Supplementary Tables

Supplementary Table 1. Relation of DUSP2 expression and various prognostic factors in 102 patients with colon cancer

Clinicopathological Characteristics	No. patient	IHC intensity 0	1	2	P-value
Age (years)					
<60	37	15 (41%)	21 (57%)	1 (3%)	0.122
>60	65	18 (28%)	38 (58%)	9 (14%)	
Sex					
Female	50	15 (30%)	29 (58%)	6 (12%)	0.722
Male	52	18 (35%)	30 (58%)	4 (8%)	
Tumor status					
T1	6	2 (33%)	4 (67%)	0 (0%)	**0.021***
T2	10	3 (30%)	6 (60%)	1 (10%)	
T3	57	15 (26%)	38 (67%)	4 (7%)	
T4	29	15 (52%)	9 (31%)	5 (17%)	
Nodal status					
N0	55	18 (33%)	27 (49%)	10 (18%)	**0.036***
N1	27	8 (30%)	19 (70%)	0 (0%)	
N2	20	7 (35%)	13 (65%)	0 (0%)	
Differentiation					
Well	11	4 (36%)	7 (64%)	0 (0%)	0.495
Moderate	83	25 (30%)	48 (58%)	10 (12%)	
Poor	8	4 (50%)	4 (50%)	0 (0%)	
Meta status					
M0	86	24 (28%)	52 (60%)	10 (12%)	0.053
M1	16	9 (56%)	7 (44%)	0 (0%)	
Pathological status					
I	13	3 (23%)	9 (69%)	1 (8%)	**0.020***
II	39	13 (33%)	18 (46%)	8 (21%)	
III	34	8 (24%)	25 (74%)	1 (3%)	
IV	16	9 (56%)	7 (44%)	0 (0%)	
Total	102				

0: negative; 1: Weak staining; 2: intermediate staining

Supplementary Table 2: List of primers used in this study

Gene	Primer sequences	Amplicon (bp)	Tm (C)
DUSP2 (Promoter)	**F:** 5'- GGGGTACC TCTCCACCCACCTCAACTTC-3'	1255	60
	R: 5'-GAAGATCTGCCTCTTCCTCTTCCTTTC G-3'		
DUSP2 (Real time PCR)	**F:** 5'- GGCCTTTGACTTCGTTAAGC-3'	105	60
	R: 5'- CCACCTCAGTGACACAGCAC-3'		
DUSP2 (cDNA)	**F:** 5'-CTCGAG GTGGCCATGGGGCTGGAG-3'	1183	62
	R: 5'-AAGCTT GTGACACAGCACCTGGGTCTCAA-3'		
DUSP2 (CHIP-PCR)	**F:** 5'-CCGAAGTCTCCAGGTCAG-3'	92	57
	R: 5'-ACCATACAAGGGCAGAGC-3'		
DUSP2 (distal CHIP-PCR)	**F:** 5'-TAGAGCGTTGGAGCCAGTGT-3'	143	60
	R: 5'-AGAGCCGATGCAGAGGAGAA-3'		
DUSP1 (Real time PCR)	**F:** 5'-CCACCATCTGCCTTGCTTAC-3'	80	60
	R: 5'-TCGCCTCTGCTTCACAAAC-3'		
DUSP4 (Real time PCR)	**F:** 5'-CGGCTCTGTTGAATGTCTCC-3'	97	60
	R: 5'-ATGTCGGCCTTGTGGTTATC-3'		
DUSP5 (Real time PCR)	**F:** 5'-CCTGAGTGTTGCGTGGATG-3'	119	60
	R: 5'-TGGTCATAAGCTGGCCTGTAG-3'		
EGR-1 (Real time PCR)	**F:** 5'-CACCTCACCACCCACATCC-3'	100	60

	R: 5'-TATGCCTCTTGCGTTCATCG-3'		
EGR-1 (promoter)	**F:** 5'-CAGTGTCCCAAGAACCAAGTG-3'	1019	60
	R: 5'-GAACACTGAGAAGCGTGCAG-3'		
CYR61(real time PCR)	**F:** 5'-CCTCGGCTGGTCAAAGTTAC-3'	143	60
	R: 5'-TTTCTCGTCAACTCCACCTC-3'		
GRP78(real time PCR)	**F:** 5'-TATGAAGCCCGTCCAGAAAG-3'	82	60
	R: 5'-CGAGCCACCAACAAGAACA-3'		
MDR-1(real time PCR)	**F:** 5'-GCTGGTTGCTGCTTACATTC-3'	109	60
	R: 5'-GCCTATCTCCTGTCGCATT-3'		
Osteopontin (real time PCR)	**F:** 5'-AAAGCGAGGAGTTGAATGGT-3'	143	60
	R: 5'-CTTGTGGCTGTGGGTTTC-3'		
DUSP2-P (core HRE mutation)	**Sense:** 5'-CAGACTGCGCCCCATTTTAGGGGCGGGGGC-3'		
	Antisense: 5'-CGCCCCCGCCCCTAAAATGGGGCGCAGTCT -3'		
DUSP2-P (flanking region mutation)	**Sense:** 5'-GCCCCACGTGGGGGGCGGGGG-3'		
	Antisense: 5'- CCCCCGCCCCCCACGTGGGGC-3'		

Supplementary Table 3: List of siRNAs used in this study.

