

SUPPLEMENTARY MATERIAL

Proteogenomic analysis of Vascular Invasion in Hepatocellular Carcinoma (HCC)

Reveals Molecular Drivers and Predictive Biomarkers

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1 Upstream regulators of proteomic changes in vascular invasion.

Top upstream transcriptional regulators of the proteomic changes in HCC with vascular invasion.

Supplementary Figure 2 Fibronectin protein mapping in LC/MS analysis of MYC-HCC

Sequence coverage Map of mouse Fn1 in MYC-HCC tumor and liver tissue samples. a) Fibronectin sequence from N-terminus to C-terminus (left to right). Quantified peptides are indicated in orange and identified but not quantified (not identified in at least 20% of the samples) are indicated in blue. b) Quantified peptides for MYC-HCC tumor and liver tissue samples. Z-scores indicate the number of standard deviations that a peptide deviated from the average across all samples. Peptides with Z-score greater and less than zero are indicated in green and red respectively.

Supplementary Figure 3 Fibronectin protein expression in murine MYC-HCC tumor

IHC for FN1 in MYC- HCC. The dotted line shows the tumor border with the surrounding liver. The brown staining indicated Fn1 expression. The bottom insert shows the FN1 cellular staining (60X) while the inserts in the top right demonstrate the stromal FN1 expression (60X).

Supplementary Figure 4 MYC promotes the transcription of FN1

a. Multiple instances of MYC binding identified in the promoter regions of FN1 in the Gene transcription regulation database (GTRD) from ChIPseq analysis. b. Motif finding analysis of eukaryotic promoter database (EPD) identifies multiple MYC binding motifs (red boxes) in the promoter region of the human FN1 gene. c. Correlation of MYC and FN1 in multiple human cancers in the TCGA database.

Supplementary Figure 5: Fibronectin promotes invasiveness of SNU182 cells.

Demonstration of FN1 knockdown in SNU182 cells transfected with scr siRNA or FN1 siRNA, using qPCR of. b. Schematic of basement membrane invasion assay and quantification of invaded SNU182 cells transfected with scr siRNA or FN1 siRNA.

Supplemental Figure 6: Fibronectin expression in human HCC tissue

a. Correlation of fibronectin tissue protein expression in the human TCGA dataset with presence of vascular invasion, tumor grade and tumor stage. b. Impact of fibronectin mRNA protein expression in the human TCGA dataset with recurrence free survival.

Supplementary Figure 7: Validation of Fibronectin expression in human HCC

Representative cores of HCC tissue with immunohistochemistry for fibronectin expression from the human cell pathology atlas (HCPA).

Supplementary Figure 8: Fibronectin expression in tumor thrombus

Representative images of three human HCC tissues with vascular invasion showing IHC staining for fibronectin expression and HepPar1 in tumor emboli within vasculature.

SUPPLEMENTARY TABLES

Supplementary Table 1

Comparison of significantly mutated genes and somatic copy number variations between HCC with or without vascular invasion.

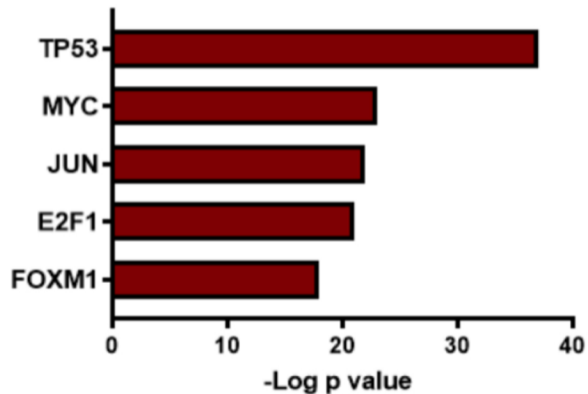
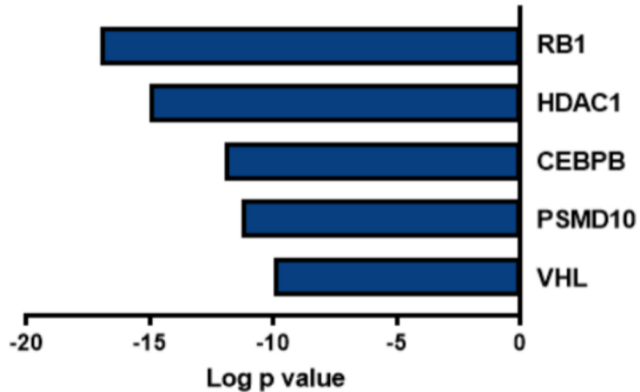
Supplementary Table 2

Differentially expressed mRNA and microRNA between HCC with or without vascular invasion.

Supplementary Table 3

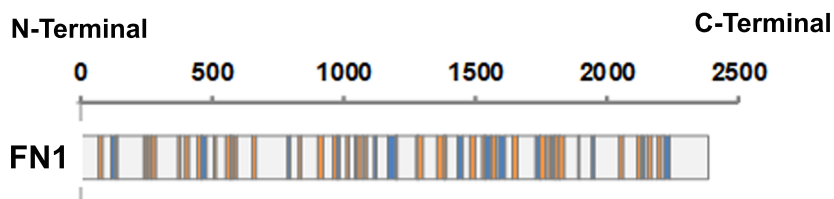
Differentially expressed proteins between HCC with or without vascular invasion.

