Supplemental Figures/Tables

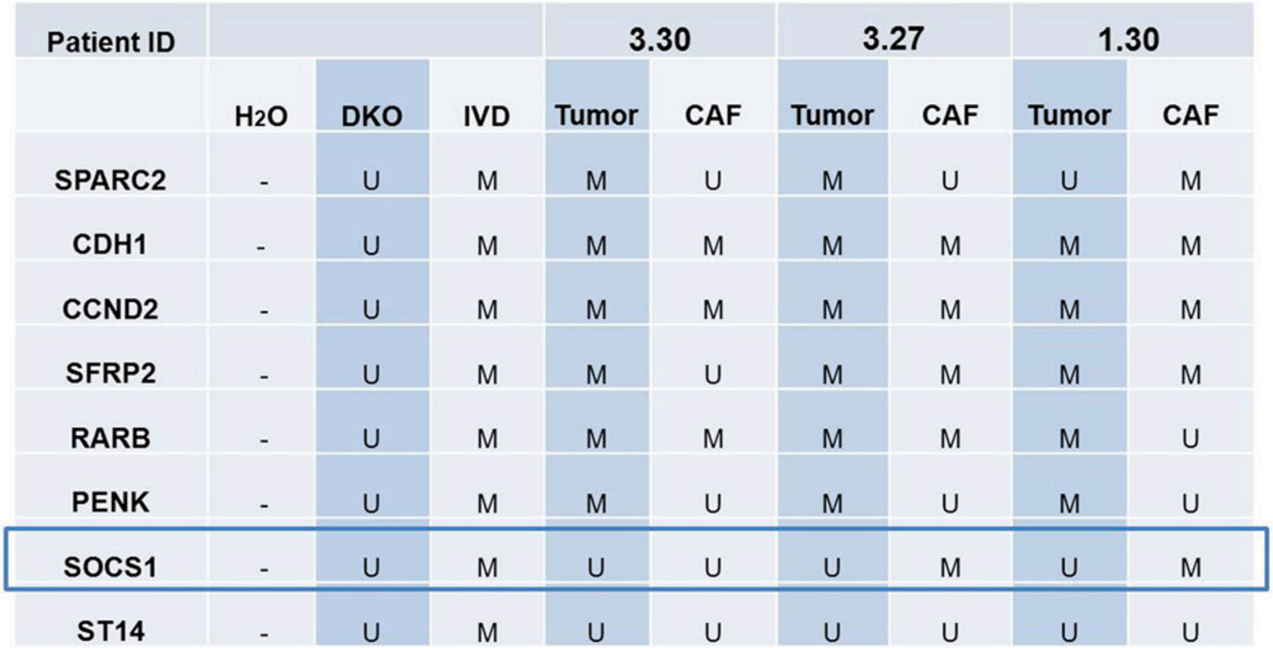
Supplementary Tables

Table S1. Primer sequences

MSP Primers		
SPARC2	Unmethylation forward	TTTTTTAGATTGTTTGGAGAGTG
	Unmethylation reverse	AACTAACAACATAAACAAAAATATC
	Methylation forward	GAGAGCGCGTTTTGTTTGTC
	Methylation reverse	AACGACGTAAACGAAAATATCG
CDH1	Unmethylation forward	TGGTTGTAGTTATGTATTTATTTTTAGTGGTGTT
	Unmethylation reverse	ACACCAAATACAATCAAATCAAACCAAA
	Methylation forward	TGTAGTTACGTATTTATTTTTAGTGGCGTC
	Methylation reverse	CGAATACGATCGAATCGAACCG
CCND2	Unmethylation forward	TGGTGTGGTTATGTTTAGTG
	Unmethylation reverse	ACAATACAACATCTAAAACCAC
	Methylation forward	TCGGTGTGGTTACGTTTAGC
	Methylation reverse	TAAAACGACGCGATACAACG
SFRP2	Unmethylation forward	GGGTGTATTTAGTTTGTAGTG
	Unmethylation reverse	CAATCCCCCCAACCAATAAC
	Methylation forward	GTCGGGGCGTATTTAGTTC
	Methylation reverse	TCCCCCCGACCAATAACG
RARB	Unmethylation forward	TTGGGATGTTGAGAATGTGAGTGATTT
	Unmethylation reverse	CTTACTCAACCAATCCAACCAAAACAA
	Methylation forward	TGTCGAGAACGCGAGCGATTC
	Methylation reverse	CGACCAATCCAACCGAAACGA
PENK	Unmethylation forward	TTGTGTGGGGAGTTATTGAGT
	Unmethylation reverse	CACCTTCACAAAAAAAATCAATC
	Methylation forward	TGTGGGGAGTTATCGAGC
	Methylation reverse	GCCTTCGCGAAAAAAATCG
SOCS1	Unmethylation forward	TTATGAGTATTTGTGTATTTTTAGGTTGGTT
	Unmethylation reverse	CACTAACAACACAACTCCTACAACAACCA
	Methylation forward	TTCGCGTGTATTTTTAGGTCGGTC
	Methylation reverse	CGACACAACTCCTACAACGACCG
ST14	Unmethylation forward	GTGTTAGGGTGAGGGTATTG
	Unmethylation reverse	ACTCACAAATCTCACAAACATC
	Methylation forward	CGTTAGGGCGAGGGTATC
	Methylation reverse	CTCGCGAATCTCACAAACG
MethySYBR Primers		
SOCS1	Methylation forward	TTCGCGTGTATTTTTAGGTCGGTC
	Methylation reverse	CGACACAACTCCTACAACGACCG

