

**Cannabidiol Enhances Cytotoxicity of Anti-cancer Drugs in Human Head and Neck Squamous Cell Carcinoma**

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## Supplementary figures and figure legends

### **Figure. S1. Anti-cancer effects of Cisplatin, 5-Fluorouracil, and Paclitaxel on FaDu cells.**

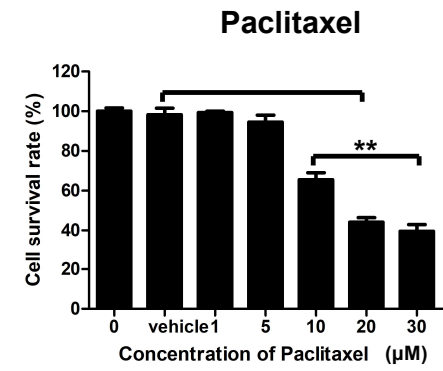
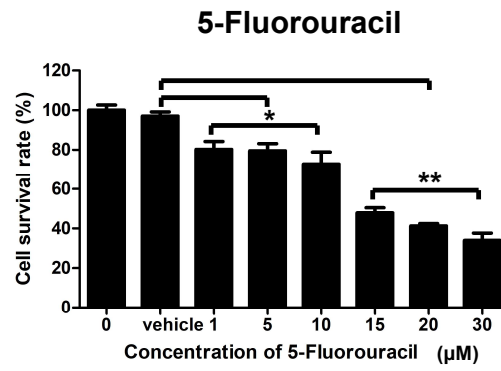
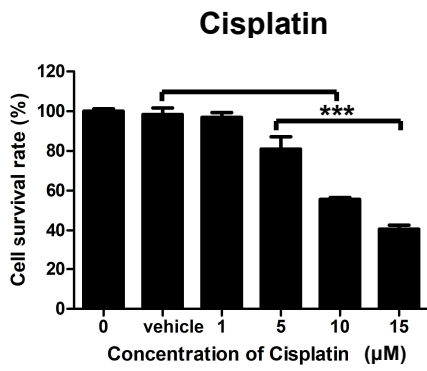
FaDu cells were treated with various concentrations of Cisplatin, 5-Fluorouracil, and Paclitaxel for 24 hours and then cell viability was determined by CCK8 assay. Error bars indicated mean and SD; \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$  compared with control.

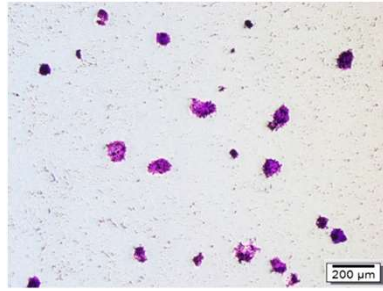
### **Figure. S2. Cannabidiol (CBD) exhibited synergistic anticancer effect with 5-Fluorouracil and Paclitaxel.**

FaDu cells were exposed to EC<sub>50</sub> value of 5-Fluorouracil (18  $\mu$ M) and Paclitaxel (15  $\mu$ M) alone or combination with 6  $\mu$ M of CBD and then cultured for 2 weeks. The cells were stained with crystal violet and visualized by a light microscopy. Scale bars, 200  $\mu$ m.

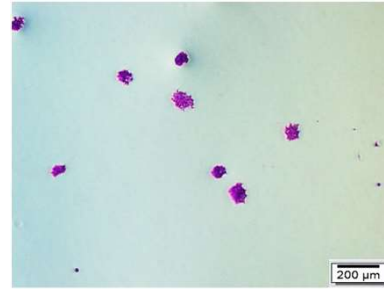
### **Figure. S3. Differentially up- and down-regulated genes in HNSCC cell line dependent**

The top 50 up- and down-regulated genes in FaDu and SCC15 were represented by images of heatmap clustering (2-fold difference with  $p < 0.05$ ). Color bar depicted the levels of color contrast for heatmap data.

