Additional File

Y-box binding protein-1 is crucial in acquired drug resistance development in metastatic clear-cell renal cell carcinoma

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Running title: YB-1/ABCB-1 in sunitinib-resistant kidney tumors

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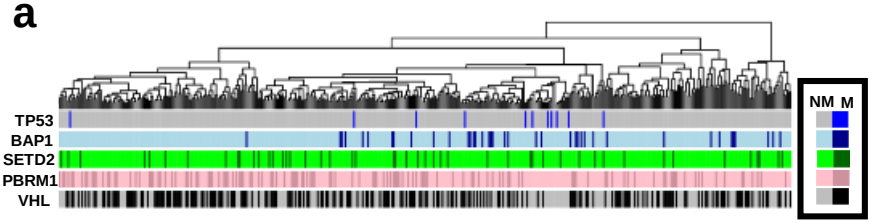
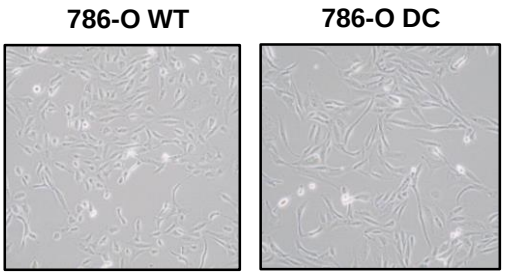
a



TCGA Dataset	Nature 2013
VHL mutated	211
VHL non-mutated	212
Total	**423**

b



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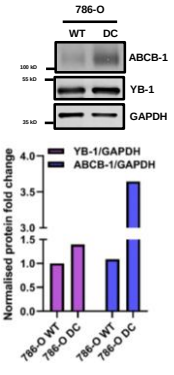

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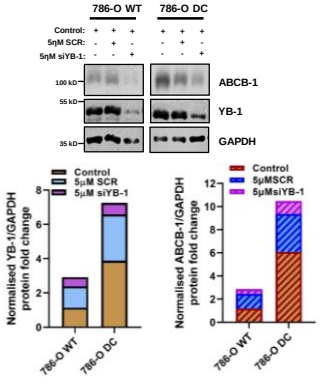

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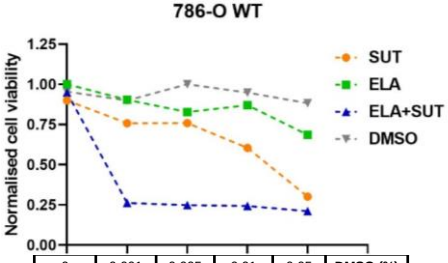
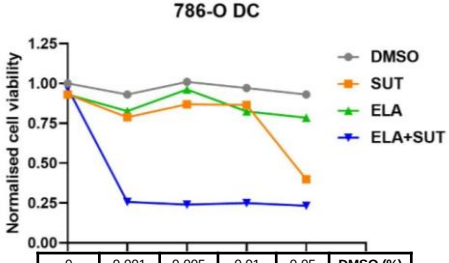
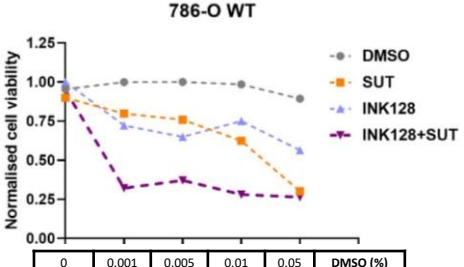


0	0.001	0.005	0.01	0.05	DMSO (%)
0	1.0	2.5	5	10	ELA (μM)
0	2.5	5	10	20	SUT (μM)
0 + 0	1.0 + 10	2.5 + 10	5 + 10	10 + 10	ELA (μM) + SUT (μM)

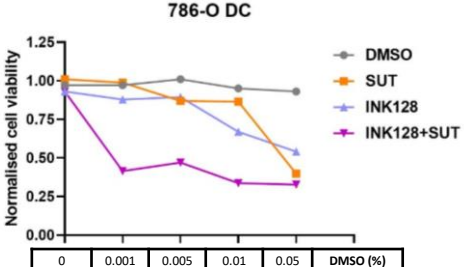


0	0.001	0.005	0.01	0.05	DMSO (%)
0	1.0	2.5	5	10	ELA (μM)
0	2.5	5	10	20	SUT (μM)
0 + 0	1.0 + 10	2.5 + 10	5 + 10	10 + 10	ELA (μM) + SUT (μM)

f



0	0.001	0.005	0.01	0.05	DMSO (%)
0	0.25	0.5	1.0	2.5	INK128 (μM)
0	2.5	5	10	20	SUT (μM)
0 + 0	0.25 + 10	0.5 + 10	1.0 + 10	2.5 + 10	INK128 (μM) + SUT (μM)



