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Supplemental Information

Modulation of mTOR and epigenetic pathways as therapeutics in gallbladder cancer

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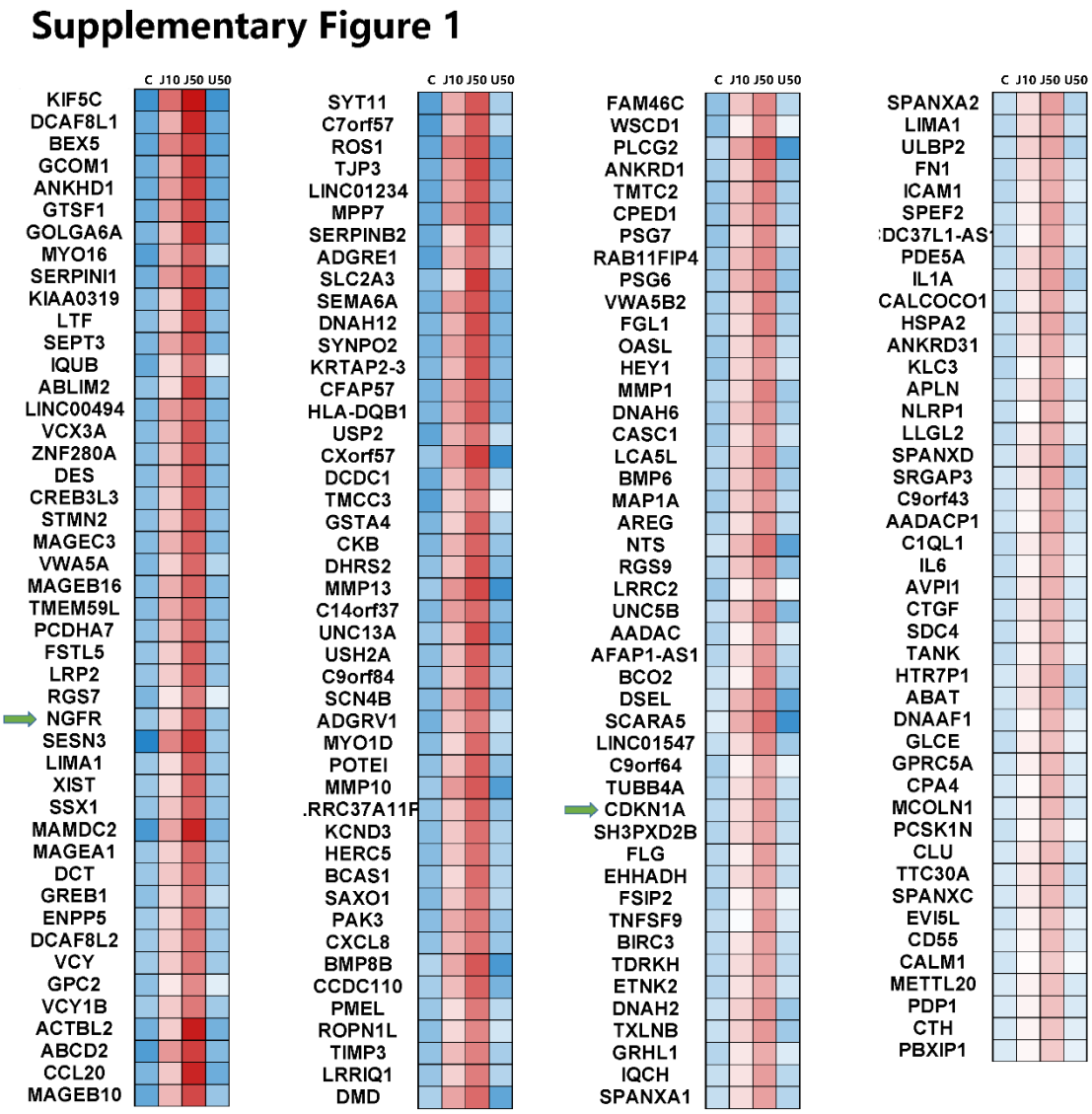
Anti-proliferative effect of JNJ-26481585 is at least partially mediated by CDKN1A and/or NGFR knockdown in gallbladder cancer.