Supplementary Figure 1: Top Upstream Regulators of VI-associated proteomic changes

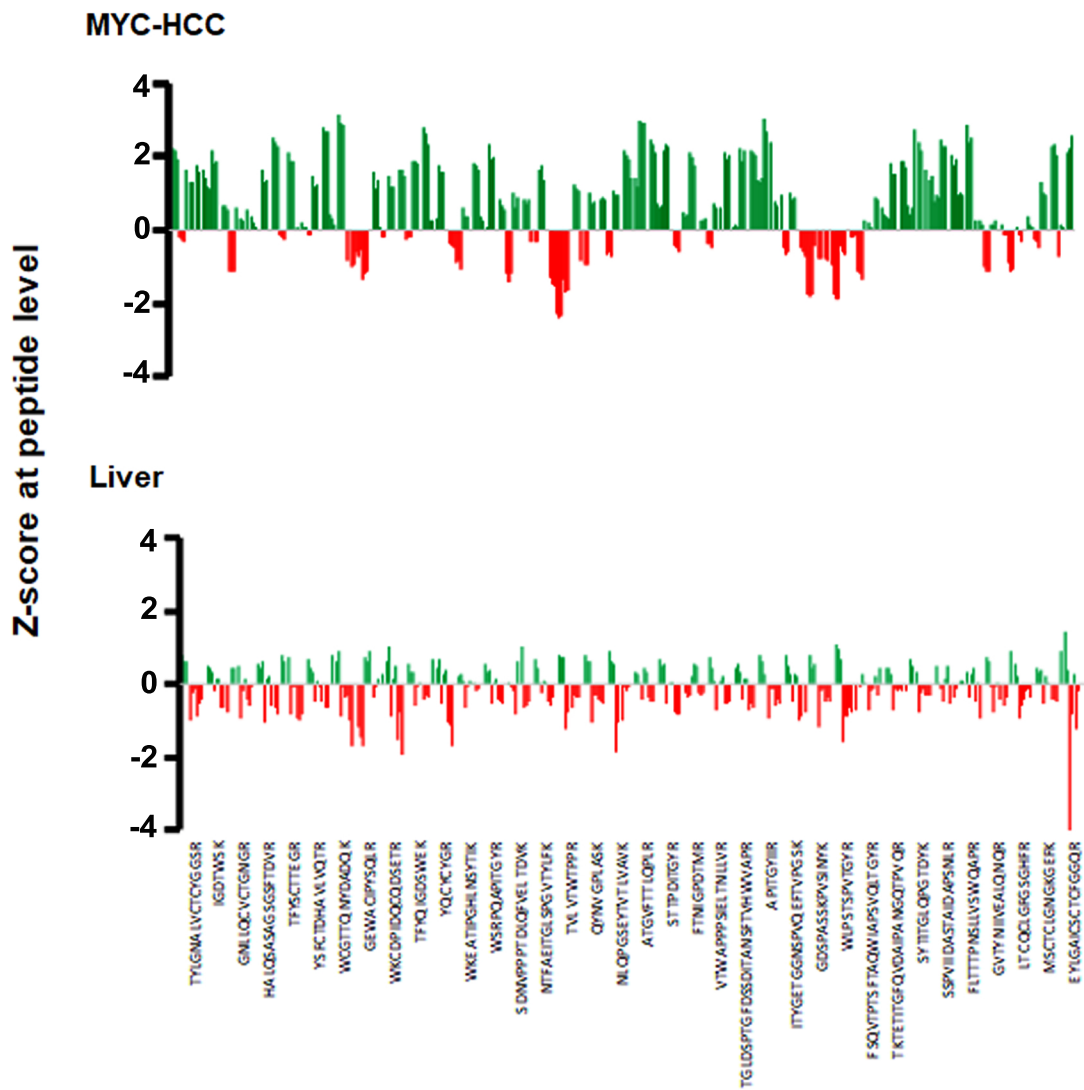


Supplementary Figure 2: Fibronectin protein expression in murine MYC-HCC

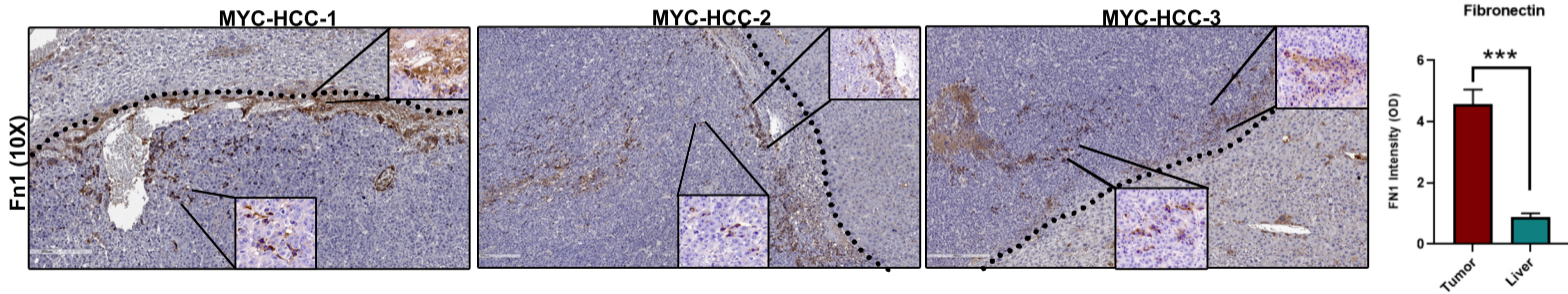
a



b

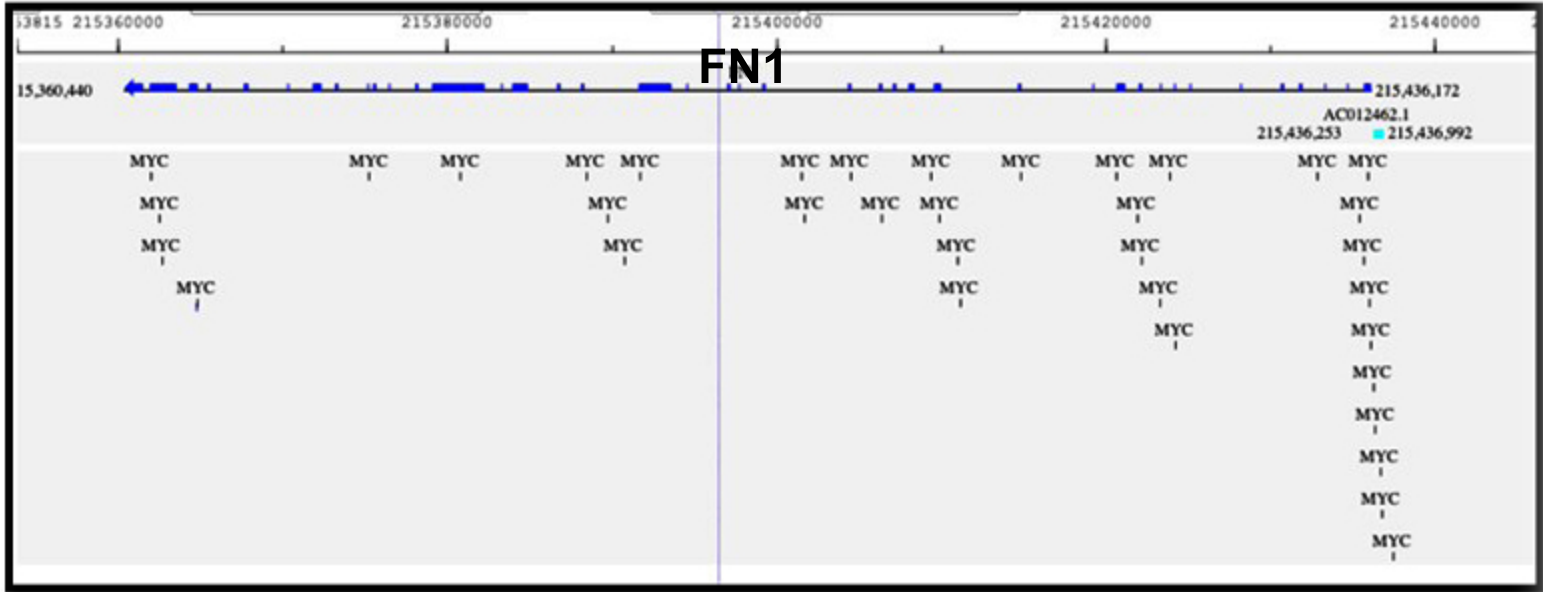