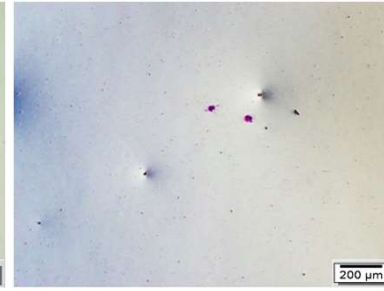




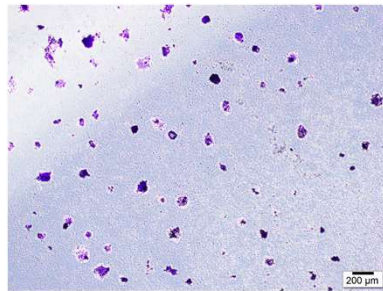
**Control**



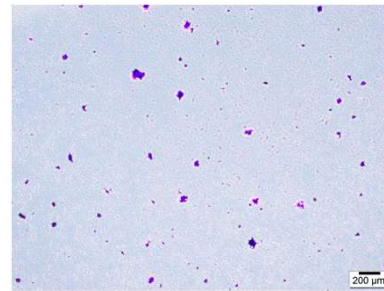
**5-FU**



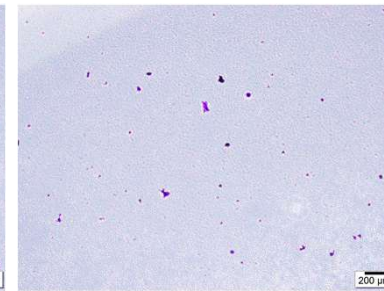
**5-FU+CBD**



**Control**



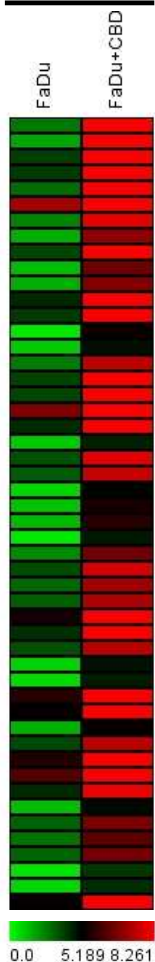
**Paclitaxel**



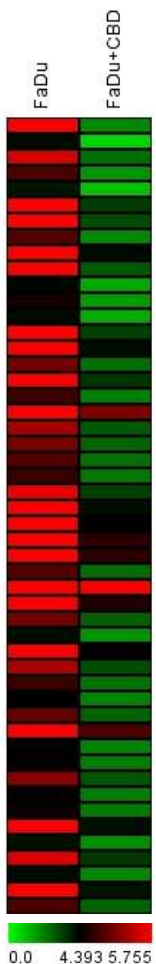
**Paclitaxel + CBD**

## FaDu

### Top 50 up-regulated genes



### Top 50 down-regulated genes

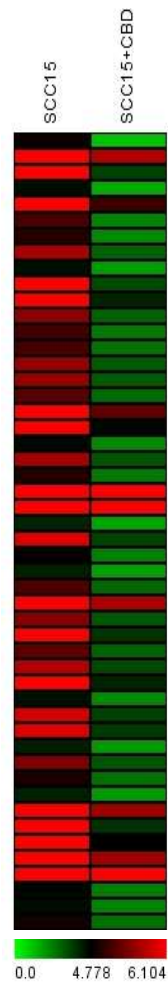


## SCC15

### Top 50 up-regulated genes



### Top 50 down-regulated genes



DUSP1  
PTGS2  
PHLDA1  
MT1E  
MT1X  
MT2A  
ATF3  
MT1JP  
IL8  
S100P  
IL6  
HBE6F  
IER3  
IL24  
SPRR2D  
CTGF  
TRIB1  
GDF15  
DDIT4  
KLF6  
CCL20  
C6orf141  
JHDM10  
TRIML2  
ERRFI1  
NUPR1  
RASD1  
FOXD1  
SLC30A1  
AR62  
LURAP1L  
GADD45A  
DUSP6  
PPP1R15A  
ALOXE3  
PNLIPRP3  
TRIB3  
DUSP5  
DUSP4  
IL1A  
STC2  
ELL2  
SESN2  
TRPV3  
LIF  
GADD45B  
PHLDB2  
CXCL3  
UPP1  
AREG

