Supplementary Figures

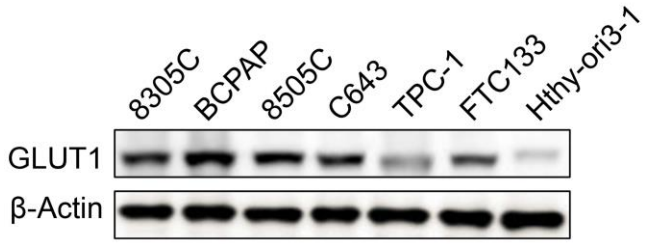

Figure S1. Western blot analysis of GLUT1 expression in a panel of thyroid cancer cell lines and human immortalized thyroid epithelial cell Hthy-ori3-1. β-Actin was used as a loading control.

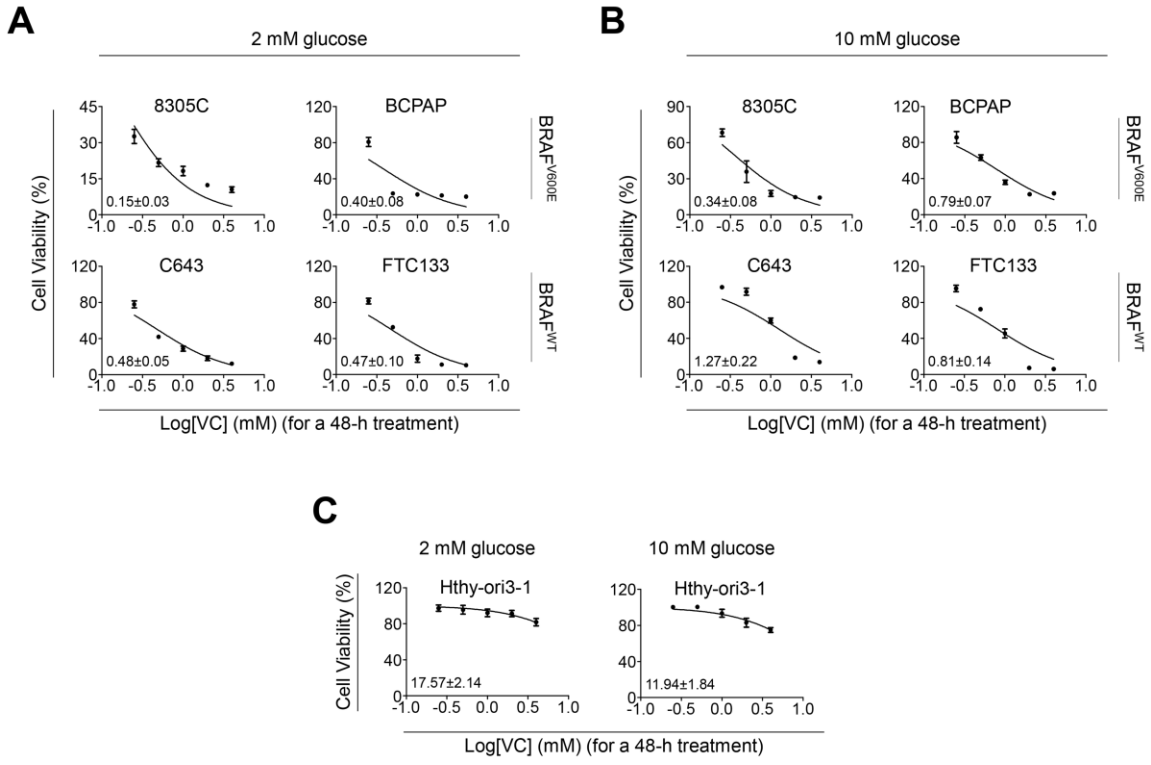

Figure S2. The above cells were treated with different doses of VC for 48 h in the medium containing 2 mM glucose (**A**) and 10 mM glucose (**B**). (**C**) The Hthy-ori3-1 cell were treated with different doses of VC for 48 h in the medium containing different glucose concentrations. Cell viability was then measured by MTT assay, and IC50 values

were calculated using the Reed-Muench method.

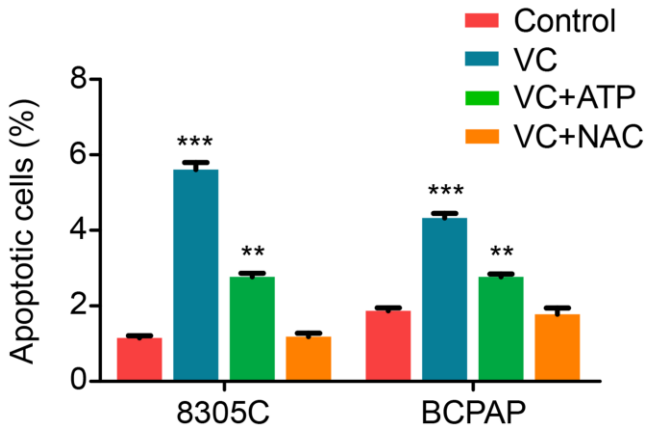

Figure S3. Cell apoptosis was analyzed by flow cytometry in 8305C and BCPAP cells with the indicated treatments. Data were presented as mean ± SD. **, *P* <0.01; ***, *P* <0.001.