Supplementary Figure 3: Fibronectin expression in murine MYC-HCC

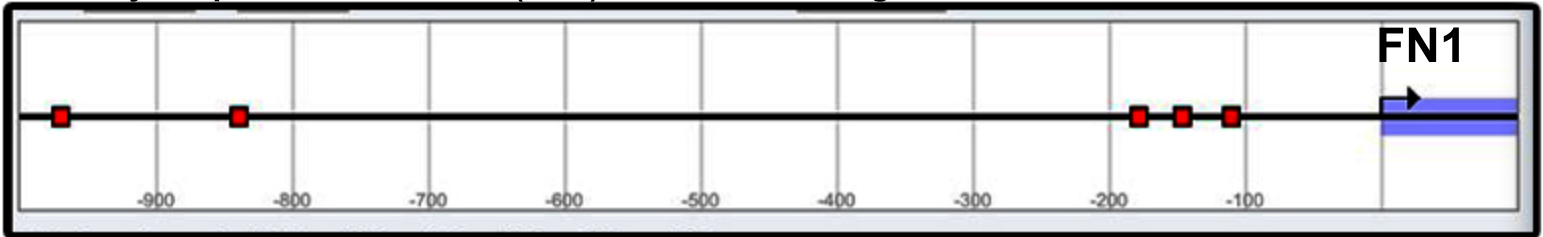


Supplementary Figure 4: MYC promotes the transcription of Fibronectin

a Gene Transcription Regulation database of Transcription Factor binding sites identified from ChIP-seq



