64	M	Tumor	Diffuse	pMMR	Primary
LYG	IIA	58	M	Tumor	Diffuse	pMMR	Primary
JXJ	IIIB	78	M	Tumor	Mixed	pMMR	Primary
LY	IIIB	71	F	Tumor	Intestinal	pMMR	Primary
LYD	IIIB	66	F	Tumor	Diffuse	pMMR	Primary

Supplementary Table 2. Signature of fibroblast clusters

C1 MMPI CAF	C2 APOD CAF	C3 POSTN CAF	C4 SDK1 CAF	C5 CFD CAF	C6 MUSTN1 CAF	C7 HHIP CAF	C8 ADGRF5 CAF	C9 SFRP2 CAF
CST1	APOD	ANGPTL6	ENSG00000286533	CFD	REGL	SOSTDC1	ADGRF5	SFRP2
MMPI1	PTGIS	ENP2	ENSG00000285744	TPP2	IRIR3	HHIP	TMEM74B	PLA2G2A
MMPI3	ENHO	POSTN	ENSG00000258399	STMN2	ENSG00000243696	HSD17B6	CD36	C7
MMPI1	CPM	ENSG00000288765	KCNQ1OT1	TMEM150C	MUSTN1	ENSG00000285783	EGFLAM	OGN
CXCL8	DMKN	BMP5	SEMA4A	TNXB	SNCG	DES	ENSG00000256540	SFRP1
CH3L1	MT1X	SLITRK6	SDK1	IGFBP3	NRGN	RIMS1	HIGD1B	CHRD12
STC1	DEFB1	AREG	ENSG00000283563	ENSG00000284686	CASQ2	MEAP5	ANGPT12	IGF1
DHBA		PCDH17	ULK4	CDKN1C	NTKR2	ACTG2	OLFML2B	FBLN2
CTHRC1		TBXAS1	NPAS3	APOE	SLC38A11	NPNT	RASD2	RSPQ3
RTGL1-TNFRSF6B		ENPP6	ENSG00000273167	PLPP3	ACAN	PCDH7	ENPEP	CCDC80
F2RL2		F3	NEAT1	CCL11	SRL	GPM6B	COX4I2	IL33
RGCC		GATM	ENSG00000285106	ADH1B	HSPB7	PRUNE2	FAM162B	HELLPAR
MFAP2		PROM1	NLGN1	LINC01082	SORBS2	DTNA	LPL	MGP
CXCL1		ENSG00000288015	SOX6	COLEC11	BCAM	ITGA9	KOSEC3	STEAP1
UBD		VSIR	MIR23AHG	CHST7	ATP1A2	MYOCD	ADAP2	KCNK3
ISLR		IL1RL1	MEG3	ABCA8	SPEG	KCNMB1	GUCY1A2	CD34
FZD8		HSD17B2	PRKN	GASK1B	MAP3K7CL	SORBS1	PDGFB	CCL19
SLC46A3		APOC1	XIST	SCARA5	MYOM1	CKB	AVPR1A	STEAP2
AGT		PTGS1	ENSG00000230490	TCF21	ITGA7	C8orf34	LZTSL1	RARRS1
BMP7		KCNK17	KAZN	FLT3LG	ENSG00000288984	WDC2	COL5A3	PRELP
VWASA		OTULINL	IL1RAPL1	FGFR2	ADIRF	HHIP-AS1	ANO1	TNFSF13B
TMEM132A		SCPEP1	GLIS3	SELENOP	PTPA43	LINC00578	RGS5-AS1	CILP
COL8A1		LOX	NFATC2	FGF10	LDB3	ADAMTS6	OLFML2A	GNMNB
TMEM158		TMEM100	PRKG1	TSPAN2	TINAGL1	NELL1	GJC1	FGI2
RGAP2		PLAT	ARHGAP24	PTPRP	NRARP	SLMAP	NOTCH3	SPHK1
FIBIN		DEFB1	GPC5	ENSG00000243004	SERPIN1	THSD4	EBF1	COL14A1
CTSK		ATP1B1	DLG2	FBLN1	SLC25A4	CNN1	LINC02202	FBLN1
ENSG00000236886		CIQL1	SAMD4A		FHL5	MYH11	STEAP4	CRLF1
COL9A1		ENHO	DOCK4		PDGFA	ASB2	RGS5	TNFAIP6