ALU	Methylation forward	GGTTAGGTATAGTGGTTTATATTTGTAATTTTAGTA
	Methylation reverse	ATTAACTAAACTAATCTTAAACTCCTAACCTCA
ST14	Methylation forward	CGTTAGGGCGAGGGTATC
	Methylation reverse	CTCGCGAATCTCACAAACG
Quantitative SYBR Green RT-PCR primers		
IGF1	Qiagen: QT00029785	
DNMT1	Qiagen: QT00034335	
DNMT3a	Qiagen: QT00090832	
DNMT3b	Qiagen: QT00032067	
Quantitative Taqman RT-PCR primers		
SOCS1	Life Technology: 4331182	
GAPDH	Life Technology: 4352934	
Pryosequencing Primers		
SOCS1	Qiagen: PM00176785	

Table S2. Promoter methylation status of 8 genes in 3 paired primary PDAC cells and CAFs measured by methylation specific PCR



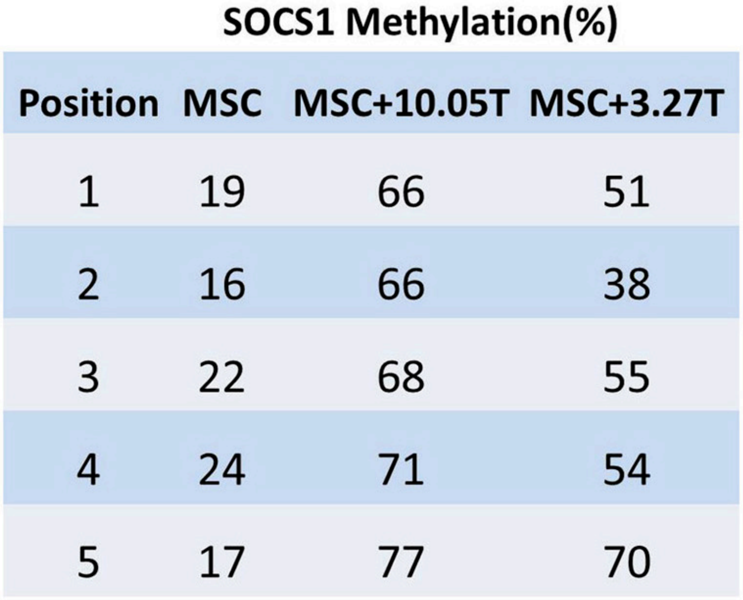
Patient ID				3.30		3.27		1.30	
	H_2O	DKO	IVD	Tumor	CAF	Tumor	CAF	Tumor	CAF
SPARC2	-	U	M	M	U	M	U	U	M
CDH1	-	U	M	M	M	M	M	M	M
CCND2	-	U	M	M	M	M	M	M	M
SFRP2	-	U	M	M	U	M	M	M	M
RARB	-	U	M	M	M	M	M	M	U
PENK	-	U	M	M	U	M	U	M	U
SOCS1	-	U	M	U	U	U	M	U	M
ST14	-	U	M	U	U	U	U	U	U

Table S3. SOCS1 methylation analyzed by pryosequencing in stroma dissected from PDAC tissues*

	SOCS1 Methylation(%)				
Samples	**Position 1**	**Position 2**	**Position 3**	**Position 4**	**Position 5**
1	86	52	81	78	29
2	32	15	52	49	47
3	5	3	18	5	5
4	54	95	19	92	95
5	50	42	51	55	48
6	67	42	49	43	16
7	45	36	59	58	53
8	47	47	48	48	50
9	56	23	42	39	50
10	54	54	53	51	57
11	48	32	58	67	30
12	65	57	77	73	69
13	37	35	48	49	44
14	35	32	96	55	80
15	64	40	82	75	65
16	22	29	45	47	50

*Intratumoral areas were circled by pathologist (R.A. Anders) to identify tumor epithelial and stromal regions suitable for microdissection. Microdissection was performed with the use of Leica LMD 7000 dissecting system (Leica Microsystems).