FAM111B  
ZNF488  
CCDC142  
DSCC1  
E2F2  
ORC1  
ZNF367  
B3GNT1  
E2F1  
XRCC2  
GPR135  
GOLGA2P5  
C21orf58  
SLC47A2  
NRARP  
LOC100129981  
ABCB6  
NGFR  
LY6D  
LYSD1  
ATAD5  
TXNIP  
EPN3  
CDT1  
FEN1  
PCNA  
NT5DC2  
SKP2  
SMO  
LOC728643  
GINS2  
CNIH2  
ZNF599  
DIDO1  
HSPA2  
ZNF348  
SLC27A3  
CASP8AP2  
TNN2  
FAM213B  
ZNF341  
APOL1  
VSNL1  
ALG6  
PCYOX1  
GGT8  
PRAME  
CCNE1  
LRRC45  
ACTRT3

SPRR2D  
IL9  
USP2  
CSF2  
DUSP1  
CXCL2  
SERPINB2  
CXCL1  
TNFAIP3  
HBE6F  
LCE1F  
MT1E  
MT1JP  
GDF15  
CXCL3  
KRTAP2-3  
PPP1R15A  
ATF3  
MT1X  
AREG  
DDIT3  
NFKBIZ  
HSPA5  
MT1F  
DDIT4  
LCN2  
NFKBIA  
IER3  
KLF6  
SPRR1B  
PRPH  
RHOV  
NR1D1  
HAS2  
UPP1  
DUSP5  
MMP10  
OSBP2  
HERPUD1  
ELF3  
ODC1  
C10orf55  
ZFP36  
LURAP1L  
RND3  
ARRDC3  
MT2A  
TRIB3  
CEBPB

PALLD  
HIST1H3C  
HIST1H3A  
HPS6  
HIST1H1B  
CCNF  
CROT  
PER3  
BMP4  
FGF1  
FEN1  
DIO2  
HIST1H4A  
ZNF488  
FITM2  
IL6  
PPP1R10  
CHST14  
HIST1H2AH  
HIST1H2AB  
DIDO1  
JPH2  
S1PR5  
HIST1H2A6  
HIST1H4B  
CDKN2C  
TRIM59  
LFNG  
SERTAD4  
B3GNT1  
HIST1H2AJ  
EID2  
FGD3  
CCDC8  
SUV39H1  
PRR5L  
HTR1D  
EPN3  
HIST1H2BI  
ST3GAL2  
SLC38A10  
DGCR8  
SLFN12  
FADD  
HIST1H2AL  
HIST1H3J  
HIST1H4D  
HIST1H2AM  
SUOX  
KLHL3  
FAM217B

## Supplemental Experimental Procedures

**Table 1. Sequences of oligonucleotide primers for quantitative RT-PCR.**

Primer	Direction	Sequence
<i>p53</i>	forward	5'-AGACCTGTGGGAAGCGAAAA-3'
	reverse	5'-TCATCCATTGCTTGGGACGG-3'
<i>Bcl-2</i>	forward	5'-TCCCTCGCTGCACAAATACTC-3'
	reverse	5'-ACGACCCGATGGCCATAGA-3'
<i>Bax</i>	forward	5'-CCAAGGTGCCGGAAGTGA-3'
	reverse	5'-CCCGGAGGAAGTCCAATGT-3'
<i>Caspase3</i>	forward	5'-ATTGTGAGGCGGTTGTAGAA-3'
	reverse	5'-CATGTATGATCTTTGGTTCCAA-3'
<i>Caspase9</i>	forward	5'-TGCTGAGCAGCGAGCTGTT-3'
	reverse	5'-AGCCTGCCCGCTGGAT-3'
<i>Beclin</i>	forward	5'-AGCTGCCGTTATACTGTTCT-3'
	reverse	5'-TGTGTCTTCAATCTTGCCTT-3'
<i>LC3</i>	forward	5'-GCAGCCTTTCTTCCAGAGAC-3'
	reverse	5'-CTGGAAAAGTGGAGGCTGAG-3'
<i>DUSP1</i>	forward	5'-CGTCTCAGCCAATTGTCCCA-3'
	reverse	5'-AACACCCTTCCTCCAGCATTC-3'
<i>KLF6</i>	forward	5'-ACGGCTGCAGGAAAGTTTACA-3'
	reverse	5'-GCTTTCGGAAGTGCCTGGTTA-3'
<i>MCM2</i>	forward	5'-ATATCAGCGCAACCGCTTTG-3'
	reverse	5'-AGGTCGTGGCTGAACTTGTT-3'
<i>PARP1</i>	forward	5'-GGTGGACATCGTTAAAGGAACCAA-3'