b Eukaryotic promoter Database (EPD) MYC Motif Finding Tool

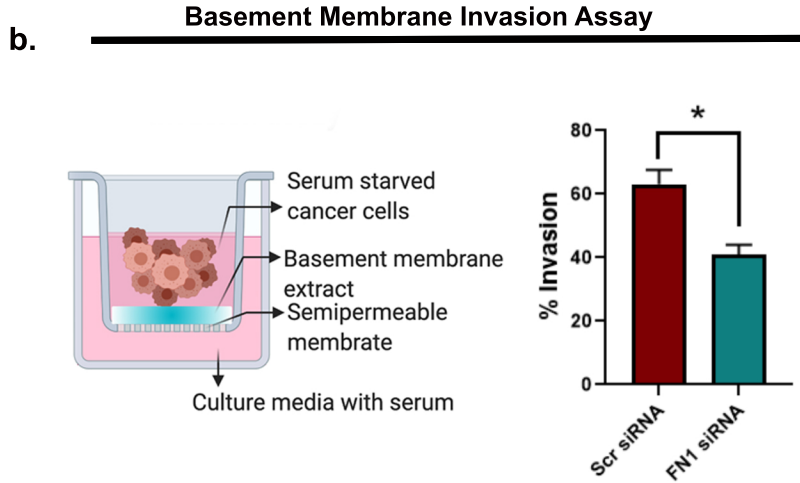
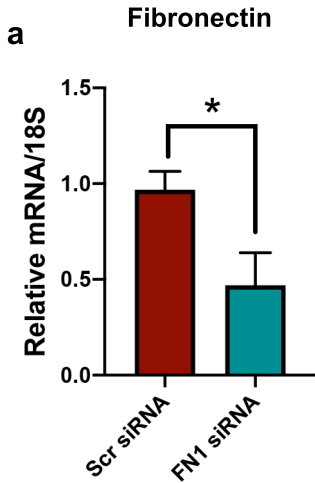


MYC (p=0.001): -116. -152. -184. -845, -976.

c Significant correlation between MYC and FN1 across human cancers in TCGA

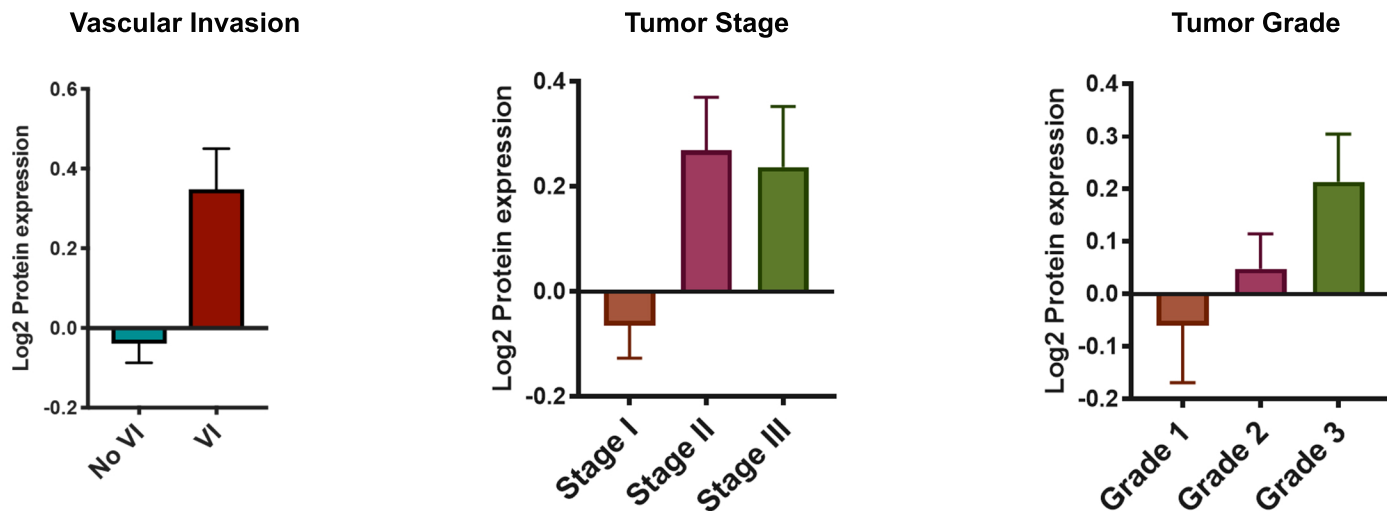
Diseases or Studies	Sample number	Correlation coefficient (r)	p-value
Kidney Clear Cell Carcinoma	603	0.4237	1.13E-27
Kidney Papillary Cell Carcinoma	321	0.393	2.68E-13
Glioblastoma Multiforme	171	0.4121	2.13E-08
Thyroid Carcinoma	571	0.2317	2.15E-08
Pheochromocytoma & Paraganglioma	185	0.3919	3.47E-08
Kidney Chromophobe	91	0.5355	4.52E-08
Adrenocortical Cancer	77	0.5733	5.04E-08
Bladder Urothelial Carcinoma	426	0.2494	1.84E-07
Testicular Germ Cell Tumor	154	0.3684	2.58E-06
Sarcoma	264	0.2431	6.55E-05
Liver Hepatocellular Carcinoma	421	0.1738	3.41E-04
Lung Adenocarcinoma	574	0.1259	2.52E-03
Uterine Corpus Endometrioid Carcinoma	204	0.1763	1.17E-02

