Antimalarial Induced Cancer Cell Death Requires Oxidative Pentose Phosphate Pathway Inhibition

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SUPPLEMENTARY MATERIALS:

SUPPLEMENTARY FIGURES:

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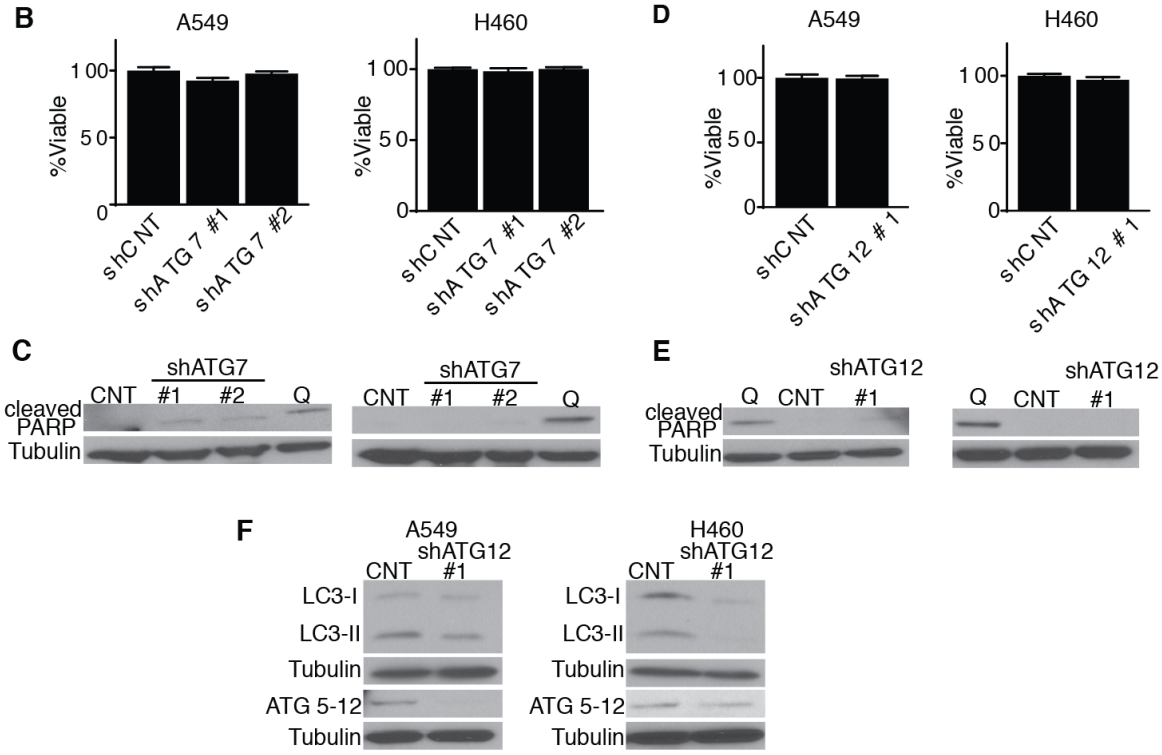
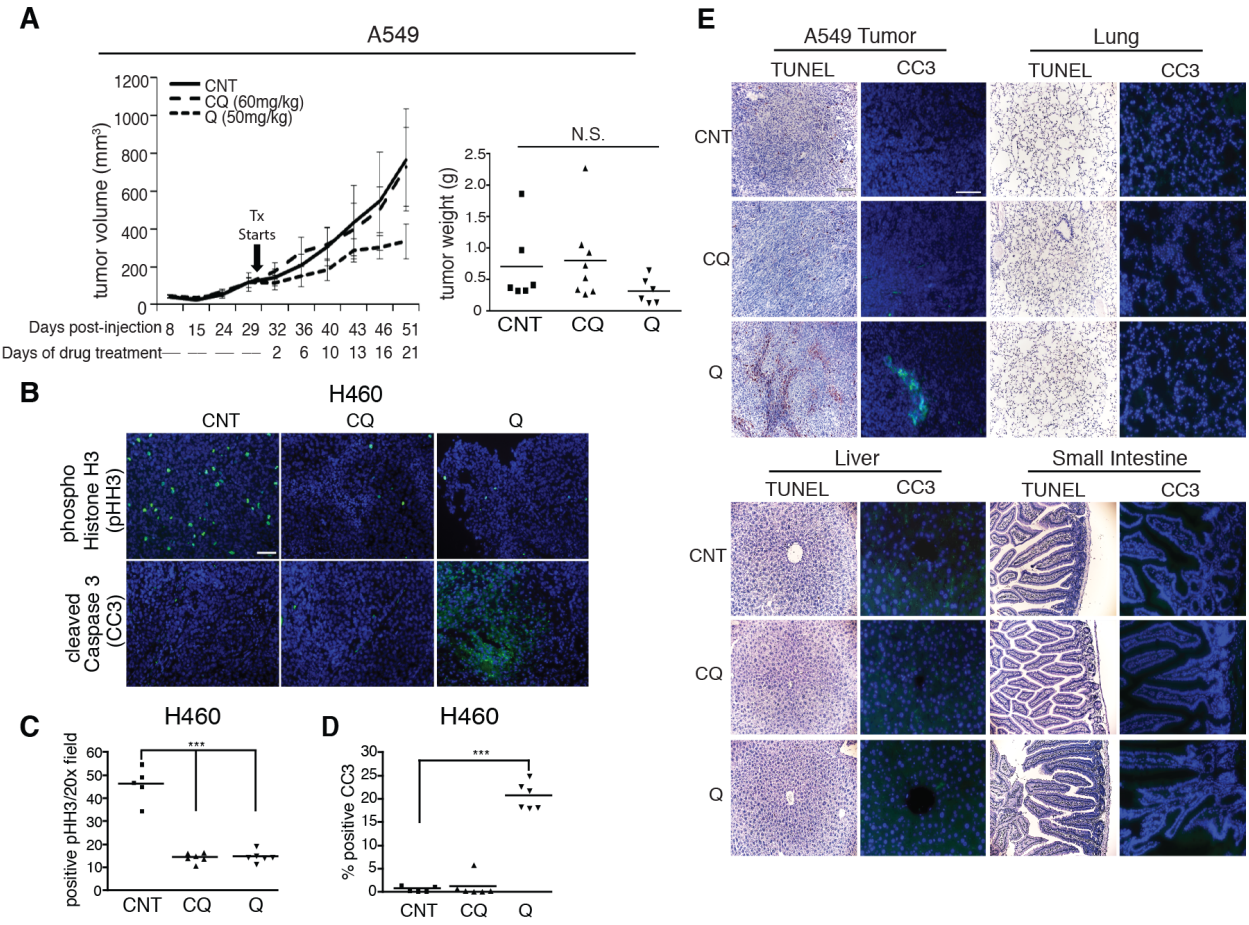
Cell Line	Tumor Type	KRAS Mutation	p53 Status
A549	Lung adenocarcinoma	G12S	Wild type
NCI-H460	Lung large cell carcinoma	Q61H	Wild type
NCI-H358	Lung bronchoalveolar carcinoma	G12C	Deleted (null)