To understand the anti-proliferative mechanisms of JNJ-26481585, we extracted total RNA from GBC-SD cell lines treated with high-concentration JNJ-26481585 (50nM), low-concentration JNJ-26481585 (10nM), high-concentration UNC1999 (50nM) or DMSO separately and performed comparable RNA-seq analysis. UNC1999 is a potent, orally bioavailable and selective inhibitor of EZH2 and showed no effect in suppressing gallbladder cancer growth (Figure 3A). We introduced UNC1999 as a control in this study to rule out other possible epigenetic effects other than HDAC inhibition. Our goal is to identify genes which are differentially expressed under JNJ-26481585 treatment with various concentrations but showed no difference between DMSO and UNC1999 groups. Among the top gene candidates (Supplementary Figure 1 & Supplementary Data_1), CDKN1A and NGFR are considered cell growth or cell cycle related[1-3]. Next, we used short hairpin RNA (shRNA) to knock down CDKN1A and NGFR and confirmed the knockdown efficiency of shRNAs (Supplementary Figure 2A-B). We generated GBC-SD cells stably expressing CDKN1A shRNA, or NGFR shRNA, or both shRNAs, or control shRNA, and treated these cells with JNJ-26481585. Knockdown of CDKN1A or NGFR partially reversed the cell death phenotype induced by JNJ-26481585 (Supplementary Figure 2C). Knockdown of both CDKN1A and NGFR completely rescues the cell death phenotype (Supplementary Figure 2C). These results indicated that CDKN1A and NGFR mediated the function of JNJ-26481585 in gallbladder cancer cells.

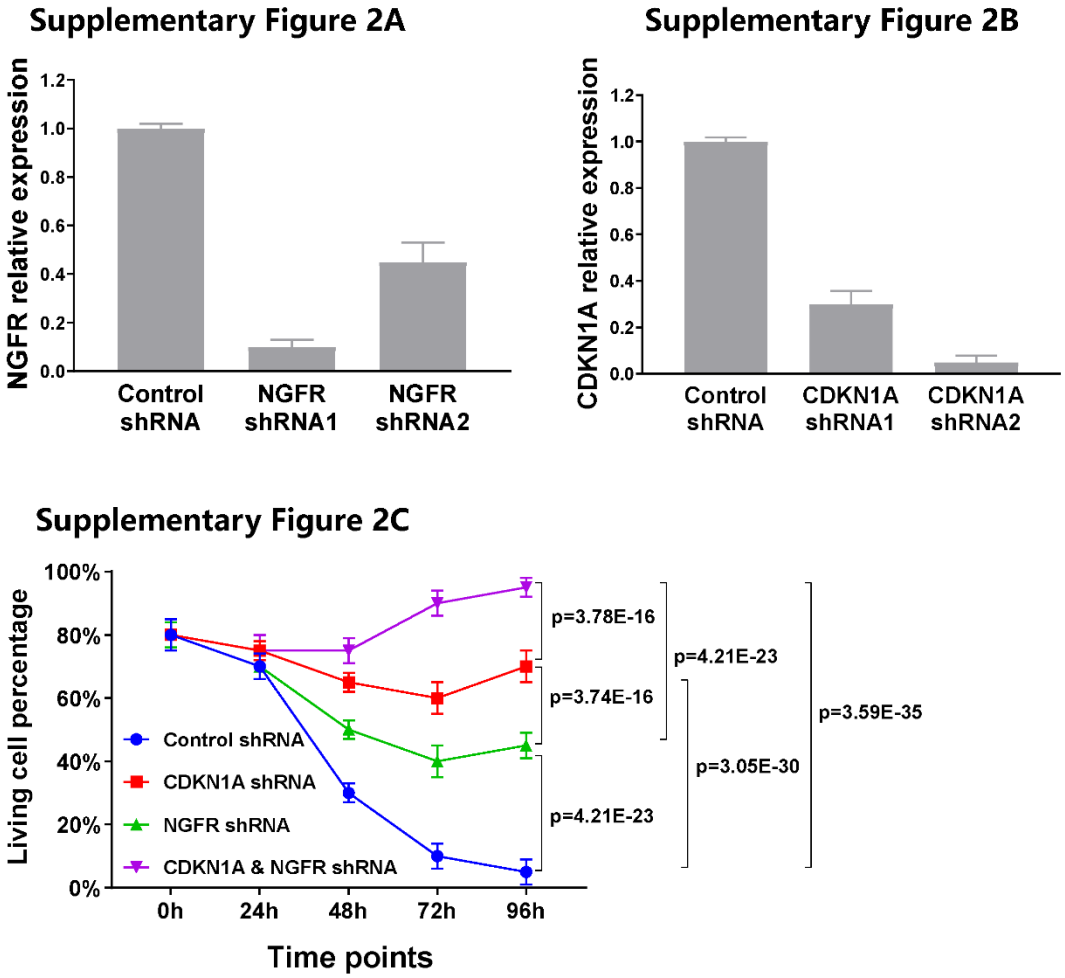
Supplementary Table. Compounds library targeting epigenetic or ErbB pathway and corresponding CIDs on PubMed