IGFBP5		NSG1	KLHL29		NET1	PLN	LRR-4B	FAP
		TSPAN12	RFX3		HEY2	ALDH1B1	ENSG00000228655	MCL1B
		HAPLN1	PTPRG		CRYAB	MYLK	RGS7BP	CYP1B1
		COL4A6	MAGI2		GJA4	ZNF536	ITGA4	CXCL12
		PDGFD	ZSWIM6		SLC7A2	SNCAIP	GPR4	RGMA
		TM4SF1	CDK17		CSR2P	LMOD1	UACA	MEDAG
		POLA2	ARHGAP24		NRARP	TTLL7	GJA4	TSTD1
		CXCL14	MCPT2		PLCB4	TRPC6	FOXS1	SERPINE2
		MT1M	MAML2		PLN	PDLIM3	ARHGAP15	PRRX1
		CITED2	NAALADL2		CRIP1	RAB23	CCDC3	PTGDS
		MT1A	RORA		ESAM	DMD	ARHGAP26	GOS2
		CCDC68	ENSG00000285848		IL6	JPH2	INPP4B	COL8A1
		PRR16	NOTCH3		NOTCH3	SLMAP	CSR4	SH3BP5
		BMP2	PDE10A		HSPB8	SYNPO2	RASGRP2	HSPB6
			PRH1		LG4	ACTA2	MYO1B	FGF7
			BICC1		JPH2	COL23A1	SLIT3	C3
			PTPRM		KCNAB1	CES1	CDH6	C1orf89
			PDZRN3		ADAMTS4	LIMS2	LETR1	ALDH1A1
			WVVOX		GPR4	PPP1R12B	NOTCH3	SPHK1
			FTX		LETR1	BAG2	PEAR1	EPHA3
			BNC2		PHLDA2	CDC42EP3	TPPP3	MUSK
			CAMK1D		MYH11	NTN1	SEPTIN4	IGFBP4
			EXT1		GPRC5C	MYLK-AS1	ENSG00000280878	PBX3
			TRIO		TAGLN	LINC02381	IGHG4	AKR1C3
			ARHGAP42		CAP2	TCEAL1	PRSS23	MIR99AHG
			FHIT		PRELP	TGFB3	HEY2	MBP
			ANKRD31B		HSPB6	CARMN	ARHGDB1B	CCL11
			MAP3K5		CDKN1A	TPM1	CRMP1	ADH1B
			CRY1		RGS16	AOC3	ESAM	CFD
			ADAMTS9-AS2		HEYL	NDE1	CSR2P	MGST1
			ENSG00000266978		SYNM	TGFB1	PRSCC1	ENSG00000273259
			DENND2A		DMPK	RGS9	HEYL	PTGFERN
			LSAMP		LBH	FLRT2	FRZB	CLU
			ENSG0000027356		EFHD1	ITGA8	EPHA3	MAPKAP1
			RIPOR3		MYL9	PGM5	CACNA1C	THY1
			NFAS2		PCDH1	PFKP	MCAM	CLSTN3
			ENSG00000265257		CXCL1	GNF7	HEF2C	FLT2
			NUP153		ITGA3	LPP	LG4	GSN
			NXN		CIQTNF1	FHL1	HIP1	PLTP
			HIVEP3		GPR20	RALGPS2	NRARP	COMMD9
			KDMA6		SYSL-DBNDD2	CAB39L	TSPAN15	PLAC9
			BKCD1		SLIT3	SCARA3	SLIT3	ZNF385A
			GALNT18		ID4	TCEAL4	PLXND1	CPXM1
			AUTS2		DACT3	FBN2	PLCL1	
			NBEA		SGCA	DIPK2A	TINAGL1	
			REFX2		FSIP1	TES	PDGFRB	
			PVT1		NDUFA4L2	SMTN	PLCE1	
			SHANK2		RBPM52	MCPT1	ATP1B2	
			GPHN		ECRG4	FBXL7	MAP2	
			SMYD3		FOXS1	CSRPI	EPB41L1	
			SOX5		CNN1	SOC3	TESC	
			GLIS1		TESC	FLNB	AFAP12	
			CACNA2D1		TGFB3	FLNA	COLEC11	
			ZHX2		FRZB	ADCY5	RNF152	
			SBF2		ACTA2	TAGLN	CENPP	
			SIK3		FADS3	OSBPL10	PDGFA	