Name	catalog	Sequence
siHIF-1α_1	12938-122	CCAUGAGGAAAUGAGAGAAAUGCUU
siHIF-1α_2	12938-122	CCACAGUGCAUUGUAUGUGUGAAUU
siHIF-2α_1	HSS103261	GGCCAGGUGAAAGUCUACAACAACU
siHIF-2α_2	HSS103263	AGAAAGAUCAUGUCGCCAUCUUGGG
shDUSP2-1	**F:**TCGAG GCATAGGCTTCATTGACTGGTTCAAGAGACCAGTCAATGAAGCCTATGTTTTTACGCGTA	
	R:AGCTTACGCGTAAAAACATAGGCTTCATTGACTGGTCTCTTGAACCAGTCAATGAAGCCTATGCC	
shDUSP2-2	**F:**TCGAGGATCTGTCTGGCATACCTCATTCAAGAGATGAGGTATGCCAGACAGATTTTTTACGCGTA	
	R:AGCTTACGCGTAAAAAATCTGTCTGGCATACCTCATCTCTTGAATGAGGTATGCCAGACAGATCC	
shLuciferase	**F:**TCGAGGTGCGTTGCTAGTACCAACTTCAAGAGAGTTGGTACTAGCAACG CACTTTTTAAGCT	
	R: AGCTTAAAAAGTGCGTTGCTAGTACCAACTCTCTTGAAGTTGGTACTAGCAACGCACCTCGA	

Supplementary figures

Legends for supplementary figures

Supplementary figure 1: Hypoxia fails to inhibit DUSP2 mRNA expression in liver cancer cell lines. A, Liver cancer cells were cultured under normoxia or hypoxia for 24 hr and levels of mRNAs coding for DUSP2 (left panel) and pyruvate dehydrogenase kinase 1 (PDK1, right panel) were quantified by RT-qPCR. *: $p < 0.05$ compared to normoxia control. B, Representative Western blot shows the upregulation of HIF-1a protein in liver cancer cells cultured under hypoxia.

Supplementary figure 2: Expression of PDK1 mRNA under normoxia and hypoxia in 10 cancer cell lines. *: $p < 0.05$ compared to normoxia control.

Supplementary figure 3: Alignment of hypoxia response element (HRE) and flanking sequences in promoter of dusp2 gene in eight mammalian species.

Supplementary figure 4: Representative Western blots of HeLa cells cultured under normoxia (Nor) or hypoxia (Hypo) for 24 hrs showing levels of phosphorylated P38 MAPK (P-p38), P38 MAPK (T-p38), phosphorylated JNK (P-JNK), total JNK (T-JNK), and HIF-1α.

Supplementary figure 5: Expression of DUSP1, DUSP4, and DUSP5 in HeLa cells cultured under hypoxia. HeLa cells were cultured under normoxia or hypoxia for 24 hr and levels of mRNAs coding for DUSP1, DUSP4, and DUSP5 were quantified by RT-qPCR. *: $p < 0.05$ compared to normoxia control.

Supplementary figure 6: Induction of the DUSP2-GFP fusion gene in stable clones of HeLa cells attenuated ERK phosphorylation. Representative Western blots from four different clones.

Supplementary figure 7: Representative pictures showing the expression of GFP (left panel) and DUSP2 (right panel) in xenografted tumors growing in mice treated with (+ Doxy) or without (-Doxy) doxycycline. Scale bars, 50 μm for low and high magnifications.

Supplementary figure 8: Induction of DUSP2 inhibited tumor growth in SCID mice (n=5 per group). Mice were inoculated with HeLa cells stably expressing the DUSP2-GFP fusion gene (clone #13) when doxycycline was added to the drinking water. *: $p < 0.05$ compared to group without doxycycline.

Supplementary figure 9: Representative pictures show tumors in mice inoculated with DUSP2 knockdown (shDUSP2_#8 and shDUSP2_#12) and control (shLuciferase) HeLa cells.

Supplementary figure 10: Dose-dependent killing of HeLa and HCT116 cells by paclitaxel, cisplatin, and oxaliplatin under normoxia.

Supplementary figure 11: Survival of HeLa cells treated with paclitaxel (Pacli), cisplatin (Cisp), or oxaliplatin (Oxali) under normoxia in the presence or absence of DFO for 24 hrs as percentage of control (without drug treatment). *: $p < 0.05$ compared to vehicle control.