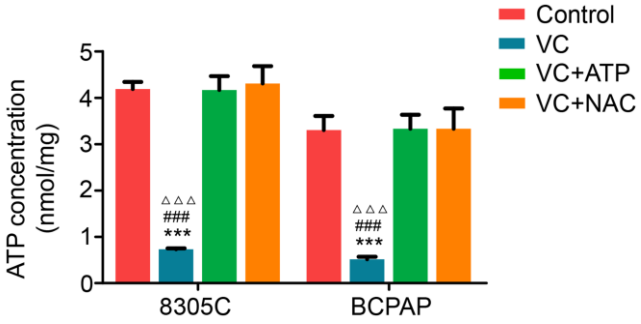

Figure S4. ATP measurement in 8305C and BCPAP cells with the indicated treatments by ELISA assay. Data were presented as mean ± SD. ***, *P* <0.001 for comparison with the control; ###, *P* <0.001 for comparison with a combined treatment of VC and ATP; △△△, *P* <0.001 for comparison with a combined treatment of VC and NAC.

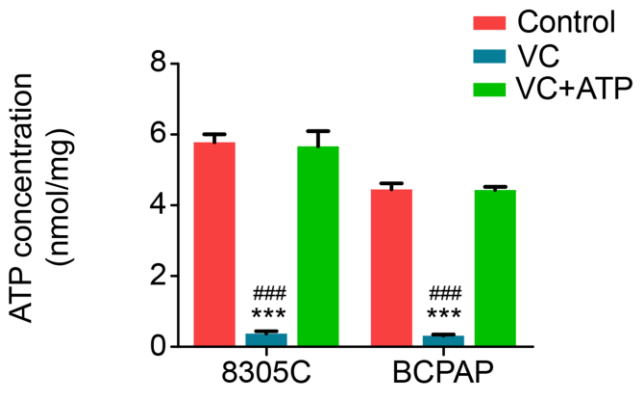

Figure S5. ATP measurement in 8305C and BCPAP cells with VC treatment alone or in combination with ATP by ELISA assay. Data were presented as mean ± SD. ***, *P* <0.001 for comparison with the control; ###, *P* <0.001 for comparison with a combined treatment of VC and ATP.

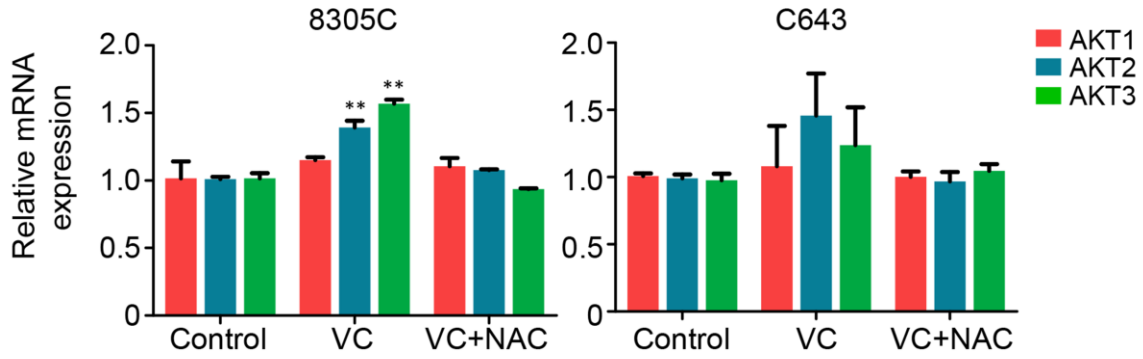

Figure S6. qRT-PCR assay of expression of three AKT isoforms in 8305C and C643 cells with the indicated treatments. *18S* rRNA was used as a reference gene. **, *P* <0.01.

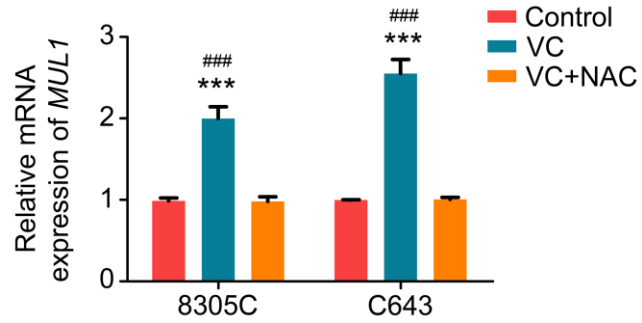

Figure S7. qRT-PCR assay of MUL1 expression in 8305C and C643 cells with VC

treatment alone or in combination with NAC. *18S* rRNA was used as a reference gene. ***, *P* <0.001 for comparison with the control; ###, *P* <0.001 for comparison with a combined treatment of VC and NAC.

