

## **Anticancer effect of deuterium depleted water - redox disbalance leads to oxidative stress**

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### ***Supplementary Figure captions:***

**Table S1.** Common identified proteins in protein abundances analysis.

**Table S2.** Top 50 up and down regulated proteins specific for DDW and the abundance ratios to control.

**Table S3.** STRING analysis result of top 50 up regulated proteins

**Table S4.** STRING analysis result of top 50 down regulated proteins

**Table S5.** Result of time course analysis.

**Table S6.** Time course analysis of proteins most regulated by MTX.

**Table S7.** Time course analysis of proteins most regulated by DDW.

**Table S8.** Time course analysis of proteins most regulated by PCTL.

**Table S9.** Common proteins identified in redox proteomics result.

**Table S10.** Common peptides identified in redox proteomics result.

**Table S11.** Top 20 oxidized and reduced proteins.

**Table S12.** Oxidation levels of p62 peptides in DDW vs NW.

**Table S13.** Common proteins in cells growing in NW or DDW medium in redox result.

**Table S14.** Common peptides in cells growing in NW or DDW medium in redox result.

**Table S15.** Result of TPP analysis.

**Table S16.** R analysis of TPP result.

**Table S17.** STRING analysis result of overlap top 50 up regulated proteins with disbalance in oxidation -reduction level or changes in stability.

**Table S18.** STRING analysis result of top 50 down regulated proteins with disbalance in oxidation-reduction level or changes in stability.

**Figure. S1.** Survival of A549 cell line grown in media with different deuterium concentrations from 17-150 ppm.

**Figure. S2.** DDW effect on cell proliferation and dynamic analysis of regulation of DDW on proteome abundance.

**Figure. S3.** Inhibitory curve of auranofin.

**Figure. S4.** Validation of DDW anticancer mechanism.

**Table S3. STRING analysis result of top 50 up regulated proteins**

	Pathway ID	Pathway description	Observed gene count	False discovery rate
Cellular Component (GO)	GO:0005776	autophagosome	4	0.02
	GO:0044754	autolysosome	2	0.0407
UniProt Keywords	KW-0131	Cell cycle	9	0.0047
	KW-0832	Ubl conjugation	16	0.0135

**Table S4. STRING analysis result of top 50 down regulated proteins**

	Pathway ID	Pathway description	Observed gene count	False discovery rate
Biological Process (GO)	GO.0009165	nucleotide biosynthetic process	6	0.0325
	GO.0010224	response to UV-B	3	0.0325
Molecular Function (GO)	GO.0004748	ribonucleoside-diphosphate reductase activity, thioredoxin disulfide as acceptor	2	0.0183
	GO.0016491	oxidoreductase activity	9	0.0183
Cellular Component (GO)	GO.0005829	cytosol	20	0.0122
	4115	p53 signaling pathway	6	3.22E-06
	480	Glutathione metabolism	4	0.000816
KEGG Pathways	1100	Metabolic pathways	10	0.0242
	5161	Hepatitis B	4	0.0242
	5214	Glioma	3	0.0242