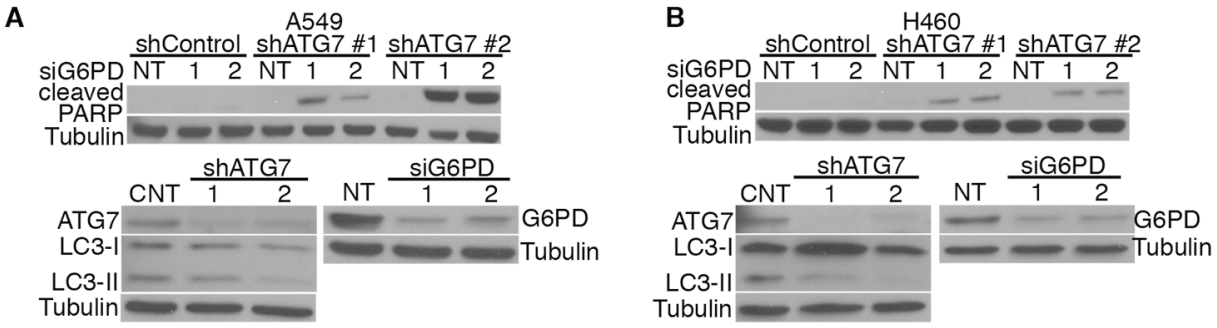
Supplementary Figure 1: ATG7 and ATG12 depletion do not induce apoptosis in NSCLC cells. (A) K-Ras mutation status and p53 status in NSCLC lines used in this study. **(B, C)** Effects of ATG7 knockdown on A549 or H460 cell survival, assayed via trypan blue exclusion. Immunoblotting for cleaved PARP shows minimal apoptosis in ATG7 depleted cells. Treatment with 5 μM Q was used as a positive control. (**D, E**) Effects of ATG12 knockdown on H460 or A549 cell survival, assayed via trypan blue exclusion. Immunoblotting for cleaved PARP shows minimal apoptosis in ATG12 depleted cells**.** Treatment with 5 μM Q served as a positive control. (**F**) Immunoblotting for LC3-II and ATG5-ATG12 complex upon depletion of ATG12. All data represent mean ± SEM from 3 independent experiments.