Supplementary Figure 5: Fibronectin promotes invasiveness of SNU182

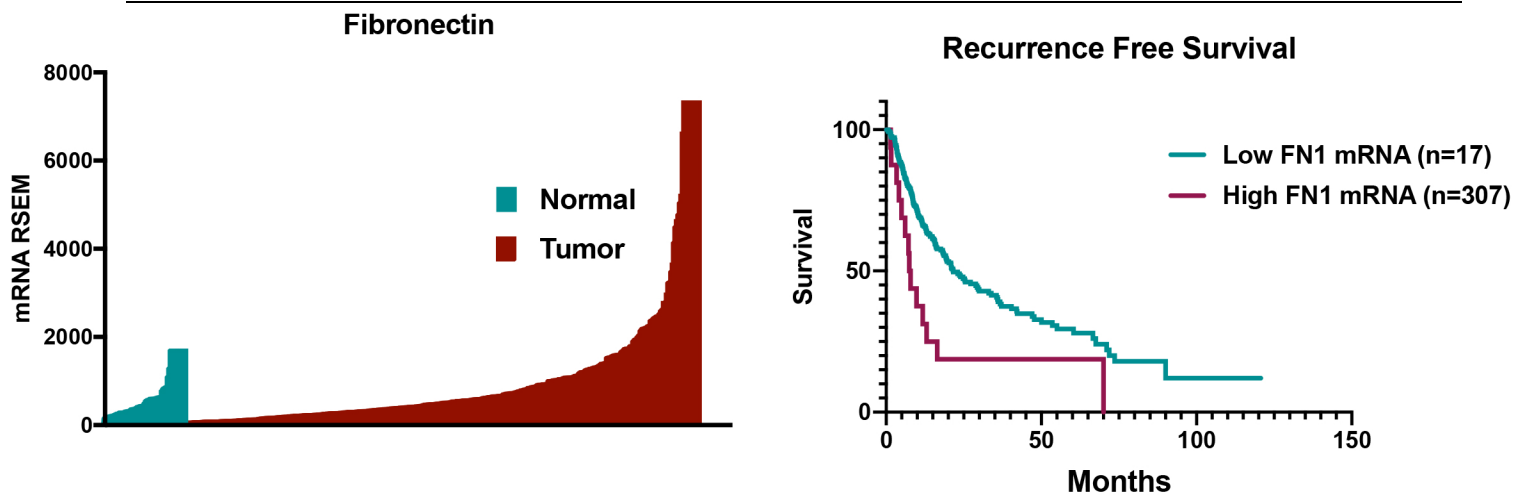


Supplementary Figure 6: Fibronectin expression in human HCC

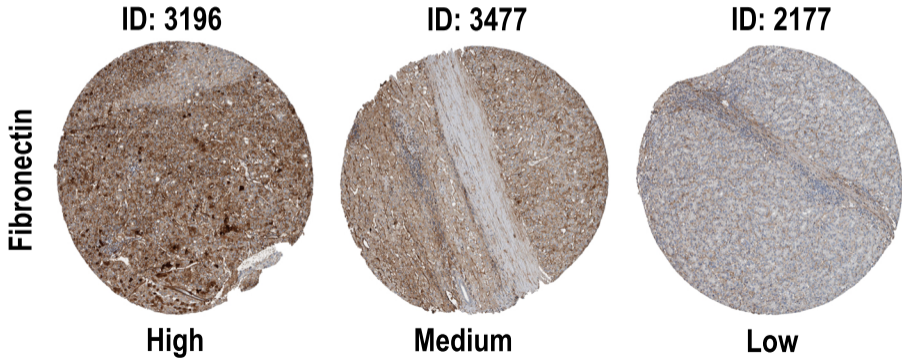
a Fibronectin Tissue Protein Expression



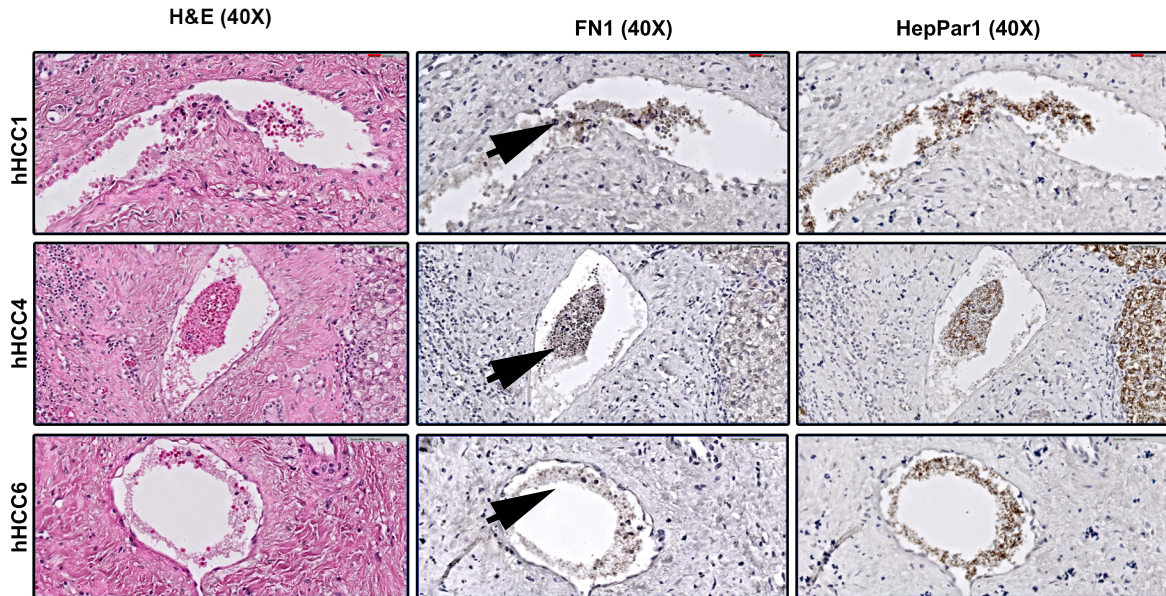
b Fibronectin Tissue mRNA Expression



Supplementary Figure 7: Fibronectin expression in HCPA repository



Supplementary Figure 8: Fibronectin expression in tumor thrombus



Supplementary Table 1: Comparison of significantly mutated genes and somatic copy number variations between HCC with or without vascular invasion.