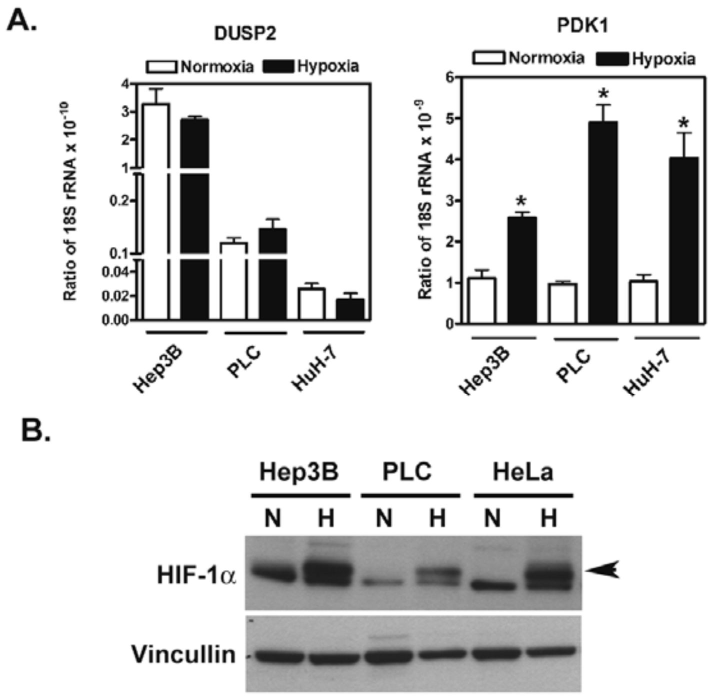
Supplementary figure 12: Knocking down DUSP2 increases drug resistance under normoxia. Stable clones of HeLa cells with DUSP2 knockdown or mock control (shLuciferase) were cultured under normoxia and treated with cisplatin (20 nM) or oxaliplatin (25 μg/ml) for 24 hrs. Percentage of cell survival was calculated by normalizing to groups without drug treatment. *: $p < 0.05$ compared to shLuciferase group.

Supplementary figure 13: Inhibition of ERK phosphorylation reverses hypoxia-mediated drug resistance. HeLa cells were cultured under normoxia, hypoxia, or hypoxia plus PD98059 (50 μM) and treated with cisplatin (a) or oxaliplatin (b) for 24 hrs. Percentage of cell survival was normalized to cells without drug treatment. *: $p < 0.05$ compared to normoxia; #: $p < 0.05$ compared to hypoxia with drug (cisplatin or oxaliplatin) treated group.

Supplementary figure 14: Representative figures of HeLa cells treated with or without Paclitaxel (50 mM) and doxycycline (2 μg/ml) under normoxia or hypoxia for 24 hrs showing cells undergoing apoptosis. Apoptotic cells show brown nuclei using TUNEL assay.

Supplementary figure 15: Induction of DUSP2 in xenograft tumors increased drug sensitivity. (a, b) Growth curves of tumors with inducible DUSP2-GFP inoculated into mice (n=7-9/group) treated with or without paclitaxel (10 or 20 mg/kg body weight) combined with or without doxycycline. Arrow indicates the day treatments started. *: $p<0.05$ compared to control group (-doxy/-pacli); #: $p <0.05$ compared to paclitaxel only group (-doxy/+pacli). (c), Representative picture shows tumors *in situ* at the time of sacrificed.

Supplementary figure 16: HIF-1α-mediated gene regulatory network. Red lines indicate suppressive effects while green lines indicate activating effects.



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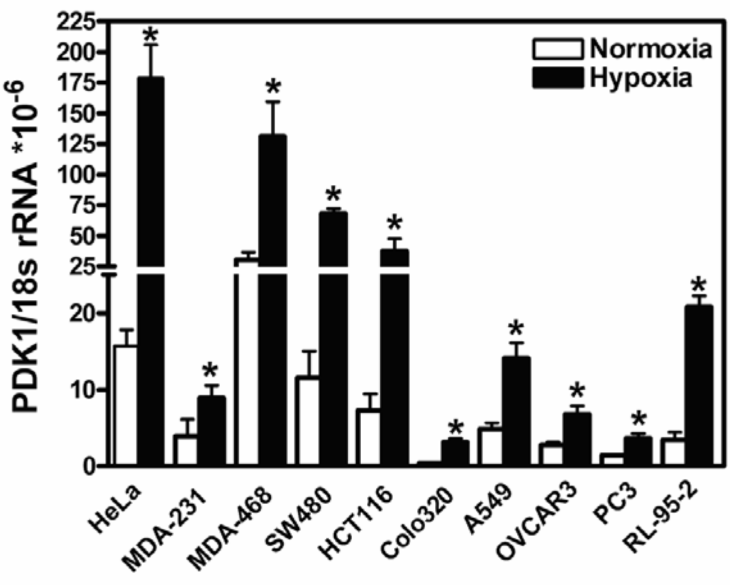

