

## **Supplementary Material**

# **Allicin Overcomes Hypoxia Mediated Cisplatin Resistance in Lung Cancer Cells through ROS Mediated Cell Death Pathway and by Suppressing Hypoxia Inducible Factors**

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## Supplementary Information

### Supplementary Methods

#### *Real-time PCR primer sequences used*

Gene	Forward primer (5'->3')	Reverse primer (5'->3')
<i>CDH1</i>	GACCCAACCCAAGAATCTATC	AGGCTGTGCCTTCCTACAGAC
<i>CDH2</i>	ACAATGCCCTCAAGTGTTAC	CATTAAGCCGAGTGATGGTCC
<i>p53</i>	AGTGGAAGGAAATTTGCGTGTGGAG	AGGATGGGCCTCCGGTTCATG
<i>p21</i>	ACCCTCTCATGCTCCAGGTG	TGTCTGACTCCTTGTTCGCTG
<i>BAX</i>	GAGTGTCTCAAGCGCATCG	CGTCAGAAAACATGTCAGCTG
<i>BCL2</i>	ACAACATCGCCCTGTGGATG	TCACTTGTGGCCAGATAGGC
<i>Tet1</i>	CAGAACCTAAACCACCCGTG	TGCTTCGTAGCGCCATTGTAA
<i>Tet2</i>	ACGCAAGCCAGGCTAAACA	GCTGGGACTGCTGCATGA
<i>Tet3</i>	TCCAGCAACTCCTAGAACTGAG	AGGCCGCTTGAATACTGACTG
<i>18s</i>	GTAACCCGTTGAACCCCAT	CCATCCAATCGGTAGTAGC

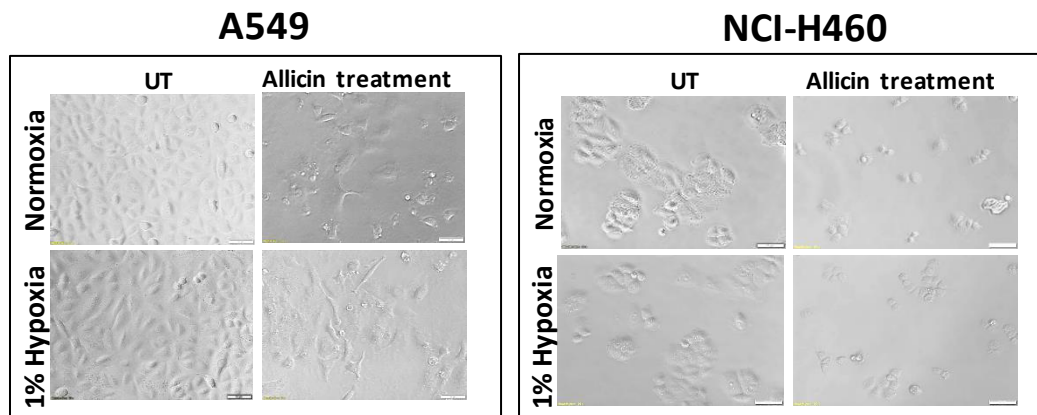
#### *Antibodies Used*

Anti-LC3A/B antibody (#ab58610) was procured from Abcam, UK. Anti HIF-1 $\alpha$  (#610959) antibody was purchased from BD Biosciences, USA and anti-p53 (#SC-126), anti-JNK (#SC-7345) and anti-p-JNK (#SC-7654) from Santa Cruz, US. Anti p38 MAPK (#9212S), anti phospho-p38 MAPK (Thr180/Tyr182) (#9211S), anti PARP (#9542S), anti-cleaved PARP (Asp214) (#9541S), anti-phospho-histone H2A.X (Ser139) (#9718S), anti-p21 Waf1/Cip1 (12D1) (#2947S) and anti HIF-2 $\alpha$  (#7096S) antibody was purchased from Cell Signaling Technology, US and  $\beta$ -actin (#NB600-501) from Novus Biological, US.

