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Vitamin C selectively kills *KRAS* and *BRAF* mutant colorectal cancer cells by targeting GAPDH

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More than half of human colorectal cancers (CRCs) carry either *KRAS* or *BRAF* mutations, and are often refractory to approved targeted therapies. We report that cultured CRC cells harboring *KRAS* or *BRAF* mutations are selectively killed when exposed to high levels of vitamin C. This effect is due to increased uptake of the oxidized form of vitamin C, dehydroascorbate (DHA), via the GLUT1 glucose transporter. Increased DHA uptake causes oxidative stress as intracellular DHA is reduced to vitamin C, depleting glutathione. Thus, ROS accumulates and inactivates glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Inhibiting GAPDH in highly glycolytic *KRAS* or *BRAF* mutant cells leads to an energetic crisis and cell death not seen in *KRAS* and *BRAF* wild-type cells. High-dose vitamin C impaired tumor growth in $Apc/Kras^{G12D}$ mutant mice. These results provide a mechanistic rationale for exploring the therapeutic use of vitamin C for CRCs with *KRAS* or *BRAF* mutations.

Activating *KRAS* and *BRAF* mutations are found in approximately 40% and 10% of human colorectal cancers (CRCs), respectively (*1*). BRAF is a direct target of KRAS and both activate the mitogen-activated protein kinase (MAPK) pathway. Clinical studies indicate that activating mutations in *KRAS* and *BRAF* predict resistance to epidermal growth factor receptor (EGFR)-targeting agents (*2*–*4*). Thus, novel therapies for *KRAS* or *BRAF*-mutant CRCs are urgently needed.

Glucose uptake, as measured by FDG-PET, correlates with *KRAS* or *BRAF* mutations and GLUT1 overexpression in CRCs (*5*, *6*) consistent with our previous finding that *KRAS* or *BRAF* mutant CRC cells rewire glucose metabolism, in part by upregulating *GLUT1* expression (*7*). These data suggest a strategy for targeting *KRAS* or *BRAF*-mutant cancers by exploiting the selective expression of *GLUT1* and the metabolic liability that comes with increased reliance on glycolysis.

Dietary vitamin C is transported across cellular membranes by sodium vitamin C cotransporters (SVCTs) and facilitative glucose transporters (GLUTs) (*8*, *9*). While SVCTs transport vitamin C directly into the cell, GLUTs—mainly GLUT1 and GLUT3—transport the oxidized form of vitamin C, dehydroascorbate (DHA). Following import, DHA is reduced to vitamin C at the expense of glutathione (GSH), thioredoxin and NADPH (*10*). Given that GLUT1 levels in *KRAS* and *BRAF* mutant cells are elevated, we hypothesized that the increase in DHA uptake could disrupt redox homeostasis and compromise cellular viability. To test our hypothesis, we used a panel of isogenic CRC cell lines harboring WT or mutant alleles of *KRAS* (HCT116 and DLD1) or *BRAF* (VACO432 and RKO) (*7*).

In cell culture media, vitamin C is oxidized to DHA (half-life ~70 min) unless reducing agents are added (fig. S1) (*11*). Using ^{14}C-radiolabeled vitamin C, we tested which form of vitamin C (reduced or oxidized DHA) is preferentially imported. Both HCT116 and VACO432 cells take up [^{14}C]-vitamin C efficiently (Fig. 1A). However, adding GSH to the media to prevent oxidation of vitamin C to DHA abrogated [^{14}C]-vitamin C uptake (Fig. 1A). Furthermore, [^{14}C]-vitamin

C uptake was significantly decreased in both HCT116 and VACO432 cells treated with a GLUT1 specific inhibitor, STF31, and in GLUT1 knockout cells (Fig. 1, A and B). Glucose competed with DHA for uptake in CRC cells (fig. S2). These results indicate that CRC cells preferentially import DHA, rather than vitamin C, and that uptake is mediated by GLUT1 (fig. S3, A and B). Given the increased expression of GLUT1 in mutant cells, we investigated whether *KRAS* or *BRAF* mutations influenced vitamin C uptake. Importantly, the mutant lines took up significantly more [^{14}C]-vitamin C than their WT counterparts (Fig. 1, B and C). Overexpressing GLUT1 in WT cells was sufficient to increase [^{14}C]-vitamin C uptake to levels commensurate with those of the mutants (Fig. 1B and fig. S3C). Moreover, *KRAS* and *BRAF* mutant cells imported DHA faster than [^{14}C]-vitamin C (fig. S4), consistent with the observation that vitamin C must first be oxidized to DHA to enter cells through GLUT1. Together, these results indicate that GLUT1 is the primary means of vitamin C uptake in CRC cells and that elevated GLUT1 expression in *KRAS* or *BRAF* mutant cells drives increased DHA uptake.