Table S4 Pyrosequencing results of five methylation sites on the *SOCS1* promoter in MSCs or CAFs* before or after co-culture with human PDAC cells.



SOCS1 Methylation(%)

Position	MSC	MSC+10.05T	MSC+3.27T
1	19	66	51
2	16	66	38
3	22	68	55
4	24	71	54
5	17	77	70

*Representative results are shown. Noted that even DNMT inhibitor treated cells would have a baseline value of methylation at 0-20% (Supplementary Fig. S1).

Table S5. A list of clusters with a minimum of 5 genes that are both methylated and downregulated in CAFs upon interacting with PDAC tumor cells

Chr*	Begin†	End‡	p-value§	Genes clustered
chr1	18306826	19878641	1.78e-02	*IGSF21 HTR6 TAS1R2 CAPZB C1orf151*
chr1	20751518	22802674	1.78e-02	*EPHA8 NBPF3 FAM43B CDC42 HSPG2 USP48*
chr1	25537397	26401619	1.78e-02	*TMEM50A GRRP1 SLC30A2 TMEM57 CATSPER4*
chr1	36462633	39725376	5.84e-04	*INPP5B RSPO1 MACF1 C1orf122 YRDC THRAP3 CDCA8 EPHA10*
chr1	114433437	116185270	1.78e-02	*VANGL1 NHLH2 SYT6 TRIM33 SYCP1*
chr1	154545375	154913813	1.78e-02	*NES CCT3 MEF2D C1orf61 RHBG*
chr10	43009989	46061009	2.63e-04	*ZNF485 C10orf25 RASGEF1A MARCH8 PTPN20B ZNF22*
chr10	59625619	69125933	3.67e-02	*ANK3 TMEM26 CTNNA3 IPMK C10orf107 ZNF365*
chr10	119291945	122339357	3.67e-02	*EMX2 PRDX3 PPAPDC1A GRK5 SEC23IP*
chr10	131523536	134889906	3.67e-02	*STK32C KNDC1 PPP2R2D INPP5A EBF3*
chr11	2355122	4071015	7.57e-04	*TRPM5 ART5 STIM1 KCNQ1 ART1 CD81 NAP1L4*
chr11	10775169	17455025	4.49e-03	*INSC C11orf58 ABCC8 PARVA PTH CALCA MICALCL EIF4G2*
chr11	33235743	34450179	2.14e-02	*ABTB2 CAT HIPK3 C11orf41 CD59*
chr11	43658718	46358680	2.14e-02	*DGKZ MAPK8IP1 EXT2 ALKBH3 GYLTL1B HSD17B12*
chr11	59353323	59994800	4.49e-03	*MS4A4A MS4A3 GIF MS4A6E MS4A7 MS4A1*
chr11	125658191	133787022	4.49e-03	*B3GAT1 GLB1L3 APLP2 ACAD8 TIRAP PRDM10 ADAMTS8 FLI1*
chr12	384938	2814450	4.23e-02	*DCP1B NINJ2 NRIP2 CACNA1C CCDC77*
chr12	6431510	7172733	1.07e-02	*RBP5 VAMP1 PTMS TAPBPL C12orf53 ATN1 GPR162 CD4 LRRC23*
chr12	9639137	10174135	1.07e-02	*CD69 CLEC2B CLEC12A CLEC1A CLEC7A KLRB1*
chr12	55768943	56297293	4.23e-02	*NAB2 LRP1 ARHGAP9 MBD6 GEFT*
chr12	100512877	102413879	4.23e-02	*CHPT1 IGF1 ASCL1 MYBPC1 C12orf42*
chr12	123374914	131256526	2.17e-03	*GALNT9 GLT1D1 NCOR2 SLC15A4 MMP17 GPR133 RIMBP2 DDX51*
chr13	47414792	51483631	2.71e-03	*RCBTB2 PHF11 INTS6 SUCLA2 ATP7B CAB39L FNDC3A RCBTB1*
chr14	80491621	89808717	3.99e-04	*FOXN3 TTC8 C14orf143 PSMC1 KCNK10 SEL1L TSHR*
chr14	91858677	93493520	1.48e-02	*PRIMA1 ASB2 SLC24A4 LGMN KIAA1409*
chr15	49802553	53487866	3.92e-02	*ONECUT1 MYO5A LYSMD2 GNB5 CCPG1*
chr15	72005841	72911189	3.92e-02	*CCDC33 CSK CPLX3 CYP11A1 LOXL1*
chr15	77041340	81744472	9.49e-03	*KIAA1199 IL16 TMC3 EFTUD1 RASGRF1 TMED3 BNC1 AP3B2*
chr16	1388307	1830173	2.99e-03	*HAGH CRAMP1L UNKL EME2 IFT140 FAHD1*
chr16	2672498	3050723	1.57e-02	*KCTD5 TCEB2 CLDN9 CLDN6 MMP25*
chr17	19081282	21263772	4.35e-03	*ALDH3A1 MAPK7 KCNJ12 EPN2 TMEM11 DHRS7B*
chr17	53295335	54417314	2.09e-02	*CUEDC1 OR4D1 OR4D2 TEX14 PPM1E*