Somatic Mutations			
Gene	No Vascular Invasion n (%)	Vascular Invasion n (%)	p value
TP53	52 (25.6%)	39 (37.5%)	0.035
HIST1H1C	1 (0.5%)	5 (4.8%)	0.018
AHCTF1	6 (3.0%)	7 (6.7%)	0.139
NFE2L2	5 (2.5%)	6 (5.8%)	0.193
CTNNB1	52 (25.6%)	33 (31.7%)	0.282
PIK3CA	9 (4.4%)	2 (1.9%)	0.344
RB1	7 (3.4%)	6 (5.8%)	0.376
AZIN1	4 (2.0%)	4 (3.8%)	0.450
EEF1A1	8 (3.9%)	2 (1.9%)	0.504
RPS6KA3	7 (3.4%)	5 (4.8%)	0.548
ARID2	9 (4.4%)	6 (5.8%)	0.588
ALB	26 (12.8%)	16 (15.4%)	0.599
BAP1	10 (4.9%)	7 (6.7%)	0.599
KRAS	4 (2.0%)	2 (1.9%)	0.665
CREB3L3	4 (2.0%)	1 (1.0%)	0.665
GPATCH4	5 (2.5%)	1 (1.0%)	0.668
APOB	23 (11.3%)	10 (9.6%)	0.702
CDKN2A	7 (3.4%)	2 (1.9%)	0.723
AXIN1	15 (7.4%)	8 (7.7%)	1.000
ARID1A	14 (6.9%)	7 (6.7%)	1.000
KEAP1	10 (4.9%)	5 (4.8%)	1.000
LZTR1	6 (3.0%)	3 (2.9%)	1.000
1L6ST	7 (3.4%)	3 (2.9%)	1.000
RP1L1	7 (3.4%)	3 (2.9%)	1.000
ACVR2A	6 (3.0%)	3 (2.9%)	1.000
NRAS	3 (1.5%)	2 (1.9%)	1.000
Copy Number Variations			
Gene	No Vascular Invasion n (%)	Vascular Invasion n (%)	p value
CCND1	14 (7%)	7 (6.7%)	0.154
FGF19	11 (5.5%)	6 (5.7%)	0.108
MYC	40 (19.9%)	17 (16.2%)	0.515
MET	5 (2.5%)	6 (5.7%)	0.151

VEGFA	17 (8.5%)	6 (5.7%)	0.066
MCL1	21 (10.4%)	15 (14.3%)	0.838
TERT	13 (6.5%)	7 (6.7%)	0.941
RB1	15 (7.5%)	7 (6.7%)	0.701
CDKN2A	10 (5.0%)	7 (6.7%)	0.113
ERRFI1	7 (3.5%)	4 (3.8%)	0.108
NCOR1	4 (2.0%)	5 (4.8%)	0.187

Supplementary Table 2 Differentially expressed mRNA and microRNA between HCC with or without vascular invasion.

mRNA Differential Expression in HCC with Vascular Invasion		
ID	Adjusted p-value	Difference
GTF2F2	0.000002	74.5
ENGASE	0.000008	151.3
RCCD1	0.000013	71.4
C12orf47	0.000025	79.1
LOC80154	0.000028	119.6
FLJ35024	0.000030	2.2
ZNF391	0.000031	7.1
OAZ2	0.000035	-342.6
ZNF681	0.000054	29.7
EXOSC8	0.000054	66.3
NUDT15	0.000064	70.4
ZMYND8	0.000069	134.6
JMJD6	0.000072	164.4
CENPJ	0.000077	43.4
EEF1E1	0.000089	79.6
CTHRC1	0.000090	203.9
COG1	0.000097	123.2
ZNF137	0.000136	11.6
FAM24B	0.000143	7.5
CNFN	0.000145	25.9
C12orf27	0.000161	46.2
TARS	0.000166	395.8
PTPDC1	0.000178	21.7
DSCR9	0.000182	1.2
DDIT3	0.000199	342.7
SLC1A7	0.000202	270.0
HOXC6	0.000205	19.9
ALDH8A1	0.000213	-865.9
WDR4	0.000225	67.9
EFNA3	0.000259	59.4
TMPRSS6	0.000264	-2210.3
ZNF85	0.000273	17.0
C5orf34	0.000285	14.7