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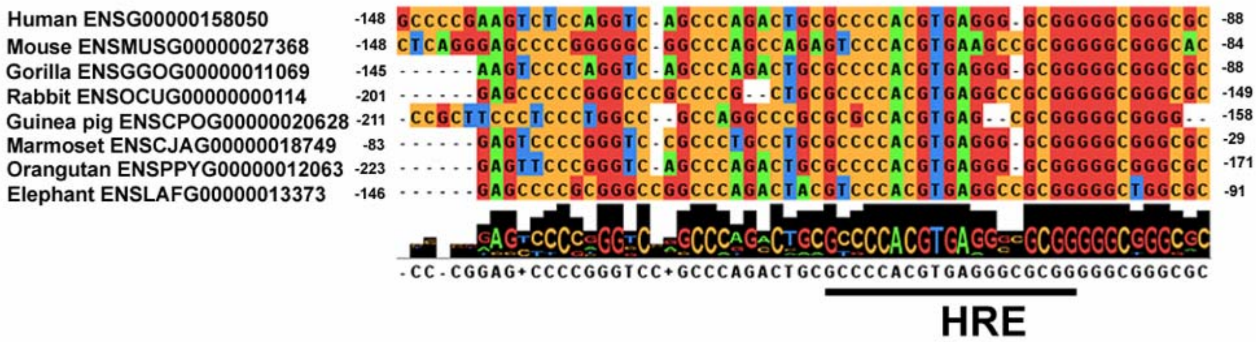

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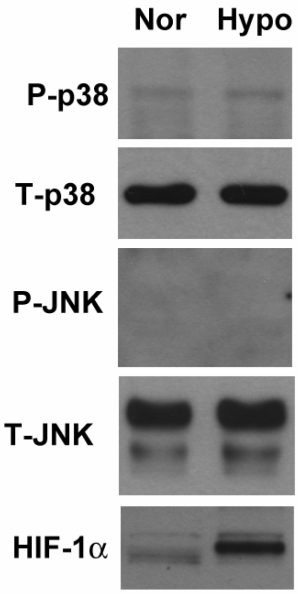

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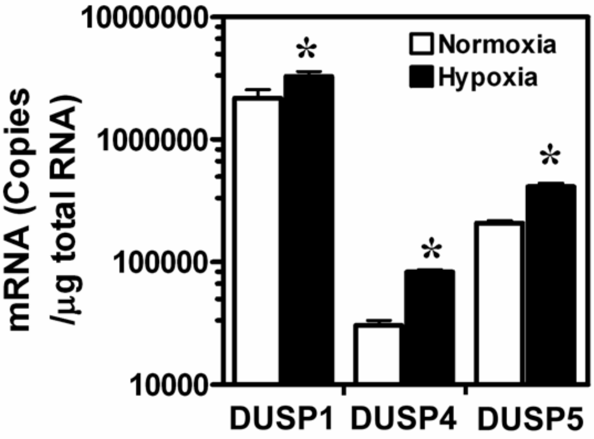

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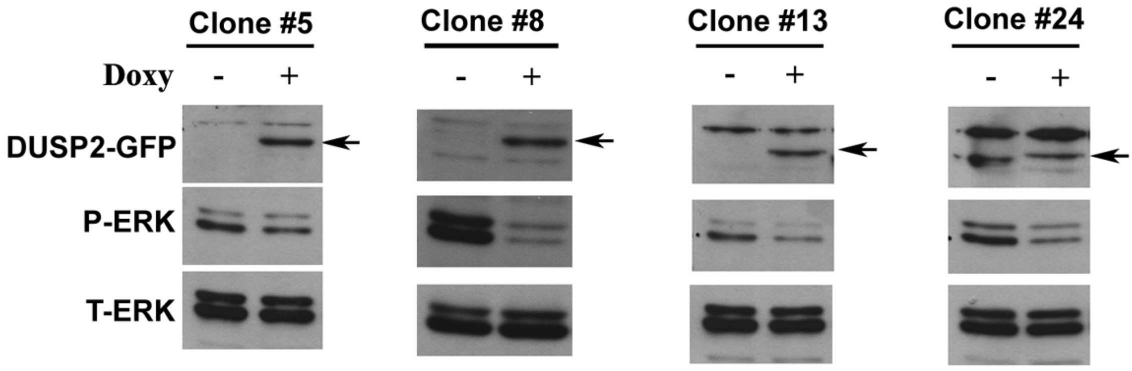