chr17	74597021	76705827	2.09e-02	*NPTX1 CCDC40 HRNBP3 BAIAP2 ENPP7*
chr19	12768634	13070609	2.22e-02	*GADD45GIP1 KLF1 MAST1 NFIX PRDX2*
chr19	38391410	40352625	2.22e-02	*KCTD15 UBA2 SLC7A10 FXYD5 HPN ZNF30 GRAMD1A*
chr19	58715988	59418771	2.22e-02	*ZNF331 CACNG8 PRPF31 TSEN34 LILRB3*
chr2	43311478	47563864	1.87e-02	*RHOQ THADA EPAS1 MSH2 PLEKHH2 SIX2 TTC7A*
chr2	100256184	102162765	1.87e-02	*NPAS2 MAP4K4 LONRF2 IL1R1 CHST10*
chr2	158097149	162808291	1.87e-02	*FAP ACVR1C SLC4A10 DPP4 RBMS1 CD302*
chr2	191818374	200033446	1.87e-02	*MOBKL3 HECW2 PGAP1 MYO1B SATB2 ANKRD44*
chr2	218790118	219566371	1.87e-02	*SLC11A1 PLCD4 CTDSP1 RQCD1 ARPC2 CRYBA2*
chr2	241174817	242261937	1.87e-02	*PPP1R7 SNED1 FARP2 ATG4B CAPN10*
chr20	16658628	18413284	3.93e-02	*PCSK2 OTOR SNRPB2 C20orf12 POLR3F*
chr20	35406501	41251971	3.93e-02	*CHD6 BPI TOP1 SRC BLCAP SLC32A1 PTPRT*
chr21	45013383	46542092	1.99e-02	*UBE2G2 PTTG1IP COL6A2 C21orf56 C21orf57*
chr3	8997277	10297900	1.72e-02	*TATDN2 OGG1 PRRT3 SRGAP3 RPUSD3 IL17RE*
chr3	172801344	180435189	1.72e-02	*NLGN1 PLD1 FNDC3B SPATA16 PIK3CA*
chr3	195596838	197422697	1.72e-02	*C3orf21 GP5 TNK2 ZDHHC19 TMEM44*
chr4	85633459	89424771	3.72e-03	*AFF1 C4orf36 ARHGAP24 NKX6-1 PPM1K SPARCL1 ABCG2*
chr4	174684227	184075624	1.87e-02	*ODZ3 HAND2 GPM6A VEGFC DCTD*
chr5	140154627	140872730	2.71e-03	*PCDHB11 PCDHA7 PCDHB2 PCDHB16 PCDHA2 PCDHA6 PCDHA12 PCDHB5 PCDHGA1 PCDHB1*
chr5	176959777	179568736	1.29e-02	*ZNF354A ADAMTS2 B4GALT7 ZNF354C GRM6 RASGEF1C COL23A1*
chr6	30621674	31029977	1.74e-02	*C6orf134 TUBB PRR3 GNL1 KIAA1949 DPCR1*
chr6	55147029	73962300	1.74e-02	*KHDRBS2 GFRAL COL9A1 HCRTR2 RIMS1 BAI3 DST BAG2 KCNQ5*
chr6	143114297	149439818	3.97e-03	*PHACTR2 FBXO30 RAB32 PLAGL1 SASH1 UST HIVEP2*
chr7	75794052	86942955	7.08e-03	*UPK3B YWHAG ABCB4 CACNA2D1 GRM3 SRCRB4D HGF SEMA3D C7orf23*
chr7	100037925	100331477	3.08e-02	*ZAN PCOLCE ACHE TFR2 ACTL6B*
chr7	102899472	106589491	3.08e-02	*RELN PIK3CG LHFPL3 SYPL1 PRKAR2B*
chr7	136203938	139366471	3.08e-02	*CHRM2 DGKI CREB3L2 ZC3HAV1 TBXAS1*
chr7	150709139	158073179	7.08e-03	*MLL3 ACTR3B PTPRN2 DPP6 PRKAG2 CNPY1 WDR86*
chr8	105570642	114518418	7.65e-03	*PKHD1L1 RSPO2 ANGPT1 CSMD3 LRP12*
chrX	49250566	51662188	3.34e-02	*NUDT11 GAGE1 CCNB3 SHROOM4 MAGED1*
chrX	54850895	56328252	3.34e-02	*MAGEH1 ALAS2 MAGED2 RRAGB KLF8*
chrX	101853995	105339604	3.34e-02	*MUM1L1 GPRASP2 MCART6 WBP5 TCEAL8 TCEAL7 NGFRAP1*