**Table S11. Top 20 oxidized and reduced proteins.** OX - oxidation percentage, C – NW, A – auronafin, D – DDW.

Oxidized or reduced	Gene names	Oxidation percentage (OX)									Rank
		A-OX-1	A-OX-2	A-OX-3	C-OX-1	C-OX-2	C-OX-3	D-OX-1	D-OX-2	D-OX-3	
Oxidized	HNRNPA1	0.4	0.6	2.8	3.0	3.6	2.5	11.2	11.5	11.0	1
	RPAP3	2.2	3.2	5.3	12.1	4.9	12.1	42.3	33.6	42.9	2
	p62	13.0	11.7	10.8	16.1	18.5	19.7	34.5	35.7	34.5	3
	RPL32	1.5	7.7	6.4	8.2	4.4	3.5	30.8	20.7	28.5	4
	CA12	57.7	60.2	57.3	65.4	61.8	61.6	76.3	75.2	77.0	5
	DDX39B	3.2	3.3	4.9	4.0	3.7	5.1	8.5	7.4	8.3	6
	PACSLIN2	16.8	12.8	14.1	16.5	14.4	9.9	25.8	27.2	24.9	7
	ANXA2	2.7	1.5	2.4	5.1	5.9	5.7	12.1	12.2	13.6	8
	FKBP4	6.0	6.2	6.8	7.0	8.0	7.4	16.5	23.7	16.3	9
	PSMD4	4.1	9.5	7.1	2.0	6.2	10.2	19.3	20.1	22.1	10
	RFC2	9.1	9.3	14.9	10.0	6.0	13.6	24.9	31.0	25.0	11
	SARS2	1.2	2.3	4.2	1.2	1.8	1.9	13.3	17.5	9.5	12
	MYO1B	7.2	8.2	7.9	7.4	9.5	9.6	15.0	12.8	16.8	13
	ACTR2	8.7	5.5	5.2	4.2	6.1	10.9	24.1	16.5	24.1	14
	HSPA9	4.6	6.3	9.1	12.6	7.0	7.4	19.5	17.6	21.9	15
	FSCN1	3.5	6.0	6.1	10.5	10.0	11.7	21.5	19.2	23.0	16
	GSK3A	4.9	2.3	8.1	10.7	8.0	9.5	25.8	17.9	26.2	17
	CTPS1	7.1	7.9	8.4	7.8	7.1	7.7	15.4	12.0	11.4	18
	TIPRL	4.3	6.5	6.9	6.4	9.5	6.0	15.9	12.6	13.1	19
	XPO5	7.6	9.6	10.8	1.7	3.8	5.8	30.1	18.3	27.8	20
Reduced	ITGAV	67.9	68.2	67.4	72.1	69.0	72.0	51.6	59.3	53.7	1
	CELF1	23.2	14.8	25.5	25.9	22.9	26.8	5.6	8.8	6.8	2
	GLG1	69.5	66.9	74.3	57.8	59.9	63.3	45.3	43.4	43.2	3
	EIF4G2	15.0	14.3	16.6	7.9	18.2	17.5	4.5	2.7	3.6	4
	JAG1	84.3	83.3	94.9	74.0	80.8	79.7	61.8	36.6	62.2	5
	UBE2M	11.4	17.6	16.8	10.9	11.1	15.3	3.9	2.4	8.1	6
	TSPAN14	74.7	73.6	82.3	63.6	42.2	48.2	30.9	24.2	26.7	7
	EWSR1	9.8	7.3	12.9	7.4	8.8	8.6	3.7	2.4	5.9	8
	EPHA2	73.8	75.8	76.4	65.5	66.4	66.3	53.9	63.0	54.9	9
	DIS3	16.8	26.7	22.3	18.6	19.2	21.3	13.2	7.3	14.2	10
	ASNS	23.0	22.5	26.6	11.4	16.8	12.1	4.9	2.5	7.3	11
	HNRNPF	6.8	10.0	16.8	16.0	16.9	17.3	2.3	5.4	4.8	12
	SMC1A	20.0	22.7	23.5	15.5	12.5	13.6	8.7	7.3	7.4	13
	BSG	51.0	51.0	46.0	78.1	80.7	87.0	23.5	18.4	35.5	14
	ALCAM	79.9	78.6	87.2	35.9	49.3	53.9	22.6	20.1	23.8	15
	LMAN2	48.5	44.1	53.2	40.9	44.4	41.3	38.6	28.2	35.7	16
	RPL36A	7.3	7.1	8.2	4.8	6.9	8.2	4.7	3.0	5.2	17
	DDB1	6.7	9.3	6.3	8.7	5.5	7.9	4.0	3.1	5.5	18
	ERGIC3	76.2	78.2	81.0	48.6	53.2	49.4	35.8	33.1	37.1	19
	RPS15A	10.5	8.9	15.1	13.1	12.1	11.6	8.1	6.0	8.8	20

**Table S12. Oxidation levels of p62 peptides in DDW vs NW.**

Sequence	Mass	Charges	Score	Peptide number		Oxidation percentage (%)						
				NW	DDW	NW-1	NW-2	NW-3	DDW-1	DDW-2	DDW-3	p value
23-46	2453.99	5;6	114.63	1	1	13.53	9.45	11.73	18.83	17.62	18.51	0.005446
119-139	2080.96	5	94.632	1	1	21.79	31.26	30.06	56.40	56.32	54.89	0.000732
151-166	1719.66	5;6	96.208	1	1	15.99	21.19	22.08	42.50	41.99	45.63	0.000437

