

SUPPLEMENTARY TABLES

Dissecting the role of the NADPH Oxidase NOX4 in TGF-beta signaling in hepatocellular carcinoma

Rut Espinosa-Sotelo^{a,b}, Noel P. Fusté^a, Irene Peñuelas-Haro^{a,b}, Ania Alay^{c,d}, Gabriel Pons^e, Xènia Almodóvar^a, Júlia Albaladejo^a, Ismael Sánchez-Vera^e, Ricard Bonilla-Amadeo^a, Francesco Dituri^f, Grazia Serino^f, Emilio Ramos^{b,g}, Teresa Serrano^{b,h}, Mariona Calvoⁱ, María Luz Martínez-Chantar^{b,j}, Gianluigi Giannelli^f, Esther Bertran^{a,b}, Isabel Fabregat^{a,b,*}.

Supplementary Table 1. Clinico-pathological characteristics of HCC patients used in this study.

Patient ID	Liver pathology	Etiology	Tumor size (cm)	Histological grade	Status	Follow-up (months)	Relapse (Yes/No)
1	Cirrhosis	Alcohol	2	2	Death		No
2	Cirrhosis	Unknown	4.5	3	Death	45	Yes
3	No Fibrosis	Unknown	6	2	Death	45	No
4	No Fibrosis	Unknown	7	2	Death	43	No
5	No Fibrosis	Unknown	20	2	Death	12	Yes
6	Cirrhosis	VHC	4.5	4	Alive	98	No
7	No Fibrosis	Alcohol	2	2	Alive	86	No
8	Cirrhosis	VHC	5	3	Alive	81	Yes
9	Cirrhosis	VHC	2.1	3	Death	42	Yes
10	No Fibrosis	VHB	5.5	3	Alive	77	Yes
11	Cirrhosis	VHC	2	3	Alive	74	No
12	Cirrhosis	VHC	3.2		Alive	72	No
13	No Fibrosis	VHC	9.5	2	Alive	71	No
14	Cirrhosis	VHB	4.9	2	Alive	71	No
15	Cirrhosis	Alcohol	2.5	2	Death	57	Yes
16	No Fibrosis	VHC	2.9	2	Alive	70	No
17	Cirrhosis	VHC	1.2	2	Alive	65	Yes
18	Cirrhosis	Alcohol	26	2	Alive	61	Yes
19	No Fibrosis	Unknown	8	3	Alive	61	No
20	Cirrhosis	VHC	4.5	2	Alive	61	No
21	Cirrhosis	Alcohol	3.8	3	Alive	60	No
22	Mild Fibrosis	Unknown	4	2	Alive	59	No
23	Mild Fibrosis	VHC	1.2	3	Alive	59	No
24	Cirrhosis	VHC	1.8	1	Alive	59	No
25	Mild Fibrosis	Unknown	4.5	3	Alive	49	No
26	Cirrhosis	VHC	5.8	3	Alive	48	Yes
27	Cirrhosis	VHC	5.5	2	Alive	22	No
28	Cirrhosis	NASH	2.5	2	Alive	38	Yes
29	Cirrhosis	Alcohol	3	3	Death	43	Yes
30	Cirrhosis	VHC	2.5	3	Alive	125	Yes
31	Mild Fibrosis	Alcohol	3	3	Alive	121	Yes
32	No Fibrosis	Unknown	27	3	Death	13	Yes
33	Mild Fibrosis	Unknown	7.5	3	Death	28	Yes

34	Cirrhosis	VHC	3	3	Death	89	No
35	Cirrhosis	Unknown	6	2	Death	109	No
36	Cirrhosis	VHC	2.5	2	Alive	111	No
37	Mild Fibrosis	VHC	3.8	2	Death	81	No
38	Cirrhosis	VHC	3.3	3	Alive	51	Yes
39	Cirrhosis	VHC	4.5	3	Death	66	Yes
40	No Fibrosis	Unknown	3	2	Alive	106	Yes
41	Mild Fibrosis	VHB	2.9	2	Alive	105	No
42	Cirrhosis	VHC	2.8	2	Alive	105	No
43	Mild Fibrosis	VHC	3.5	3	Alive	94	No
44	Cirrhosis	VHC	3.5	3	Death	35	No
45	Cirrhosis	VHC	5	2	Alive	94	Yes
46	No Fibrosis	Unknown	6.5	2	Alive	92	No
47	No Fibrosis	Unknown	20	1	Alive	87	No
48	Cirrhosis	VHC	2.5	2	Death	47	Yes
49	No Fibrosis	VHB	9	3	Death	13	Yes
50	Mild Fibrosis	NASH	4.2	3	Death	30	Yes
51	No Fibrosis	Unknown	8.3	3	Death	8	Yes
52	Cirrhosis	Alcohol	2.6	2	Alive	77	No
53	Cirrhosis	Alcohol	2.3	2	Death	60	No
54	Cirrhosis	VHC	2.3	2	Alive	29	Yes
55	Mild Fibrosis	Alcohol	1.9	2	Alive	67	No
56	No Fibrosis	VHC	4.7	2	Alive	64	No
57	Cirrhosis	VHC	2.5	4	Alive	64	Yes
58	Cirrhosis	VHC	3	2	Alive	64	Yes
59	Cirrhosis	Unknown	2.8	2	Alive	62	No
60	Cirrhosis	VHC	2.5	2	Death	13	Yes
61	Cirrhosis	VHB	4.9	4	Death	6	Yes
62	Cirrhosis	VHC	8.5	3	Death	15	Yes
63	No Fibrosis	VHC	2.5	4	Alive	75	No
64	Cirrhosis	VHC	5.5	3	Alive	75	Yes
65	Cirrhosis	Alcohol	1.4	2	Alive	73	No
66	No Fibrosis	Alcohol	7	2	Alive	53	Yes
67	Cirrhosis	Alcohol	16	2	Alive	22	Yes
68	No Fibrosis	Unknown	3.5	1	Alive	45	No
69	Cirrhosis	VHC	1.5	3	Alive	44	No
70	Mild Fibrosis	VHC	9	3	Alive	41	No