We next asked whether the increased uptake of DHA in *KRAS* and *BRAF* mutant cells could affect their survival and growth. When plated at a low density and grown in low glucose media (2 mM), all cell lines grew at similar rates and formed colonies (fig. S5). However, 24 to 48 hours of vitamin C treatment inhibited *KRAS* and *BRAF* mutant cell growth and colony formation with reduced effects on their WT counterparts (Fig. 2A and fig. S5). Due to the competitive nature of DHA import, mutant lines were most sensitive to vitamin C under low glucose conditions (2 mM). Nevertheless, selective cytotoxicity against the mutant lines was achieved even under higher glucose conditions (5-20 mM) when treating with less than 1 mM vitamin C (fig. S6), indicating that vitamin C can selectively kill mutant cells under physiological glucose concentration (5-10 mM). Importantly, plasma vitamin C concentrations greater than 10 mM are easily achieved in humans and in our murine pharmacokinetic study (fig. S7) without significant toxicity (*12*, *13*). Vitamin C was cytotoxic rather than cytostatic as evidenced by increased staining for the apoptotic marker Annexin V in the mutants (fig. S8A). Adding GSH to the culture medium was sufficient to rescue the death of each mutant line (Fig. 2A). *PIK3CA* is one of three frequently mutated oncogenes in CRCs in addition to *KRAS* and *BRAF*. Unlike *KRAS* or *BRAF*, the *PIK3CA* genotype did not predict vitamin C sensitivity (fig. S8B). Notably, although the overexpression of *GLUT1* in WT cells increased vitamin C uptake (Fig. 1B), it did not sensitize WT cells to vitamin C (fig. S8C) indicating that high GLUT1 expression alone, without oncogene induced metabolic reprograming, is not sufficient to make cells susceptible to vitamin C-dependent toxicity.

We next explored whether vitamin C altered the growth of *KRAS* and *BRAF* mutant CRC in mice. Mice bearing established xenografts derived from parental HCT116 and VACO432 cell lines were treated twice a day via intraperitoneal (IP) injection of high-dose vitamin C (4 g/kg) or PBS (vehicle control), for 3-4 weeks, at which point control mice had to be sacrificed due to tumor size. Vitamin C treatment significantly reduced tumor growth compared to vehicle control treatment (Fig. 2B). *KRAS* and *BRAF* wild-type isogenic HCT116 and VACO432 cell lines cannot form xenograft tumors in mice. To directly test the impact of *Kras* mutation on the sensitivity of tumors to vitamin C treatment, we generated a transgenic model of intestinal cancer, driven by either *Apc* mutation, or combined *Apc* and *Kras* (G12D) mutations. Compound mutant mice were generated by crossing available Apc^{flox} mice (*14*), $LSL\text{-}Kras^{G12D}$ mice (*15*), and $Lgr5\text{-}EGFP\text{-}creER^{T2}$ (*16*) animals, enabling intestinal restricted alteration of *Apc* and *Kras*. Tumors were induced with a single IP injection of low-dose tamoxifen (20 mg/kg) and treated daily thereafter with high-dose vitamin C (IP, 4 g/kg) for 5-7 weeks. While $Apc^{flox/flox}$ mice showed no difference in polyp burden following vitamin C treatment, $Apc^{flox/flox/}Kras^{G12D}$ mice had significantly fewer and smaller small intestine polyps (76 vs 165 in control group) confirming that vitamin C selectively affected *Kras* mutant tumors (Fig. 2C and fig. S9). Consistent with experiments in CRC lines, tumors from $Apc^{flox/flox}/Kras^{G12D}$ mice showed higher GLUT1 expression and greater vitamin C uptake than tumors from $Apc^{flox/flox}$ mice (Fig. 2, D and E, and fig. S10).

To investigate the mechanism by which vitamin C is selectively toxic to *KRAS* and *BRAF* mutant cells, we used LC-MS/MS based metabolomics to profile metabolic changes following vitamin C treatment (*17*). In untreated *KRAS* and *BRAF* mutant lines, the relative intracellular metabolite levels of glycolysis and the non-oxidative arm of the pentose phosphate pathway (PPP) were increased compared to their isogenic WT counterparts (fig. S11). Addition of a MEK1/2 inhibitor to the parental *KRAS* or *BRAF* mutant cells also decreased glycolytic and PPP metabolite levels indicating that the increased metabolite levels were driven by oncogene-induced MAPK activity (fig. S12) (*18*). Notably, within an hour of vitamin C treatment, the metabolic profile of the mutant cells changed dramatically. Glycolytic intermediates upstream of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) accumulated while those downstream were depleted suggesting that GAPDH was inhibited (Fig. 3A and fig. S13). Also, oxidative PPP metabolites increased (Fig. 3A and fig. S13), indicating that the blockage may shift glycolytic flux into the oxidative PPP. Indeed, vitamin C treatment stimulated oxidative PPP-dependent $^{14}CO_2$ production from [1-^{14}C] glucose in both *KRAS* and *BRAF* mutant cells, and to a lesser degree in WT cells (fig. S14A). Decreased NADPH/NADP$^+$ ratios are known to activate glucose-6-phosphate dehydrogenase allosterically to enhance oxidative PPP flux. The increased flux is an attempt to restore cytosolic NADPH back to homeostasis to mitigate oxidative stress (*19*). We reasoned that DHA uptake may deplete cellular GSH and NADPH as they are consumed in reducing DHA to vitamin C. If the capacity of this pathway to restore GSH levels is exceeded, cellular reactive oxygen species (ROS) increase because GSH is the major cellular antioxidant (*20*). Indeed, the ratio of reduced to oxidized glutathione decreased as intracellular vitamin C increased (Fig. 3B and fig. S14B). Cysteine, the major limiting precursor for GSH biosynthesis, was also dramatically depleted following vitamin C treatment (fig. S13). As expected, vitamin C treatment induced a substantial increase in endogenous ROS in *KRAS* and *BRAF* mutant cells (Fig. 3C).