OTUD3	0.000304	30.2
VPS45	0.000305	123.6
FAM104A	0.000308	85.6
APLNR	0.000309	-275.9
DHX37	0.000312	101.8
SKA1	0.000319	65.0
FAM64A	0.000321	27.8
C4orf19	0.000327	-196.5
PROZ	0.000350	-251.3
CSTL1	0.000351	2.0
AAAS	0.000362	106.7
F9	0.000375	-2719.8
DUSP15	0.000382	22.0
GPC4	0.000385	180.7
PLEKHA9	0.000386	15.6
SLC25A19	0.000391	59.9
ZNF273	0.000392	14.4
TMC5	0.000392	287.1
TMPRSS3	0.000401	244.5
HGS	0.000425	461.4
FLJ35776	0.000429	57.0
WDR75	0.000430	94.1
ZNF408	0.000437	94.0
TRIM7	0.000447	24.7
CDC25B	0.000467	458.0
ATF4	0.000471	1432.2
PYCR1	0.000471	502.6
CRIP3	0.000481	79.2
EPRS	0.000508	489.8
CDC20	0.000520	246.2
MX2	0.000522	119.2
C13orf23	0.000535	77.0
ZNF738	0.000549	22.6
UTP6	0.000550	113.7
ZNF389	0.000550	4.3
ZNF208	0.000572	43.9

ERBB2	0.000584	-944.9
ZNF204P	0.000613	38.9
RALA	0.000629	130.8
KRTAP5-7	0.000645	2.9
C2orf27A	0.000662	7.1
XPOT	0.000680	345.6
HOXD9	0.000703	34.4
ZNF193	0.000721	28.7
INO80C	0.000721	101.2
GGPS1	0.000742	98.0
SPATA1	0.000743	-1.6
PCDHA1	0.000746	83.9
PA2G4	0.000780	556.8
B3GNTL1	0.000797	73.1
DDX54	0.000798	286.5
ZNF296	0.000805	18.7
ANAPC7	0.000815	134.2
LOC440356	0.000825	2.6
LOC399815	0.000842	2.6
CCNC	0.000853	-246.4
LPCAT1	0.000859	613.2
WASH7P	0.000868	266.2
TIGD7	0.000869	37.2
WASH5P	0.000881	57.6
FAM27C	0.000882	17.0
GAS5	0.000891	908.5
C17orf90	0.000905	170.4
CBX8	0.000907	66.0
NAT9	0.000949	165.4
AKAP8L	0.000959	175.8
SIRT7	0.000980	122.3
FARSB	0.000989	136.9
IQCD	0.000995	15.2

Micro RNA Differential Expression in HCC with Vascular Invasion

ID	Adjusted p-value	Difference
hsa-mir-122	0.0158	-4399.4

hsa-mir-100	0.0295	-1598.2
hsa-let-7a-2	0.0374	-1539.3
hsa-let-7a-3	0.0389	-766.9
hsa-let-7a-1	0.0405	-757.8
hsa-mir-126	0.0437	-685.0
hsa-let-7c	0.0444	-399.8
hsa-mir-27b	0.0419	-276.4
hsa-mir-23b	0.0154	-222.3
hsa-let-7g	0.0237	-99.9
hsa-mir-152	0.0045	-77.0
hsa-mir-139	0.0392	-28.4
hsa-mir-378c	0.0092	-6.9
hsa-mir-24-1	0.0236	-4.7
hsa-mir-1538	0.0267	-0.1
hsa-mir-3152	0.0096	-0.1
hsa-mir-3622a	0.0250	-0.1
hsa-mir-133a-2	0.0395	0.0
hsa-mir-623	0.0271	0.0
hsa-mir-3650	0.0071	0.0
hsa-mir-548aa-2	0.0215	0.0
hsa-mir-320b-1	0.0087	0.0
hsa-mir-548u	0.0016	0.0
hsa-mir-3675	0.0090	0.0
hsa-mir-448	0.0422	0.0
hsa-mir-1290	0.0419	0.0
hsa-mir-3910-2	0.0345	0.0
hsa-mir-1246	0.0101	0.0
hsa-mir-548a-3	0.0289	0.0
hsa-mir-1243	0.0305	0.0
hsa-mir-1264	0.0421	0.1
hsa-mir-3150	0.0077	0.1
hsa-mir-3684	0.0069	0.1
hsa-mir-1537	0.0022	0.1
hsa-mir-1250	0.0386	0.1
hsa-mir-3680	0.0146	0.1
hsa-mir-3941	0.0242	0.1

hsa-mir-3691	0.0116	0.2
hsa-mir-488	0.0403	0.2
hsa-mir-3136	0.0142	0.2
hsa-mir-1254	0.0029	0.2
hsa-mir-3651	0.0253	0.3
hsa-mir-934	0.0319	0.3
hsa-mir-34b	0.0163	0.4
hsa-mir-1912	0.0063	0.4
hsa-mir-3660	0.0363	0.5
hsa-mir-3127	0.0496	0.6
hsa-mir-642a	0.0404	0.7
hsa-mir-550a-1	0.0166	0.7
hsa-mir-548y	0.0076	0.7
hsa-mir-212	0.0352	0.7
hsa-mir-9-3	0.0251	0.8
hsa-mir-1298	0.0047	0.9
hsa-mir-1270-1	0.0112	1.0
hsa-mir-1270-2	0.0125	1.1
hsa-mir-942	0.0461	1.1
hsa-mir-616	0.0482	1.6
hsa-mir-1911	0.0066	2.3
hsa-mir-301a	0.0484	2.6
hsa-mir-34c	0.0200	2.7
hsa-mir-1180	0.0201	8.8
hsa-mir-423	0.0227	21.0
hsa-mir-629	0.0076	23.2
hsa-mir-15a	0.0289	29.7
hsa-mir-452	0.0163	85.6
hsa-mir-582	0.0045	119.3
hsa-mir-223	0.0298	122.3
hsa-mir-9-2	0.0084	504.1
hsa-mir-9-1	0.0086	505.5
hsa-mir-21	0.0061	35419.3

Supplementary Table 3 Differentially expressed proteins between HCC with or without vascular invasion.