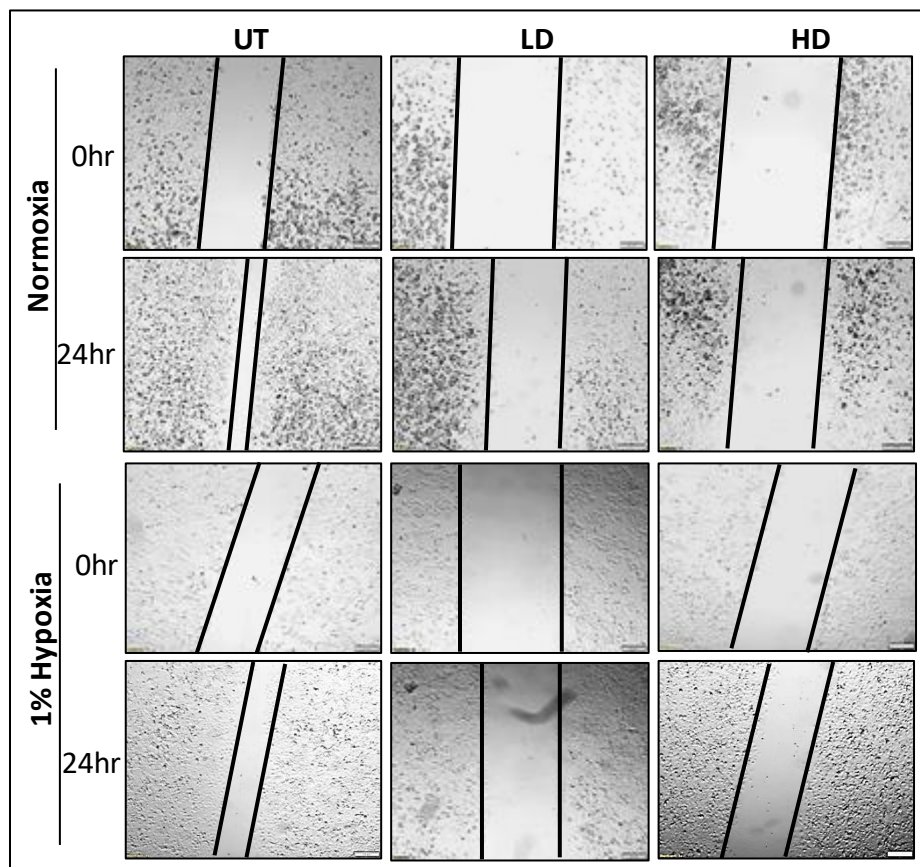
#### *Synergism calculation*

Synergism was determined by calculating the ratio index (RI) as described by Kern et al. (1998). The RI is calculated as the ratio of expected cell survival to the observed cell survival, where expected cell survival is defined as the product of the viability observed with drug A alone and the survival observed with drug B alone and observed cell survival is the viability obtained for the combination of A and B. The RI>1.5 signifies synergism.