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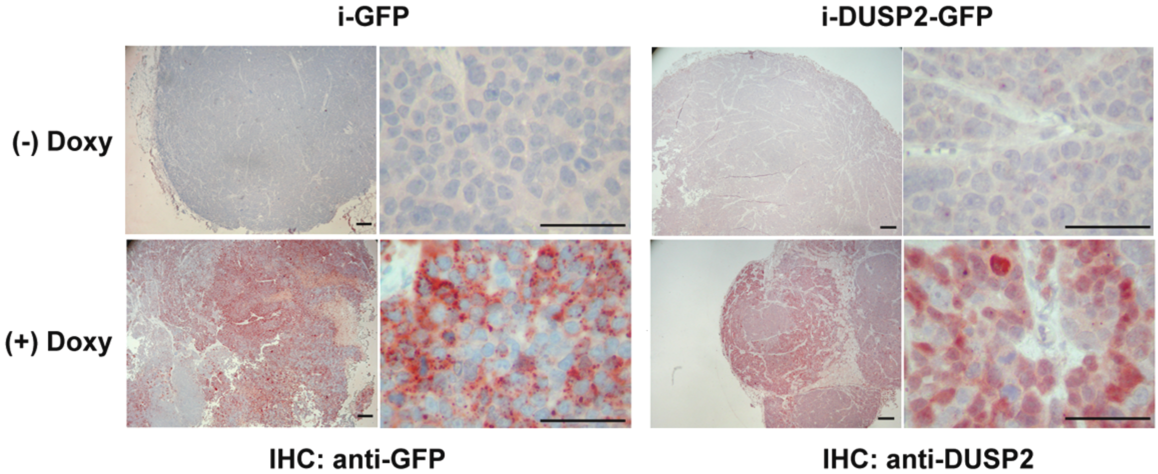

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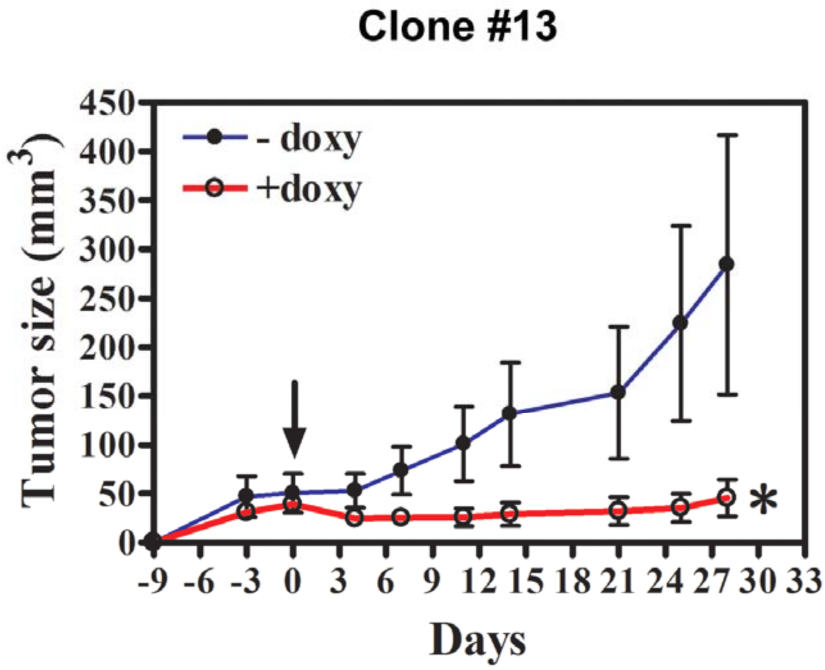

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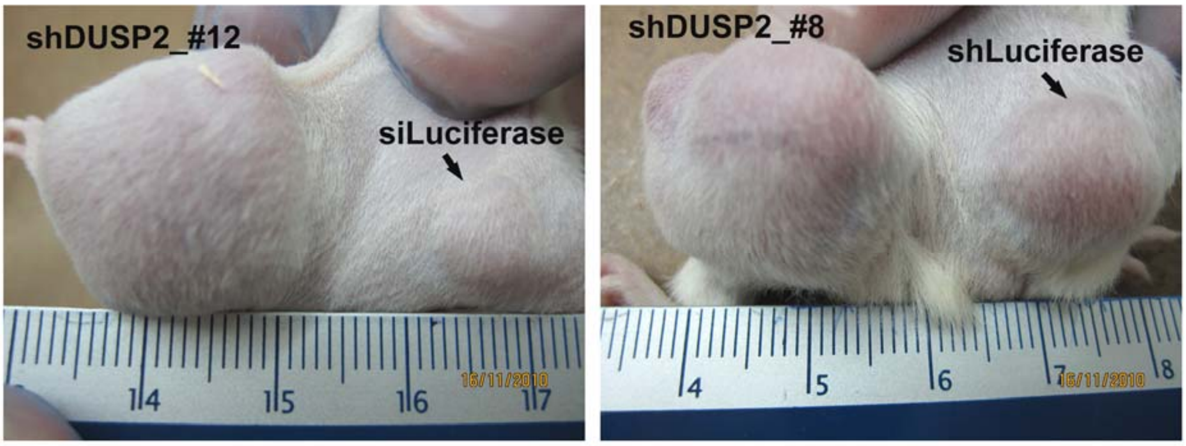