Statistical significance was calculated using ANOVA followed by Tukey's HSD and found to be non-significant.

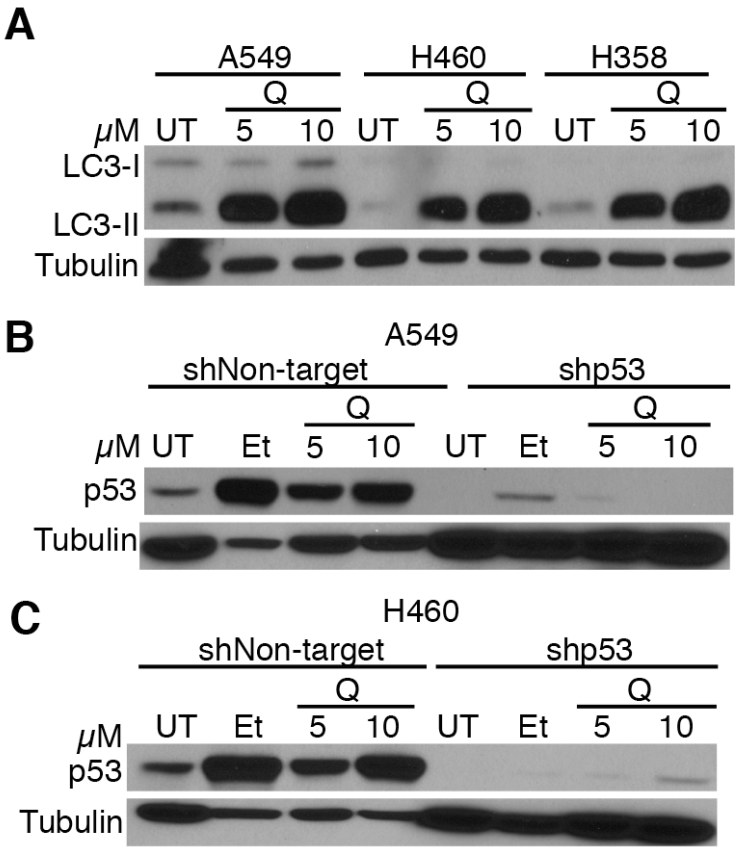


Supplementary Figure 2: Analysis of the effects of antimalarial treatment on xenograft tumors and normal tissues *in vivo*. (A) Left: Tumor volume was measured on the indicated days in A549 xenograft tumors treated with vehicle (CNT, water), CQ and Q. Right: Tumor weights from individual mice following 21 days of treatment. Data represent mean ± SEM from 6 independent tumors for each cohort. Statistical significance was calculated using ANOVA followed by Tukey's HSD. N.S.=nonsignificant. (B) Immunostaining for phospho-histone H3 (pHH3, proliferation marker) and cleaved caspase-3 (CC3, apoptosis marker) of H460 lung tumor xenografts treated with CQ and Q. Bar, 100μm. **(C, D)** Quantification of pHH3 (**C**) and CC3 (**D**) positive cells. Data are the mean ± SEM from 6 independent tumors for each condition. Statistical significance was calculated using ANOVA followed by Tukey's HSD. ***P≤0.001. (**E**) TUNEL staining and immunostaining for cleaved caspase-3 (CC3) in A549