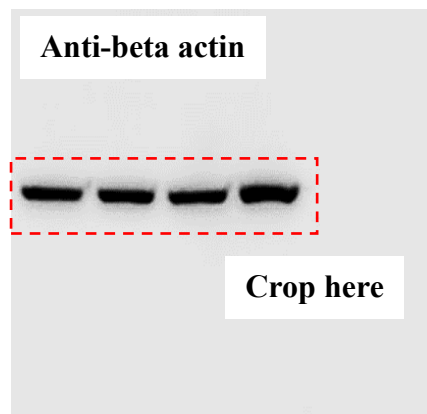
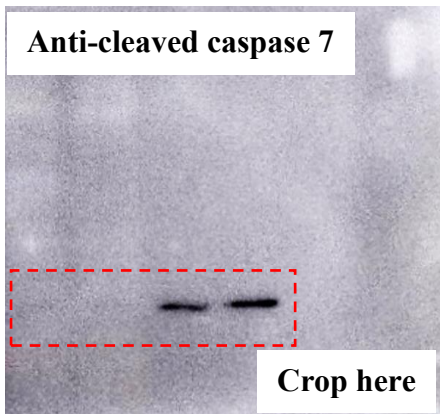
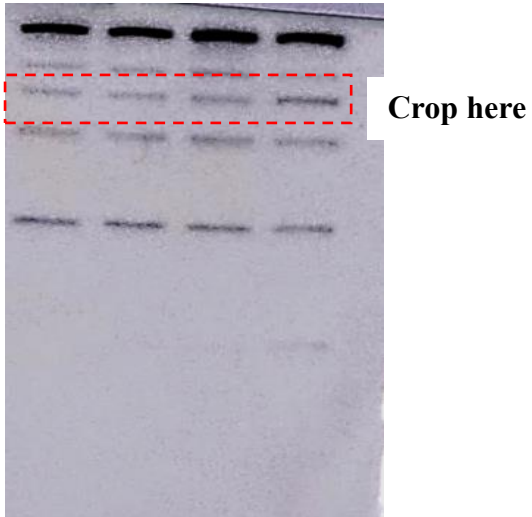
5'-TCCTCCTTGGACGGCATCT-3'

<i>BRCA1</i>	forward	5'-CTGAAAGCCAGGGAGTTGGT-3'
	reverse	5'-CTTGTTTCACTCTCACACCCAGAT-3'
<i>GAPDH</i>	forward	5'-TCGCCCCACTTGATTTTGG-3'
	reverse	5'-GCAAATTCATGGCACCGT-3'

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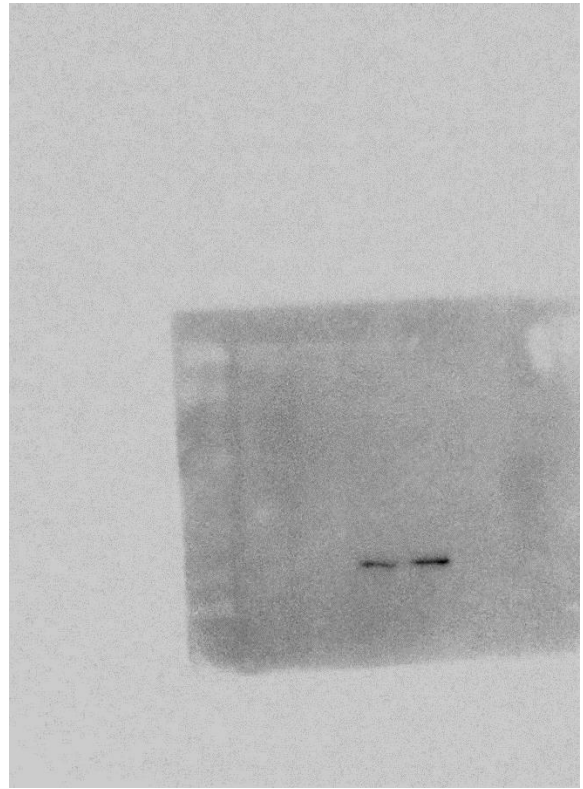
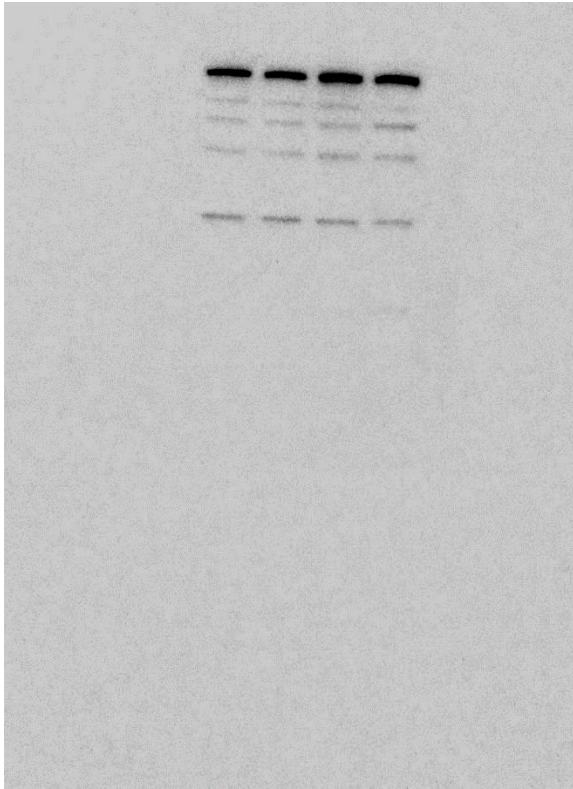
## Full-length blots in Figure 4B