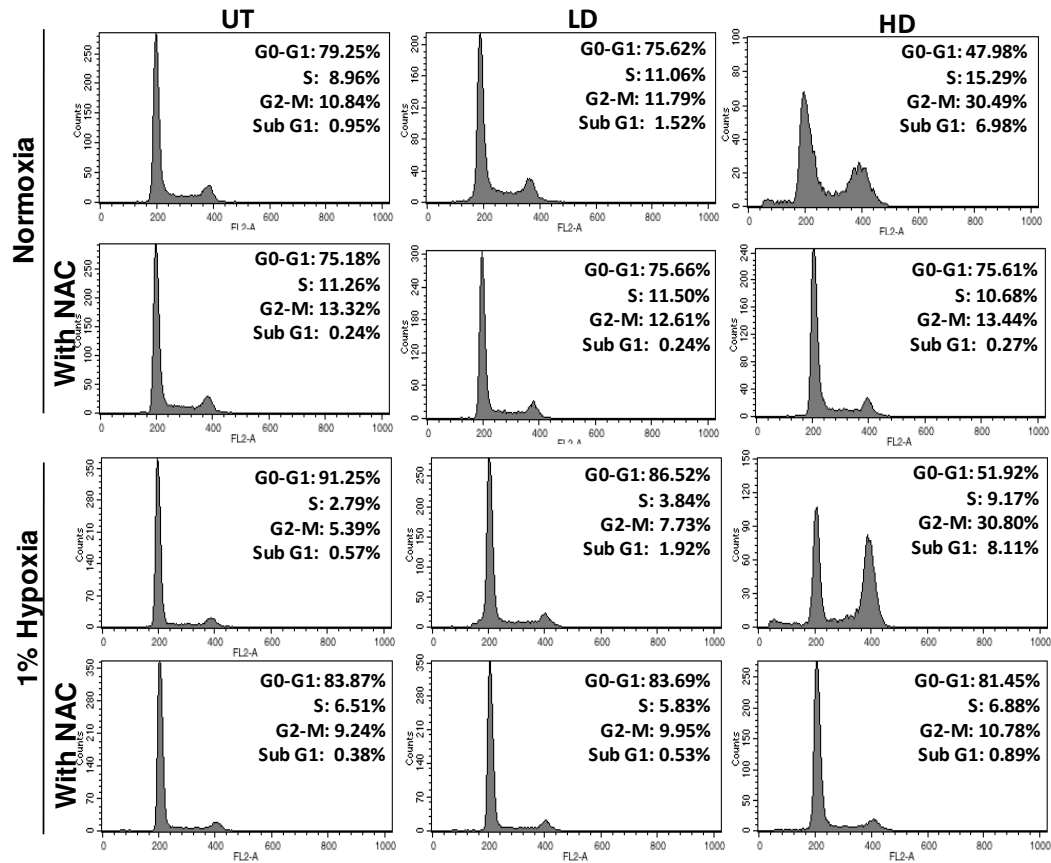
## Supplementary Figures



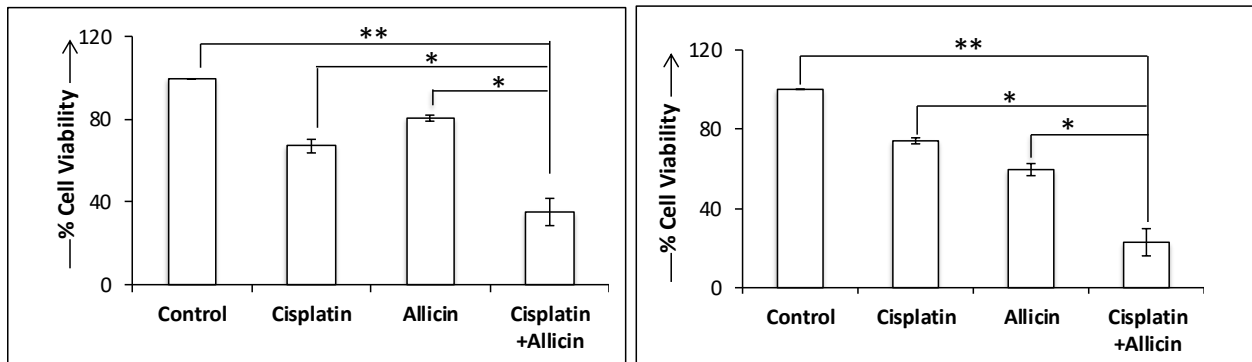
**Supplementary Figure 1:** Cellular morphological changes in A549 and NCI-H460 cell lines after allicin exposure for 48hr. Allicin treated cells were shrunk, rounded off and partially detached from the surface



**Supplementary Figure 2:** Photomicrograph of wounded A549 cells treated with 10µg/ml (LD) and 40µg/ml(HD) allicin cultured in normoxic and hypoxic condition and captured at 0hr and 24hr



**Supplementary Figure 3:** Cell cycle distribution of A549 cells after treatment with allicin in absence and presence of antioxidant NAC for 48hr in normoxic and hypoxic condition as determined by flow cytometry. UT= untreated, LD=10µg/ml allicin and HD=40µg/ml allicin.



**Supplementary Figure 4:** Cytotoxic effect of 2µg/ml cisplatin, 10µg/ml allicin and combination of 2µg/ml cisplatin and 10µg/ml allicin on A549 cells cultured in normoxia and 1% hypoxia for 72hrs. The bars represent the mean  $\pm$  standard deviation calculated from three independent experimental values. \* represents  $p < 0.05$  and \*\* represents  $p < 0.005$ .