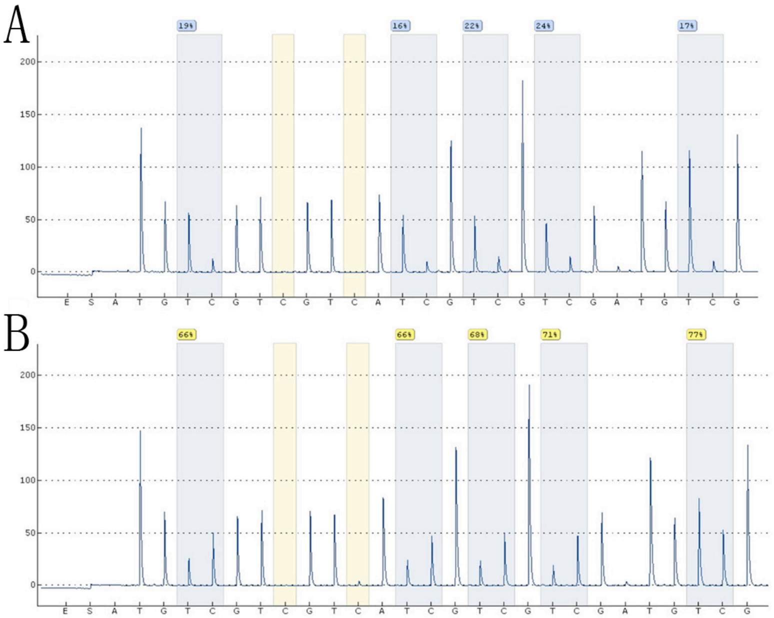
*Chr, Chromosome; †Beginning site of the cluster; ‡Ending site of the cluster; §p values indicate the significance of the clustering.

Table S6. Summary of genes with expression alterations (fold of change >1.5) in MSC cocultured with Panc10.05T vs. singly cultured MSC in both methylated genes (beta value > 0.5) and unmethylated genes (beta value < 0.5), respectively

Methylation status	**Gene Expression Alteration (p<0.001)**		
	Fold of Increase > 1.5	Fold of Decrease <1.5	No Significant Alteration
Methylated genes (n=36426)	7325	13234	15867
Unmethylated (n=36416)	11329	12740	12347

Supplementary Figures

Figure S1



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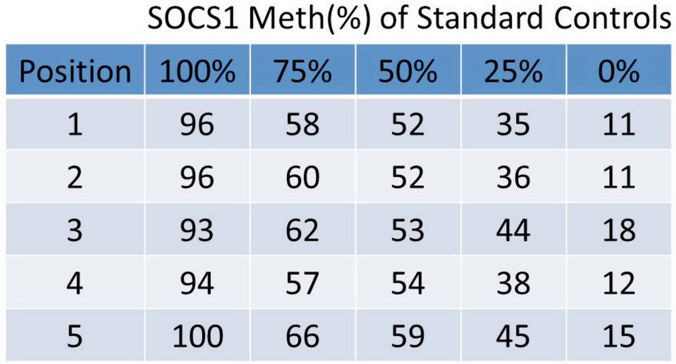