### Anti-cleaved PARP

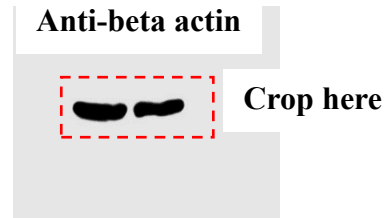
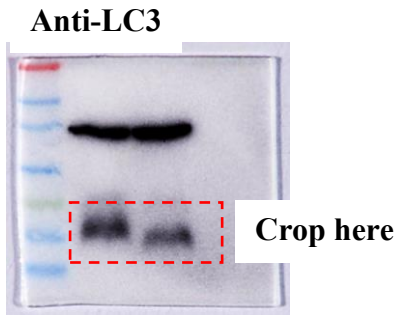




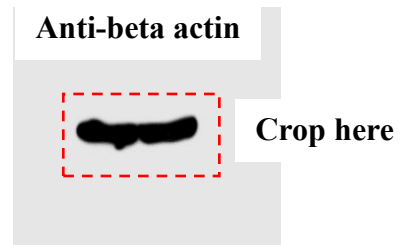
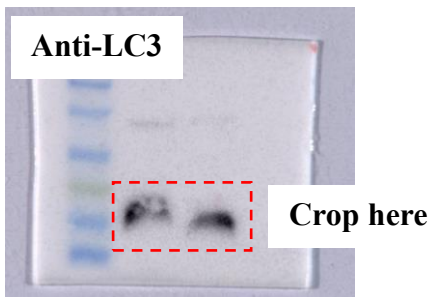
**Original blots**



**Full-length blots in Figure 4C**



<FaDu>



<SCC15>

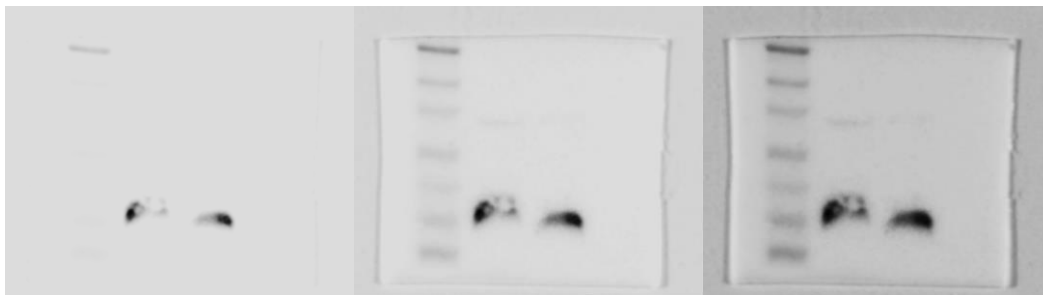
**Original blots with multiple exposure images**



**<FaDu anti-LC3>**



**<FaDu anti-actin>**



**<SCC15 anti-LC3>**



**<SCC15 anti-actin>**