NAME	p-value	q-value	Log2 Fold change
Fibronectin	0.000156061	0.006454424	1.31
PAI-1	0.002965332	0.013311046	1.31
PEA15_pS116	0.006283621	0.021513413	1.22
Ku80	0.003757161	0.015178929	1.14
ERK2	0.001476784	0.009227338	1.13
C Raf	0.00296315	0.013311046	1.13
Smad3	0.00045974	0.006454424	1.12
PEA15	0.001553116	0.009227338	1.12
Smac	0.004687466	0.017865438	1.12
AMPK_alpha	0.005687312	0.020155034	1.12
4E BP1_pT20	0.0008379	0.006863774	1.11
Bax	0.001412655	0.009205043	1.11
S6	0.002687144	0.0132391	1.11
4EBP1_pT37_T46	0.018193405	0.046371851	1.11
YB1	0.019067075	0.046970112	1.11
Bim	0.005294348	0.019444696	1.1
VEGFR2	0.013498676	0.037871285	1.1
4E-BP1_pS65	0.000482863	0.006454424	1.09
LKB1	0.000899383	0.006863774	1.09
eEF2	0.012529766	0.036681345	1.09
RBM15	0.012946087	0.03702472	1.09
TSC1	0.019835649	0.047700014	1.09
14-3-3_zeta	0.021387717	0.049658838	1.09
Annexin_VII	0.001291842	0.008698406	1.08
Rictor_pT1135	0.002393188	0.012700725	1.08
Bid	0.003033903	0.013322791	1.08
GSK3-alpha-beta	0.007297236	0.024164616	1.08
Raptor	0.00772864	0.024780717	1.08
PI3K-p85	0.018365089	0.046371851	1.08
Collagen_VI	0.020150637	0.047887396	1.08
HER3_pY1289	0.002346162	0.012700725	1.07
NF2	0.007466903	0.024327652	1.07
YAP	0.002947793	0.013311046	1.06
PDK1_pS241	0.017925317	0.046371851	1.06

4EBP1	0.020635641	0.048469761	1.06
PDK1	0.00245212	0.012700725	1.05
IRS1	0.012338773	0.036653414	1.05
ciAP	0.00842476	0.026590647	1.04
Stathmin	0.010623641	0.03251478	1.04
YB1_pS102	0.019603407	0.047700014	1.04
EGFR_pY1173	0.014966938	0.040310952	1.03
ADAR1	0.017129215	0.044936381	1.03
Chk1_pS345	0.01901539	0.046970112	0.97
Notch1	0.003311756	0.013936973	0.96
14-3-3_epsilon	0.014928847	0.040310952	0.96
FOXO3a_pS318_S321	0.000676782	0.006454424	0.95
PKC delta_pS664	0.001537112	0.009227338	0.95
ACVRL1	0.002545762	0.012856097	0.95
p27	0.003282489	0.013936973	0.95
PR	0.004190262	0.016549346	0.95
HER2_pY1248	0.012136428	0.036590424	0.95
cJun_pS73	0.005545778	0.020004415	0.94
ARaf_pS299	0.006737936	0.022684386	0.94
STAT3_pY705	0.000645392	0.006454424	0.93
mTOR_pS2448	0.000269827	0.006454424	0.93
Beclin	0.002882264	0.013311046	0.93
FoxM1	0.0057912	0.020169352	0.93
Akt	0.014234861	0.039389616	0.93
p90RSK_pT359_S363	0.000877583	0.006863774	0.92
PARP_cleaved	0.001992947	0.011502149	0.92
CD31	0.00369182	0.015178929	0.92
Tuberin_pT1462	0.005214248	0.019444696	0.92
SCD1	0.008691526	0.027010588	0.92
TTF1	0.000702957	0.006454424	0.91
Chk1	0.004260228	0.016549346	0.91
GSK3_pS9	0.013013639	0.03702472	0.91
SF2	0.000677749	0.006454424	0.9
Snail	0.000677479	0.006454424	0.9
p70S6K_pT389	0.000557078	0.006454424	0.9
Bcl2	0.000211847	0.006454424	0.9

Rad51	0.000951414	0.006863774	0.89
XBP1	0.000817714	0.006863774	0.89
c Met	0.002233519	0.012532521	0.89
PREX1	0.0000294	0.002973207	0.88
Annexin_1	0.000330374	0.006454424	0.88
INPP4B	0.000147511	0.006454424	0.88
MSH2	0.000640662	0.006454424	0.87
EGFR_pY1068	0.000526732	0.006454424	0.87
Src_pY416	0.00032104	0.006454424	0.87
VHL	0.016017704	0.042573371	0.87
ER alpha	0.000606394	0.006454424	0.86
FOXO3a	0.000454781	0.006454424	0.86
JAB1	0.000947978	0.006863774	0.86
p53	0.001123584	0.007826346	0.86
ARHI	0.000685413	0.006454424	0.85
MAPK_pT202_Y204	0.0000228	0.002973207	0.84
Akt_pS473	0.000213226	0.006454424	0.84