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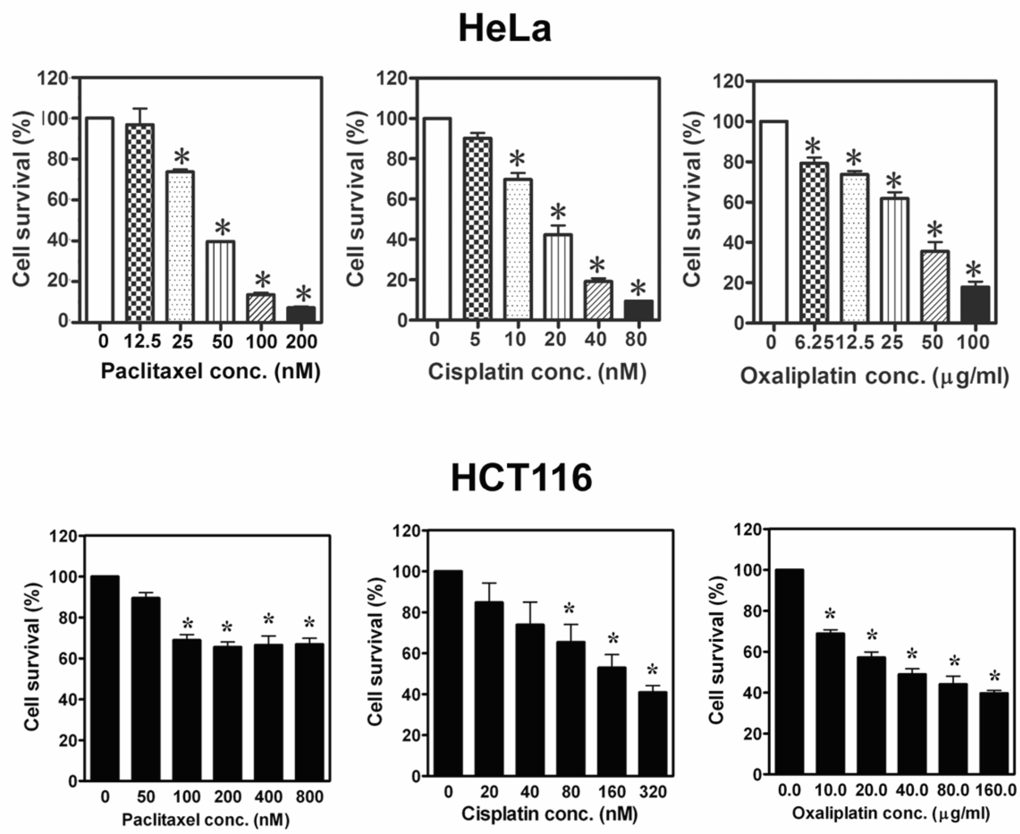

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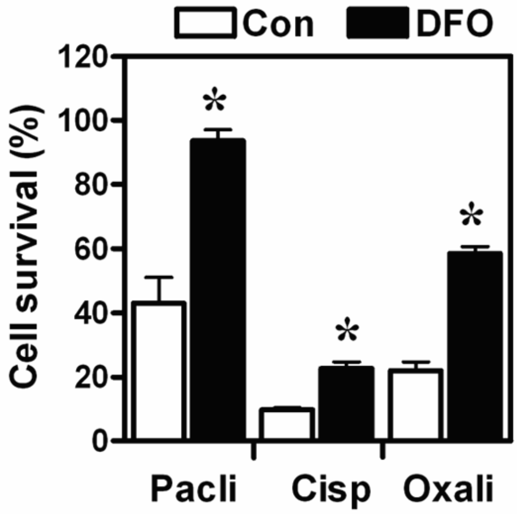

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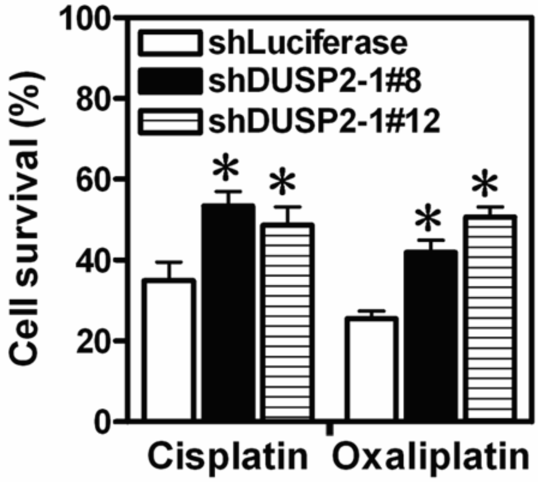