SOCS1 Meth(%) of Standard Controls

Position	100%	75%	50%	25%	0%
1	96	58	52	35	11
2	96	60	52	36	11
3	93	62	53	44	18
4	94	57	54	38	12
5	100	66	59	45	15

Figure S2

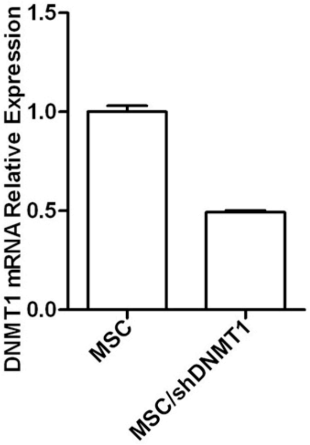
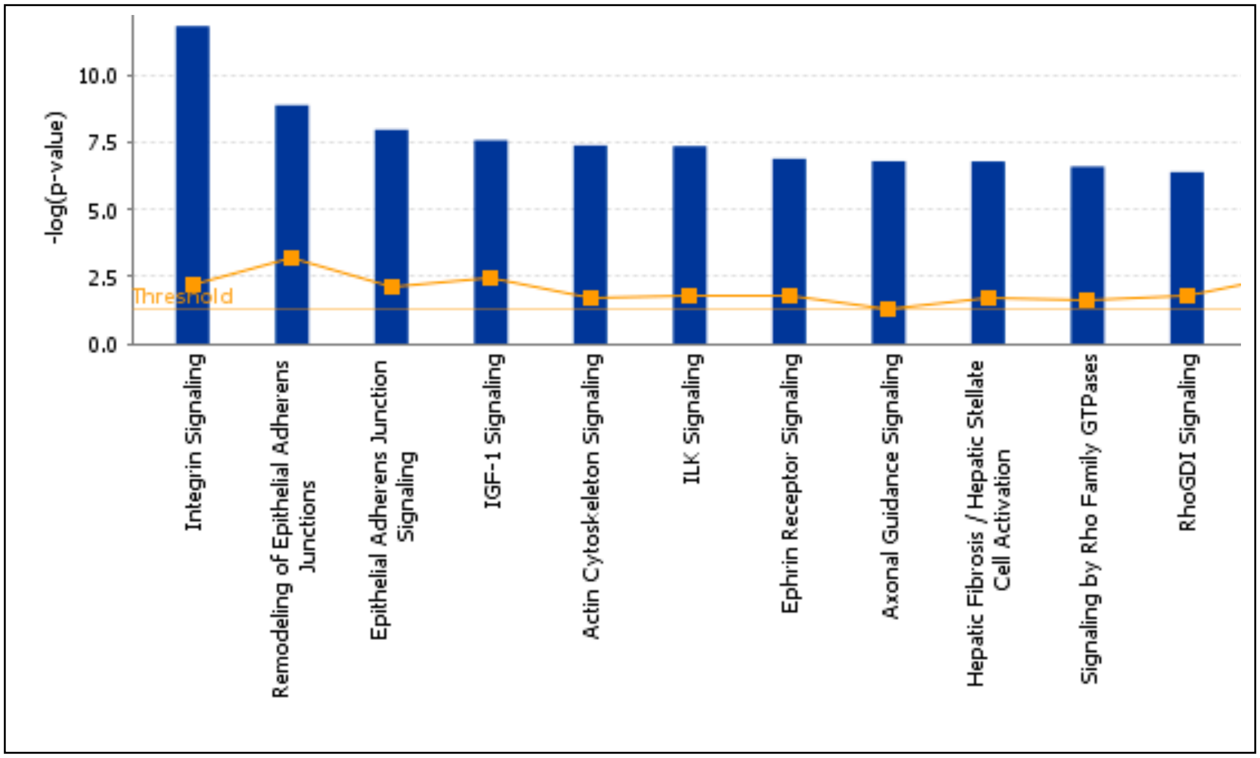


Figure S3



-log(p-value)
10.0
7.5
5.0
2.5
0.0
Threshold
Integrin Signaling
Remodeling of Epithelial Adherens Junctions
Epithelial Adherens Junction Signaling
IGF-1 Signaling
Actin Cytoskeleton Signaling
ILK Signaling
Ephrin Receptor Signaling
Axonal Guidance Signaling
Hepatic Fibrosis / Hepatic Stellate Cell Activation
Signaling by Rho Family GTPases
RhoGDI Signaling

Figure S4