71	No Fibrosis	Unknown	4.8	3	Death	16	Yes
72	Cirrhosis	VHC	2.8	4	Death	23	Yes
73	Cirrhosis	VHC	3.7	2	Alive		No
74	Cirrhosis	VHB	3.3	2	Death	12	Yes
75	Cirrhosis	Alcohol	2.6	1	Alive	25	No
76	No Fibrosis	Unknown					
77	No Fibrosis	Unknown	12	2	Alive	35	No
78	Cirrhosis	Alcohol	2.8	2	Alive	20	Yes
79	No Fibrosis	Unknown	11	2	Alive	20	Yes
80	Cirrhosis	Unknown	4.5	2	Alive	18	No
81	No Fibrosis	Unknown	3.5	2	Alive	18	No
82	Mild Fibrosis	Unknown	7.5	3	Alive	17	Yes
83	Cirrhosis	Alcohol	4	2	Alive	16	No
84	No Fibrosis	Unknown	8.6	2	Alive	16	No
85	Cirrhosis	Alcohol	2	2	Alive	15	No
86	Mild Fibrosis	Alcohol	3	3	Alive	15	Yes
87	Cirrhosis	Alcohol	3.7	3	Alive	15	Yes
88	No Fibrosis	Unknown	5.5	2	Alive	13	No
89	Cirrhosis	VHC	2.5	2	Alive	9	No
90	Cirrhosis	Alcohol	1	2	Alive	80	Yes
91	No Fibrosis	VHC	3.5	2	Alive		Yes
92	Cirrhosis	VHC	2.5	1	Alive	45	No
93	Cirrhosis	VHC	3.8	2	Alive	44	No
94	Cirrhosis	VHC	2.2	2	Alive	42	No
95	Cirrhosis	VHC	1.2	2	Alive	38	No
96	Cirrhosis	Alcohol	1.7	2	Alive	37	No
97	Cirrhosis	VHB	3	3	Death	7	Yes
98	Mild Fibrosis	VHC	5.2	3	Alive	35	Yes
99	Mild Fibrosis	Alcohol	6	4	Alive	35	No
100	Cirrhosis	VHB	3	4	Alive	29	Yes
101	Cirrhosis	VHC	1.8	2	Alive	27	Yes
102	Cirrhosis	VHC	3.5	3	Alive	24	Yes
103	Cirrhosis	Alcohol	2	2	Alive	21	No
104	No Fibrosis	Unknown	6.5	2	Alive	21	No
105	Cirrhosis	Alcohol	4.5	3	Alive	21	No
106	No Fibrosis	Unknown	6.7	2	Alive	20	No
107	Cirrhosis	VHB	4.7	3	Alive	19	Yes

108	Mild Fibrosis	Unknown	8	2	Alive	17	No
109	Mild Fibrosis	Unknown	9	2	Alive	17	Yes
110	Cirrhosis	VHC	1.5	2	Alive	15	No
111	No Fibrosis	Unknown	7.5	1	Alive	14	No
112	No Fibrosis	Unknown	5	1	Alive	13	No
113	Cirrhosis	NASH	2.5	2	Alive	13	No
114	No Fibrosis	Unknown	4.5	2	Alive	10	No
115	Cirrhosis	VHC	2.8	2	Alive	12	No
116	No Fibrosis	Unknown	9	2	Alive	9	No
117	No Fibrosis	VHB	3.8	2	Alive	9	No
118	Cirrhosis	VHC	1.3	2	Alive	10	No
119	No Fibrosis	Unknown	4	2	Alive	10	No
120	Cirrhosis	VHC	4.3	2	Alive	8	Yes
121	Cirrhosis	VHC	4	2	Alive	8	No

HBV: Hepatitis B virus; **HCV:** Hepatitis C virus; **NASH:** Non Alcoholic SteatoHepatitis

Supplementary Table 2. Antibodies and conditions used in this study.