Epigenetic Compounds	CIDs	ErbB pathway Compounds	CIDs	ErbB pathway Compounds	CIDs
GSK2801	73010930	10-DEBC	10521421	INK128	45375953
Bromosporine	72943187	API-1	24773090	KU0063794	16736978
JQ1/SGCBD01	46907787	API-2	56684105	OSI027	44224160
PFI-1	58969684	FPA124	56972210	PP242	25243800
I-CBP112	90488984	SC66	6018993	CGM097	53240420
SGC-CBP30	72201027	SBI-0640756	121241171	GSK2334470	46215815
SGC0946	56962337	PHT427	44240850	3-Methyladenine	1673
GSK343	71268957	GSK690693	16725726	ETP45658	25229608
UNC1999	72551585	obatoclax	16681698	BKM120	16654980
A-366	76285486	AMN107	644241	KU0060648	11964036
UNC0638	46224516	GSK2118436	44462760	PF04691502	25033539
UNC0642	53315878	PLX4032	42611257	PF05212384	44516953
LAQ824	6445533	SCH727965	46926350	BAG956	24882589
JNJ-26481585	25067557	Crizotinib	11626560	GDC0941	17755052
LBH589	6918837	PI103	9884685	LY294002	3973
CI-994	2746	GDC0994	71727581	PI828	25181195
GSK-J4	71729975	SCH772984	24866313	TG100713	17751063
UNC1215	57339144	GSK1120212	11707110	WORTMANNIN	312145
GSK-LSD1	91663353	PD0325901	9826528	ZSTK474	11647372
C646	1285941	Torin2	51358113	AS252424	11630874
IOX1	459617	COMPOUND 401	10039361	AS605240	5289247
olaparib	23725625	Torin1	49836027	CZC24832	42623951
IOX2	54685215	WYE687	25229450	GSK1059615	23582824
PFI-2	71300326	XL388	59604787	PIT1	3664359
PFI-3	78243717	RAPAMYCIN	5284616	PP121	24905142
LLY-507	91623361	AZD8055	25262965	NICLOSAMIDE	4477
				AG879	5487525



Supplementary Figure 1. RNA-seq heatmap in GBC-SD cell line

GBC-SD cells were treated with high-concentration JNJ-26481585 (50nM), low-concentration JNJ-26481585 (10nM), high-concentration UNC1999 (50nM) or DMSO. CDKN1A and NGFR serve as two potential candidates mediating cancer cell death in the process of HDAC inhibition.



Supplementary Figure 2. Effects of Knockdown of CDKN1A and NGFR in gallbladder cancer

(A-B) Knockdown of CDKN1A and NGFR by shRNAs decreases the expression of these two genes. Two independent shRNA against CDKN1A or NGFR were introduced into GBC-SD cells. CDKN1A and NGFR expression were measured by quantitative real-time PCR. (C) Knockdown of CDKN1A and NGFR rescues the cell death phenotype induced by JNJ-26481585. GBC-SD cells stably expressing CDKN1A shRNA, or NGFR shRNA, or CDKN1A

and NGFR shRNA, or a control shRNA, were treated with JNJ-26481585. Cell numbers were evaluated at 0hr, 24hr, 48 hr, 72hr, and 96hr after treatment.

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