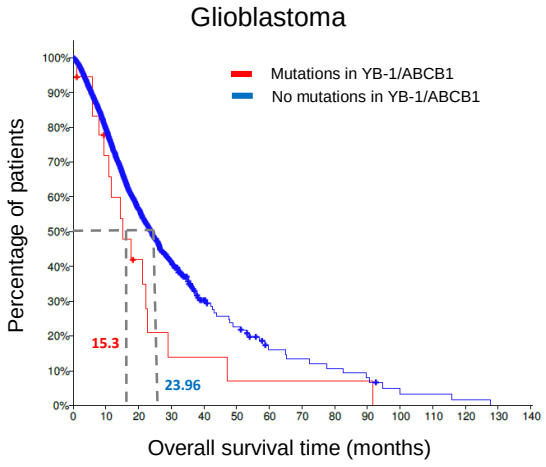
0	0.001	0.005	0.01	0.05	DMSO (%)
0	0.25	0.5	1.0	2.5	INK128 (μM)
0	2.5	5	10	20	SUT (μM)
0 + 0	0.25 + 10	0.5 + 10	1.0 + 10	2.5 + 10	INK128 (μM) + SUT (μM)

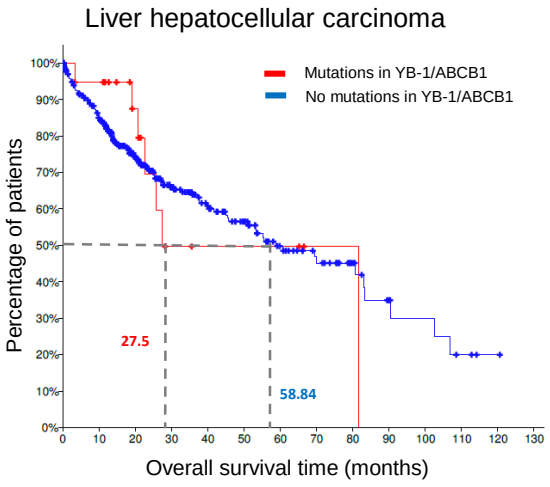
Figure 1

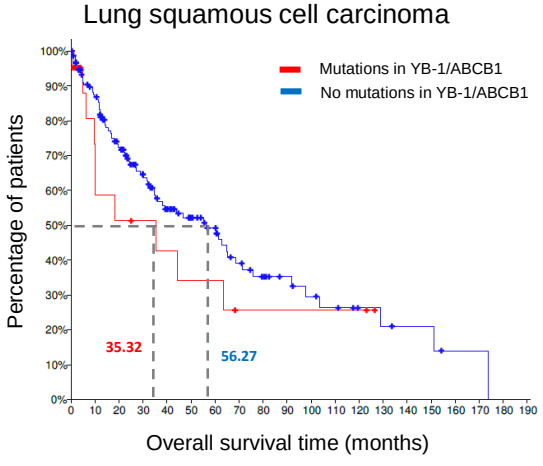
Additional File. Figure 1: VHL mutated cell-line 786-O also develop acquired sunitinib-resistance by YB-1/ABCB-1 pathway*.* a) Gene mutation data from TCGA KIRC dataset showing 212 ccRCC patients are VHL mutated and 211 non-mutated (total ccRCC patient = 423, shown in the table), b) morphological changes observed in 786-O WT and DC, c) YB-1 and ABCB-1 protein levels in 786-O WT and DC, d) knocking down YB-1 with siYB-1 also decreased ABCB-1 protein level, e) treatment response of 786-O WT and DC to ABCB-1 blocker, elacridar, with/without sunitinib, and f) with mTOR inhibitor, INK128. SUT: sunitinib, ELA: elacridar, SCR: esiEGFP, siYB-1: esiYB1.

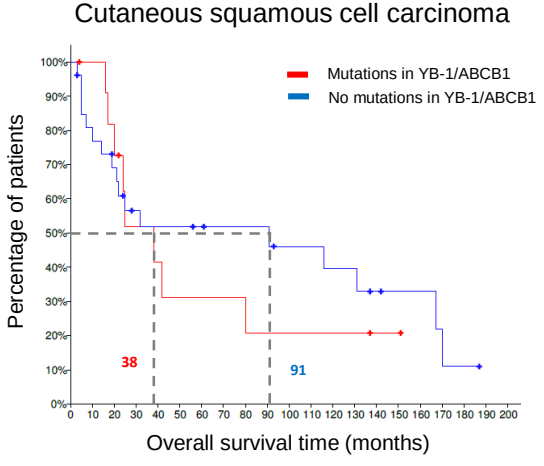
Gene	Accession#	Name	Peptide#	Log FC_80 mg/kg	t	Score	p-value	FDR
ABCB1	P08183	Multidrug resistance protein-1	26	-0.45323	-2.29642	0.053957	4.10E-11	9.55E-09
ABCB10	Q9NRK6	ATP-bonding cassette sub-family B member 10	5	0.267708	1.8302	0.108369	0.010748	0.152629
ABCC4	O15439	Multidrug resistance-associated protein 4	12	0.473752	2.73992	0.027898	6.12E-08	7.98E-06
ABCD1	P33897	ATP-bonding cassette sub-family D member 1	6	-0.30498	-1.41874	0.197421	0.042111	0.34299
ABCD3	P28288	ATP-bonding cassette sub-family D member 3	12	-0.22344	-1.26589	0.244637	0.02586	0.262226
ABCE1	P61221	ATP-bonding cassette sub-family E member 1	16	0.195736	1.60078	0.151901	0.000465	0.016489
ABCF2	Q9UG63	ATP-bonding cassette sub-family F member 2	20	0.23689	1.651535	0.14105	5.74E-05	0.003047