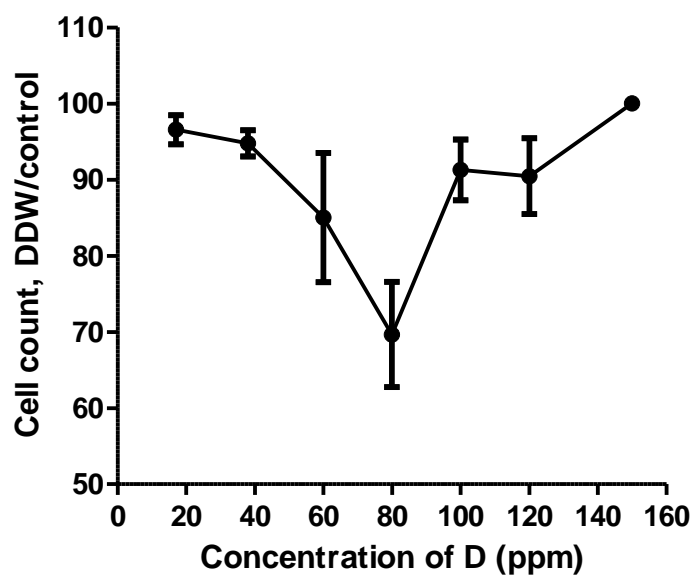
**Table S17. STRING analysis result of overlap top 50 up regulated proteins with disbalance in oxidation -reduction level or changes in stability**

	Pathway ID	Pathway description	Observed gene count	False discovery rate
KEGG Pathways	5203	Viral carcinogenesis	4	0.00738

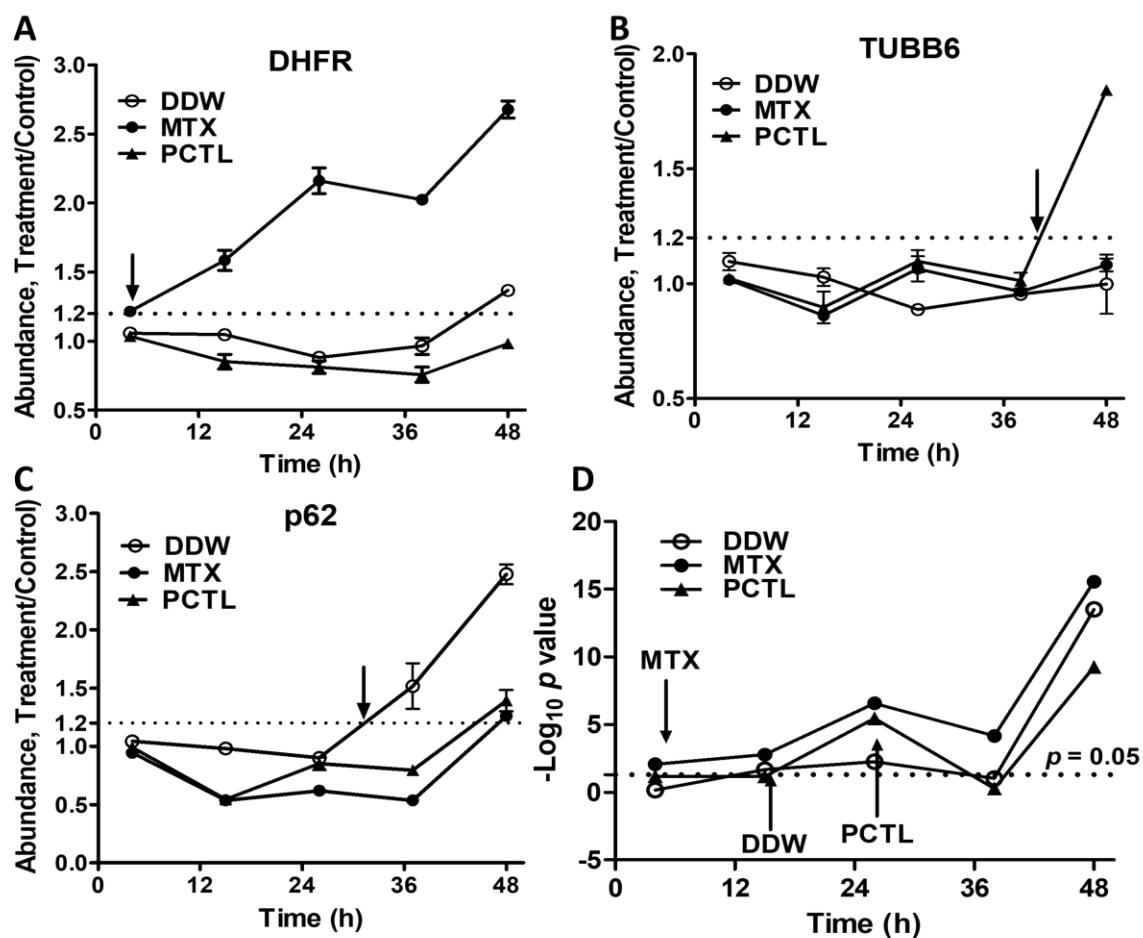
**Table S18. STRING analysis result of top 50 down regulated proteins with disbalance in oxidation-reduction level or changes in stability**