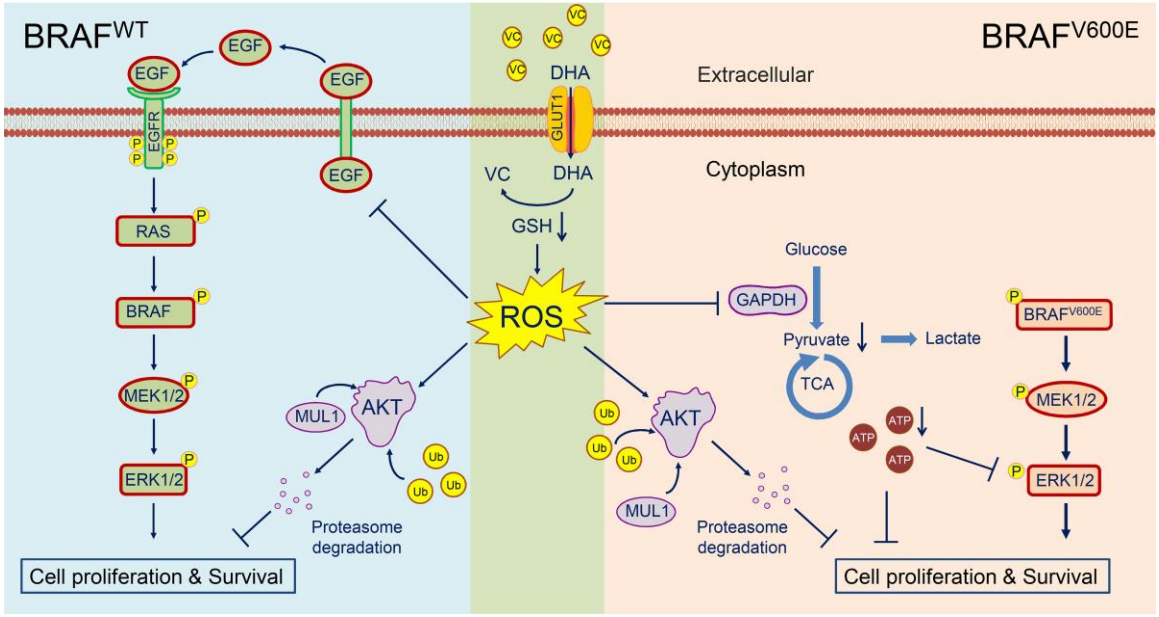

Figure S8. A schematic model illustrating the mechanism of vitamin C killing thyroid cancer cells. Generally, oxidized form of vitamin C, DHA, was taken up by thyroid cancer cells via GLUT1, thereby increasing ROS production by consuming cellular glutathione (GSH). Increased ROS inhibits ERK phosphorylation in BRAF mutant thyroid cancer cells through decreasing ATP production via targeting GAPDH, while increased ROS inhibits the activity of EGF/EGFR-MAPK/ERK cascade in BRAF wild-type thyroid cancer cells through diminishing EGF release. In addition, increased ROS also can promote AKT ubiquitination and proteasome degradation in both BRAF mutant and wild-type thyroid cancer cells probably through upregulating MUL1 expression via a ROS-dependent pathway. As a result, vitamin C ROS-dependently kills thyroid cancer

cells through blocking the activities of MAPK/ERK and PI3K/AKT pathways.

Supplementary Tables

Supplementary Table S1. The antibodies used in this study

Antibodies	Catalog#	Source
anti-Ubiquitin	Ab7780	Abcam
anti-p-EGFR	Ab32578	Abcam
anti-GlUT1	D260433	BBI Life Sciences
anti-Ki67	550609	BD Pharmingen
anti-t-AKT	BS1810	Bioworld Technology
anti- p-AKT	BS4009	Bioworld Technology
anti-t-ERK	#4695	Cell Signaling Technology
anti-AKT (IP)	#2920	Cell Signaling Technology
anti-p-ERK1/2	#4370	Cell Signaling Technology
anti-EGFR	#4267	Cell Signaling Technology
anti-Actin	sc-1616	Santa Cruz

Supplementary Table S2. The primers used in this study

Genes	Forward (5'-3')	Reverse (5'-3')
AKT1	ATGAGCGACGTGGCTATTGT	TGAAGGTGCCATCATTCTTG
AKT2	AACACAAGGAAAGGGAACCA	AGGAGGCACCGTGGACA
AKT3	GCTCAGAGGGGAGTCATCAT	GGAAGTATCTTGGCCTCCAG
MUL1	CACAAGATGGTGTGGAATCG	TCAGCATCTCCTCGGTCTCT
18S	CGCCGCTAGAGGTGAAATTC	CTTTCGCTCTGGTCCGTCTT