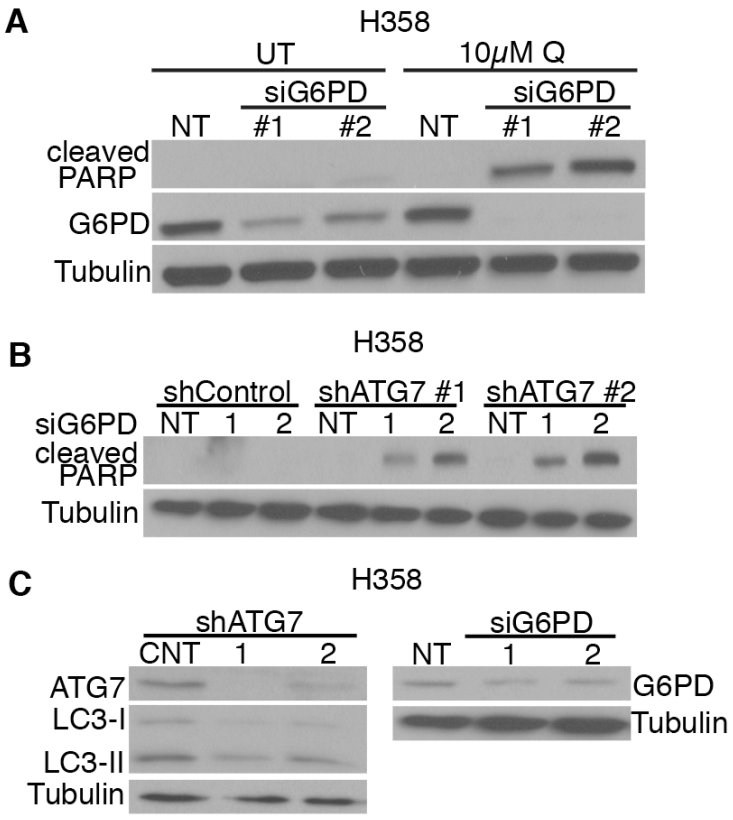
xenograft tumors, lung, liver and small intestine from tumor-bearing mice treated with vehicle (CNT, water), CQ and Q. Bar, 100μm.



Supplementary Figure 3: Validation of ATG7 and G6PD knockdown in NSCLC cells. (A, B) Immunoblotting for cleaved PARP, ATG7, G6PD and LC3-II after siRNA-mediated depletion of G6PD and shRNA knockdown of ATG7 in A549 (**A**) and H460 (**B**) cells.



Supplementary Figure 4: Effects of Q on LC3-II accumulation and p53 induction in NSCLC cells. **(A)** LC3-II accumulation following 18 h Q treatment at the indicated doses in A549, H460 and H358 cells. **(B, C)** p53 protein levels following shRNA mediated p53 knockdown in A549 (**B**) and H460 (**C**) cells in response to the indicated doses of Q for 18 h. Et=50μM etoposide (positive control).



Supplementary Figure 5: Apoptosis upon combined knockdown of ATG7 and G6PD in p53 null NSCLC cells. (A) Immunoblotting for cleaved PARP in H358 cells following siRNA-mediated knockdown of G6PD and treatment with 10 μM Q for 18 h. UT=untreated. **(B)** Immunoblotting for cleaved PARP after RNAi-mediated knockdown of G6PD and shRNA depletion of ATG7 in H358 cells. **(C)** Immunoblotting for ATG7, LC3-II and G6PD in H358 cells.