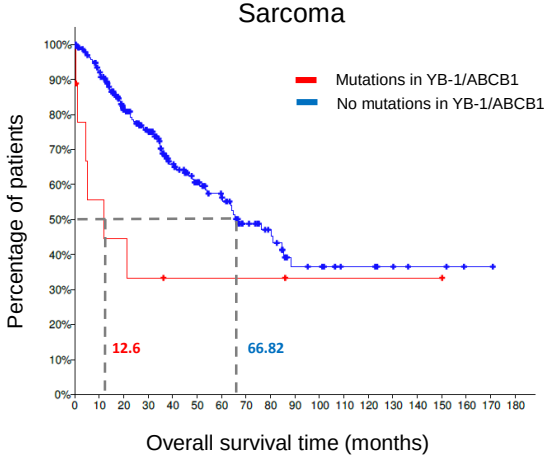
Additional File. Figure 2: Proteome data analysis. Tumors from sunitinb-sensitive and resistant rodents were analysed for difference in protein expression pattern. Significant upregulation of ATP-binding cassette transporter proteins were observed in sunitinib-resistant tumors compared to the sensitive tumors. ABCB-1 was the most markedly upregulated among other transporters from the same family of proteins. Individual p-values were calculated using Student's t-test, n=3 animals/group.


Glioblastoma
Mutations in YB-1/ABCB1
No mutations in YB-1/ABCB1
Percentage of patients
15.3
23.96
Overall survival time (months)


Liver hepatocellular carcinoma
Mutations in YB-1/ABCB1
No mutations in YB-1/ABCB1
Percentage of patients
27.5
58.84
Overall survival time (months)


Lung squamous cell carcinoma
Mutations in YB-1/ABCB1
No mutations in YB-1/ABCB1
Percentage of patients
35.32
56.27
Overall survival time (months)


Cutaneous squamous cell carcinoma
Mutations in YB-1/ABCB1
No mutations in YB-1/ABCB1
Percentage of patients
38
91
Overall survival time (months)


Sarcoma
Mutations in YB-1/ABCB1
No mutations in YB-1/ABCB1
Percentage of patients
12.6
66.82
Overall survival time (months)

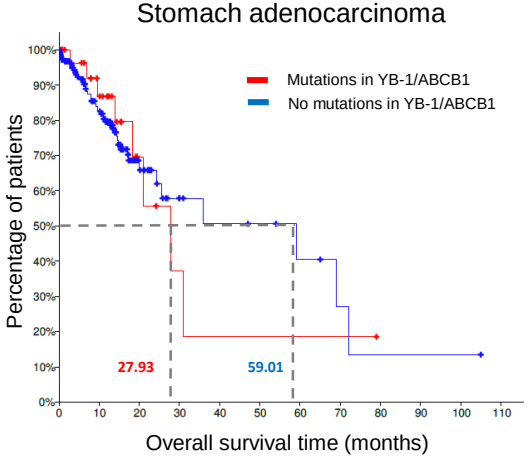

Stomach adenocarcinoma
Mutations in YB-1/ABCB1
No mutations in YB-1/ABCB1
Percentage of patients
27.93
59.01
Overall survival time (months)

Figure 3

Additional File. Figure 3: Mutation of YBX1 and ABCB1 gene in other types of cancer and the overall survival in patients. To investigate the relevance of YBX1/ABCB1 pathway in other cancer types, we analyzed patient data from TCGA cBioPortal for overall survival in patients. As shown in the Kaplan-Meier curves, drastic decrease in survival time is observed in glioblastoma, hepatocellular carcinoma, lung squamous cell carcinoma, cutaneous squamous cell carcinoma, sarcoma and stomach adenocarcinoma patients when tumors had mutation in YBX1 and ABCB1 genes (red) compared to the tumors with no mutation (blue).