Primary antibody	Secondary antibody	Working dilution	Application	Reference
AKT	Rabbit	1:1000	WB	Cell Signaling #9272
ATPb	Mouse	1:1000	WB	Abcam #ab556467
Bcl-xL	Mouse	1:1000	WB	BD Biosciences #610746
Calreticulin	Mouse	1:1000	WB	Abcam #ab22683
CYCLIN D1	Mouse	1:500	WB	BD Biosciences #556470
E-Cadherin	Mouse	1:50	IF	BD Biosciences #610182
EGFR	Rabbit	1:1000	WB	Cell Signaling #2232
GAPDH	Mouse	1:1000	WB	Milipore #MAB374
Hic-5	Mouse	1:50	IF	BD Biosciences #611165
Histone 3	Rabbit	1:1000	WB	Abcam # ab1791
Hsp27	Mouse	1:1000	WB	Cell Signaling #2402
MCL1	Mouse	1:1000	WB	Santa Cruz #sc-12756
MYC	Rabbit	1:1000	WB	Abcam #ab32072
NOX4	Rabbit	1:1000	WB	Prosci #7927
NOX4	Rabbit	1:1000	WB/IF	Abcam #ab62352
pAKT (Ser473)	Rabbit	1:1000	WB	Cell Signaling #4060
pEGFR (Tyr1068)	Rabbit	1:1000	WB	Cell Signaling #3777
Phalloidin	-	1:500	IF	Sigma #P1951
pSMAD 2 (Ser465/467)	Rabbit	1:1000	WB	Cell Signaling #3108
pSMAD 3 (Ser423/425)	Rabbit	1:1000	WB	Milipore #07-1389
SMAD 2	Mouse	1:1000	WB	Cell Signaling #3103
SMAD 3	Rabbit	1:1000	WB	Abcam #ab40854
Vinculin	Mouse	1:50	IF	Sigma #V9131
β-ACTIN	Mouse	1:3000	WB	Sigma #A5441

Supplementary Table 3. Primer sequences used in LightCycler 480 Sybr Green System quantitative PCR.

Gene	Forward (5'-3')	Reverse (3'-5')
BCL2L1	CCTGCCTGCCTTTGCCTAA	CCCGGTTGCTCTGAGACATT
BCL2L11	TAAGTTCTGAGTGTGACCGAGA	GCTCTGTCTGTAGGGAGGTAGG
BMF	CAAATCTGAACAAGCCCAAGTCTCCAG	CACACAGCTTAGTGAGCAGAACACAA
CCND1	AGCTGTGCATCTACACCGAC	GAAATCGTGCGGGGTCATTG
CDC42	CAGGGCAAGAGGATTATGACAG	GTTATCTCAGGCACCCACTT
HPRT1	CCTGGCGTCGTGATTAGTGA	CGAGCAAGACGTTCAAGTCCT
L32	AACGTCAAGGAGCTGGAAG	GGGTTGGTGACTCTGATGG
MCL1	TGCTTCGGAAACTGGACATCA	TAGCCACAAAGGCACCAAAAG
MMP9	ACCTCGAACTTTGACAGCGACA	GATGCCATTCACGTCGTCCTTA
MYC	CCCGCTTCTCTGAAAGGCTCTC	CTCTGCTGCTGCTGCTGGTAG
NOX4	GCAGGAGAACCAGGAGATTG	CACTGAGAAGTTGAGGGCATT
RAC1	GCTTTTCCCTTGTGAGTCCTG	CCTTCAGTTTCTCGATCGTGTC
RHOA	AGCTGGGCAGGAAGATTATG	CGTTGGGACAGAAATGCTTG
RHOC	CAAGACGAGCACACCAGG	AGCACTCAAGGTAGCCAAAG
SERPINE1	TCTGCCCTCACCAACATTC	GGTCATTCCCAGGTTCTCTAG
SMAD7	CGGACAACAAGAGTCAGCTGGT	GTCCTGGAGTCCGGGTTGTC
SNAI1	GCTGCAGGACTCTAATCCAGAGTT	GACAGAGTCCAGATGAGCATTG
SNAI2	ACACATTAGAACTCACACGGG	TGGAGAAGTTTTGGAGCAG
TBP	CCGCCGGCTGTAACTTC	AGAAACAGTGATGCTGGGTCA
VIM	CGTGAATACCAAGACCTGCTC	GGAAAAGTTTTGGAAGAGGCAG

Supplementary Table 4. TGF- β signaling from Hallmarks of Cancer Signature gene members.

ACVR1	CDK9	HIPK2	LTBP2	RAB31	SMAD6	THBS1
APC	CDKN1C	ID1	MAP3K7	RHOA	SMAD7	TJP1
ARID4B	CTNNB1	ID2	NCOR2	SERPINE1	SMURF1	TRIM33
BCAR3	ENG	ID3	NOG	SKI	SMURF2	UBE2D3
BMP2	FKBP1A	IFNGR2	PMEPA1	SKIL	SPTBN1	WWTR1
BMPR1A	FNTA	JUNB	PPM1A	SLC20A1	TGFB1	XIAP
BMPR2	FURIN	KLF10	PPP1CA	SMAD1	TGFBR1	
CDH1	HDAC1	LEFTY2	PPP1R15A	SMAD3	TGIF1	