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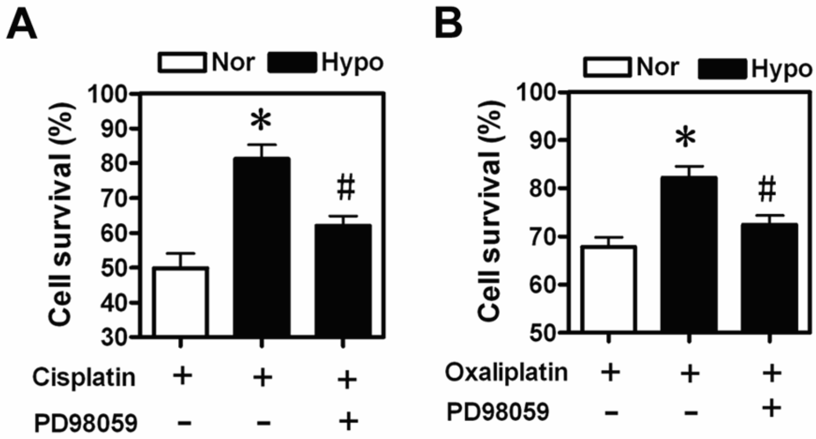

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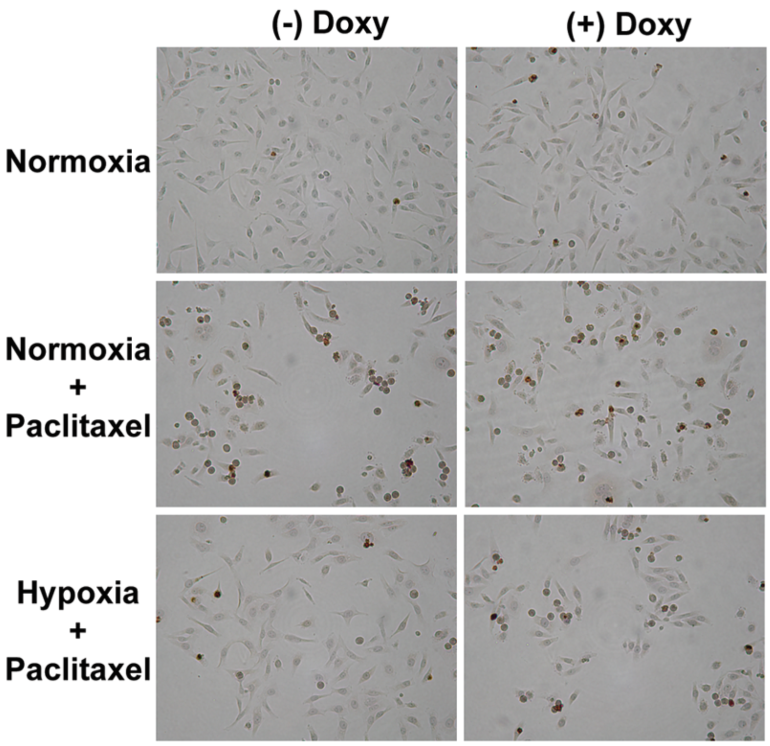

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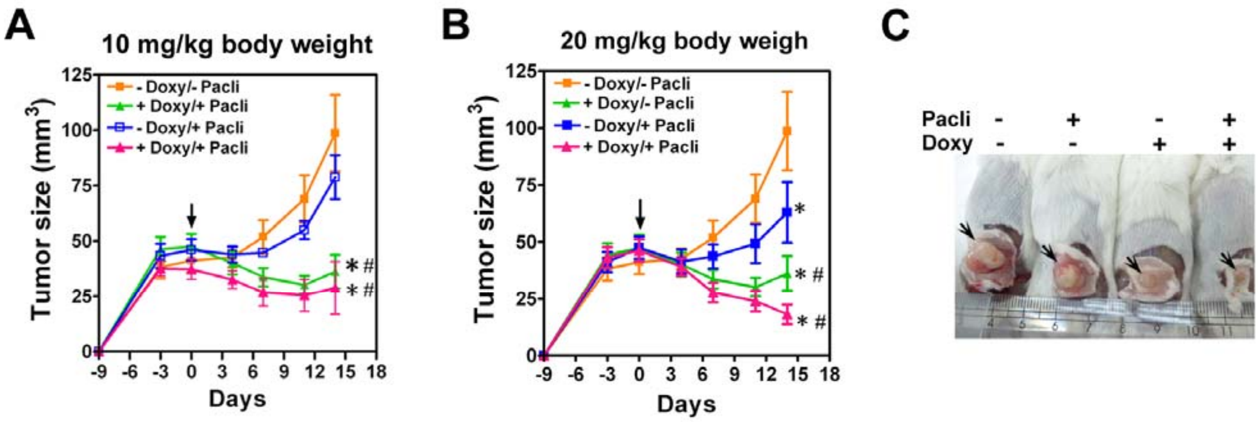
A
10 mg/kg body weight
Tumor size (mm3)
- Doxy/- Pacli
+ Doxy/+ Pacli
- Doxy/+ Pacli
+ Doxy/+ Pacli
Days
B
20 mg/kg body weigh
Tumor size (mm3)
- Doxy/- Pacli
+ Doxy/- Pacli
- Doxy/+ Pacli
+ Doxy/+ Pacli
Days
C
Pacli - + - +
Doxy - - + +