	Pathway ID	Pathway description	Observed gene count	False discovery rate
Molecular Function (GO)	GO.0016491	oxidoreductase activity	8	0.00167
	GO.0004748	ribonucleoside-diphosphate reductase activity, thioredoxin disulfide as acceptor	2	0.00407
	GO.0003824	catalytic activity	16	0.00774
KEGG Pathways	480	Glutathione metabolism	4	8.55E-05
	4115	p53 signaling pathway	4	0.000144
	1100	Metabolic pathways	8	0.00326
	240	Pyrimidine metabolism	3	0.0153

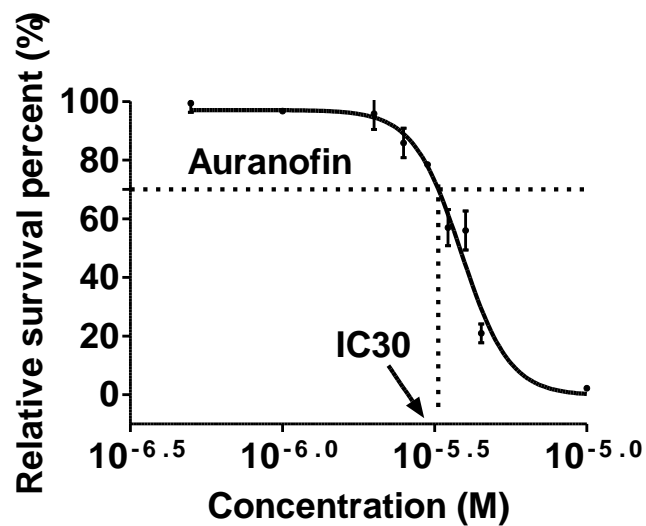




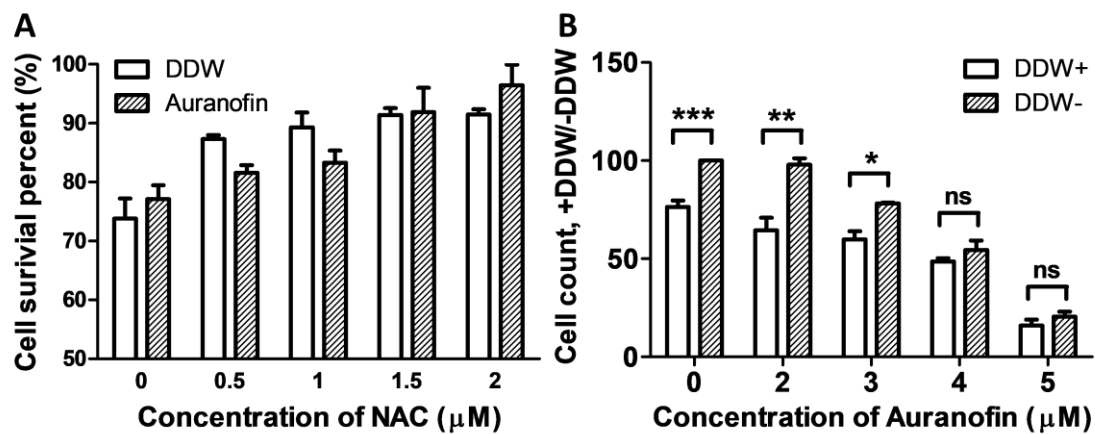
**FIG. S1.** Survival of A549 cell line grown in media with different deuterium concentrations from 17-150 ppm



**FIG. S2.** DDW effect on cell proliferation and dynamic analysis of regulation of DDW on proteome abundance. Abundance dynamic changes of DHFR (**A**), TUBB6 (**B** and p62 (**C**)). **D** Statistical significance analysis of top 50 up and down regulated proteins in FITeXP for each treatment at each time point. Error bars represent SEM,  $n = 2$ .



**FIG. S3.** Inhibitory curve of auranofin using Prism following logarithmic transformation of concentration and fitting curve using equation of log(inhibitor) vs. response – Variable slop (four parameters). Error bars represent SEM, n = 3.



**FIG. S4. Validation of DDW anticancer mechanism.** *A*, Combined effect of DDW and Auranofin on cell survival. *B*, Effect of DDW and Auranofin on cell survival in presence of NAC. Error bars represent SEM with triplicates measurements, ns  $P > 0.05$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.005$  (two-tailed unpaired t-tests).