			ZNF407		PCSK7	MALAT1	HOPX	
			INTS6		ENSG00000259768	DACT3	CD248	
			NEGR1		PDE3A	PAWR	LURAP1L	
			AF3		SNHG15	CFL2	RERG	
			STAG1		CLMN	CD9	PGF	
			IMMP2L		FCMR	PREB	HOXB-AS1	
			FBXL7		SNTA1	SYTL2	ARHGEF17	
			ATP13A3		PLCE1	PDGFC	ITGA1	
			TTC28		RASGRP2	LITB1	NEURL1B	
			BTBD9		MTHEF2	PDI45	LINC01091	
			UST		NIBAN1	SHROOM3	COL4A1	
			ARL15		SOD3	CNTN4	RASD1	
			PAN3		ISYNA1	PDLIM5	JAG1	
			DENND4A		C1orf54	LDLRAD3	BMP8A	
			CDKAL1		MCAM	HMG20B	MIR4455-2HG	
			CAMKMT		ATP1B2	TPM2	KCNK4	
			TNFRSF10B		JAG1	CORO1C	TRIB2	
			RIPK2-DT		KLHL23	MSRB3	MARCKS	
			VPS13B		GADD45B	DSN	C1orf54	
			STARD13		AKAP1	P2RY14	ARHGAP29	
			ELMO1		CRP2	FMO3	ADCY3	
			NFATC1		KLF2	ROBO2	IL34	
			BTA1		NFASC	ENSG00000258137	LINC01943	
			MAPK10		S1PR3	PCSK7	C2orf27	
			GALNT17		THBS1	SORT1	NREP	

	PRKCA	NOL3	DIXDC1	CCDC102B
	KIF1B	HSPA6	ARHGAP28	CYTOR
	ATP11A	C11orf96	NEXN	FABP5
	MTHFD1L	ADAMTS1	LARGE1	BGN
	NRG1	MEF2C	TMEM47	APOLD1
	ABL2	FILIP1	B3GALNT2	MOC51
	NFAT5	MAP3K20	CCDC69	SIPR3
	SIPA1L1	TNFRSF12A	TCEAL3	GUCY1B1
	PSME4	TMEM38B	ADAM19	COL18A1
	LDIB2	SYNP0	MINDY2	NT5DC2
	JMJD1C	ADAP2	CUEDC1	KALRN
	ABCC1	HBEGF	CD55	NR2F2
	PTBP2	TNS1	WEDC1	ARHGEF7
	ANK3	COX4I2	NEO1	ASPN
	PLEKHG1	SNHG12	PALLD	ITGA7
	PDE4D	CDH6	ITGB3	KCNJ8
	LINC00513	SPARCL1	PDLIM7	NFASC
	SMURF2	CSPG4	MYL9	COL4A2
	ENSG00000284461	ARID5A	UTRN	IMP2
	PRICKLE2	ZFH3	PDE4D	DUSP14
	AGAP1	AOC3	PTN	LRRC32
	FAF1	RAB20	ENSG00000243004	CHN1
	ADAMTSL1	TBC1D1	CDC42BPA	PDE3A
	ASAP1	ATF3	BDKRB2	TRPC6
	ABCA1	CXCL2	SAMD11	THY1
	PAR3B	LIMS2	SSC5D	FIX1
	NHS	HEXIM1	LMCD1	FAM13C
	LRMDA	TP53INP2	CCDC107	ADAMTS12
	SLC9A9	CAVIN3	KLHL42	FRMD4A
	RBM6	GAMT	KANK1	PLXNC1
	ROR2	EBF1	ACTN1	MAP1B
	BTRC	IER2	NDUFA4	MARCKSL1
	SNTB1	SERTAD1	LBR	NES
	ROBO1	TPM2	KIF13B	ND2
	ELL	SMOC2	TSPAN2	CACNA1H
	SH3RF3	INPP4B	FERMT2	ADAMTS4
	NAV2	C1QTNF2	PLS3	KIAA0040
	HIVEP2	EGR3		ISYNA1
	UBR3	HSPB1		SYNAM
	UBR5	INPP5A		APBB2
	JAK2	ANGPTL4		CAMK2N1
	ZFPM2-AS1	FLNA		RASL12
	DDAH1	ZNF503		CHCHD10
	KSR1	MSRB3		CLMN
	PARD3	ADPCY5		EDNRA
	BRAF	PPP1R12B		PELO
	GSE1	HCFC1R1		SEMA4C
	FMNL2	NDE1		ECM2