Figure S15

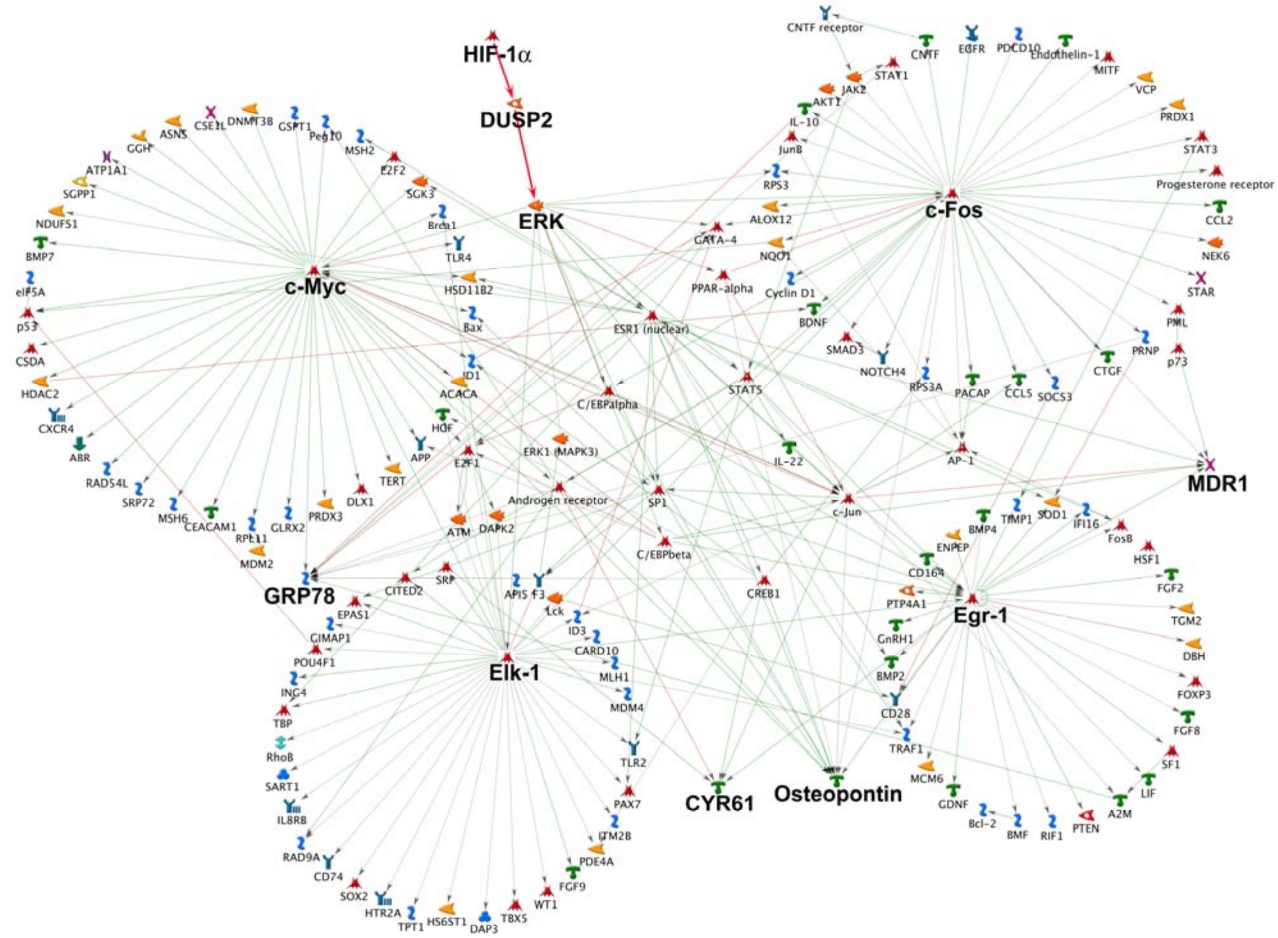
HIF-1α
DUSP2
ERK
c-Myc
c-Fos
GRP78
Elk-1
Egr-1
MDR1
CYR61
Osteopontin

Figure S16