	EXOC4	NR4A1		EFHD1
	STK39	NR4A3		SYTL2
	ANKRD44	DSTN		TBX2
	RELB	APOLD1		IGFBP2
	SCFD2	CALHM2		ADCY9
	COP1	MAP1B		IRP1
	ATG7	HOXB-AS1		DGKD
	ZNF609	PPP1R12C		ITGA3
	ENSG00000273217	ARPC1A		PTP4A3
	FNDC3B	MOB2		TRAF4
	ST7	ZNF331		IFT3
	RLF	RASD1		MGP
	FTO	GBP2		UBE2E2
	GHR	ITPK1		SORBS3
	MAST2	CSRP1		QAZ2
	MAP4K3	ANO1		SYT1-DBNDD2
	MED13L	PHILDB2		TRAK1
	CACHD1	HSPB2		TNFAIP1
	NFKB1	SYNE2		CDS2
	SPSB1	ARHGEF9		PLEKHG2
	PSB3	NR2F2		C1QTNF1
	XPR1	CVCS		B3GNT2
	ABCC4	ITFG2		NCK2
	CEMP2	KCNE4		MPRIIP
	TMT2C	CPE		CDS9
	MRTFA	KCNMB1		USP2-AS1
	BIRC6	CREM		ENSG00000267009
	ARIH1	MGST3		ADAMTSL2
	SLC24A3	CCDC107		INKA2
	FBXO11	ADCY6		EPAS1
	TLN2	H2AX		SEPTIN8
	CBLB	TUBB4B		PAK1
	DENND5A	RHOB		PLEKHH3
	THADA	DDIT3		ETS1
	OBSN	JUNB		C15orf39
	SIPA1L3	NES		RASSF1
	CASC15	GUCY1B1		DCBLD2
	UNC5C	IFT43		VASP
	KDM4C	TOB1		ENSG00000238018
	TNFRSF10D	SEPTIN4		COL14A1
	STX3	PGM5		ID4
	PDE7B	ENDOD1		UBA2
	AKT3	MAPRE2		ENSG00000286458
	ZFAND3	F10		TBC1D1
	NDRG1	KLF11		SPAP2
	ANKRD28	CCNL1		SPARC
	CDK14	DHRS3		IGFBP7
	PLCG2	CACNA1H		PDERA
	SMURF1	TBX2-AS1		RASAL2
	SPDR	CLU		SLC03A1
	ATP9B	RASL12		SLC25A24
	GBE1	MIDN		LIMD2
	EXOC6B	MIR22HG		SASH1
	CHST11	RASL11A		CD276
	CLIP2	INF2		EP8
	SEC24A	MAFF		TBX2-AS1
	RCOR1	SMIMI2		DLG1
	ADGRL3	CHCHD10		C1QTNF6
	USP32	RG5		SCRN1
	GAS7	S100A4		TGFB2
	TCT12	HSPA8		BCAR1
	RALGAP1	MAP2K3		PTP4A1
	ARID2	VASN		ACTN4

CUL3	CD9	SGIP1
MYO10		OSTF1
DENND2B		WFDL1
MAPK8		GDF11
UBR1		DGKH
CLASP2		CRIP1
TANC1		ENSG00000284969
HDAC4		PACSLN2
ENSG00000282278		
FBXW7		
CASK		
TSHZ2		
SGIP1		
LRBA		
MBD5		
SSBP2		
PITPNC1		
DLG1		
TOX		
PCCA		
ELL2		
UBE2E2		
DIP2C		
SLC23A2		
ARMH3		

Supplementary Table 3. List of oligonucleotide primers for qRT-PCR.

Genes	Forward (+)	Reverse (-)
ACTB	GCACTCTTCCAGCCTTCCTTCC	GCGGATGTCCACGTCACACTTC
TCF12	GCAGTACCAGCAGTTCACCT	TGTCTGTGAGCTTCCAGCAG
RFX1	CAGTGCCAGCCAGTCTTACTCTC	CCTCAGCCGTCTCATAGTTGTCC
SP3	ATACCACAGATCCAGTCAGCAGATG	AAGGTGTTCCAGAGGCAAGTAAGG
ZNF253	TTCAACCGATCCACAGACCTTACTAC	TTGCCACATTCTTCACATTTGTAGGG
HIVEP3	AAGCCGAAGAAGGAACCAGTGAC	AGGTCTGAGTCTGAGTCCGAGTC