Given that cancer cells with *KRAS* or *BRAF* mutations are heavily dependent on glycolysis for survival and growth and that pyruvate, the end product of glycolysis, is a major carbon source for the mitochondrial TCA cycle (*7*, *21*), we hypothesized that inhibition of glycolysis at GAPDH might deplete ATP and thereby induce an energetic crisis ultimately leading to cell death. Vitamin C treatment caused a rapid decrease in the glycolytic rate of *KRAS* and *BRAF* mutant cells, but not in WT cells, as determined by the extracellular acidification rate (ECAR), a proxy for lactate production (Fig. 3D and fig. S15). Accordingly, vitamin C induced a significant drop in ATP levels with a concomitant increase in AMP levels (Fig. 3E and fig. S16A). Within one hour, AMPK, a marker for energy stress, was activated and activation was strongest in the mutant lines (Fig. 3F). The cell permeable reducing agent and glutathione precursor N-acetyl-cysteine (NAC) rescued both AMPK activation and cell death in the mutant lines (Fig. 3, F and G). Consistent with the in vitro results, supplementing drinking water with NAC over the course of vitamin C treatment abolished ability of vitamin C to reduce xenograft growth (Fig. 3H). Similarly, pyruvate and oxaloacetate, both of which can enter the TCA cycle and thus provide ATP, or trolox (a water-soluble analog of the antioxidant vitamin E) rescued energy stress and cell death (Fig. 3G and fig. S16, B and C). Rotenone, a complex I inhibitor, attenuated the ability of pyruvate to rescue vitamin C-induced cytotoxicity (fig. S17), indicating that the lack of mitochondrial substrates caused by glycolytic inhibition also contributes to ATP depletion in mutant cells (*21*).

We next sought to determine the mechanism by which vitamin C inhibits GAPDH. GAPDH is known to have an active-site cysteine (C152) that is targeted by ROS (*22*). The active-site cysteine can undergo reversible S-glutathionylation in which the oxidized cysteine forms a mixed disulfide with GSH (Cys-GSH), or undergo further irreversible oxidations that include sulfonic acid (Cys-SO_3H) (*23*, *24*). Both cases result in loss of GAPDH activity. We measured GAPDH S-glutathionylation following vitamin C treatment by immunoprecipitating endogenous GAPDH and blotting with an antibody that recognizes S-glutathionylation under non-reducing conditions. In both *KRAS* and *BRAF* mutant lines, GAPDH S-glutathionylation levels were two to three fold higher in vitamin C treated cells compared to vehicle treated cells (Fig. 4A). However, GAPDH sulfonylation was not detected with a GAPDH-SO_3H antibody (Fig. 4B). GAPDH activity was assayed in lysates of vitamin C treated cells to confirm inhibition by S-glutathionylation (fig. S18). A one-hour vitamin C treatment decreased GAPDH activity by 50% in both *KRAS* and *BRAF* mutant cells. Combining NAC with vitamin C fully rescued GAPDH activity (fig. S18).

We reasoned that the 50% reduction in GAPDH activity following vitamin C treatment could be explained by S-glutathionylation (Fig. 4A). However, given that the GAPDH substrates were added to the lysates to perform the activity assay, and the striking accumulation of the GAPDH substrate glyceraldehyde-3-phosphate (G3P) - up to 19 fold (Fig. 3A and fig. S13), we suspected that additional mechanisms may contribute to GAPDH inhibition. This led us to examine the levels of the NAD^+ substrate required for GAPDH-dependent oxidation of G3P. In contrast to G3P levels, intracellular NAD^+ levels were significantly diminished following vitamin C treatment (fig. S19). PARP activation due to ROS-induced DNA damage consumes NAD^+ to form ADP-ribose polymers on acceptor proteins. We observed PARP activation and phosphorylation of H2AX, a marker of DNA damage, shortly after vitamin C treatment (Fig. 4C), suggesting that PARP activation may diminish NAD^+ levels thereby further inhibiting GAPDH activity by depleting substrate availability (*25*). To investigate whether PARP activation or NAD^+ depletion contributes to vitamin C-induced cytotoxicity in *KRAS* and *BRAF* mutant cells, we treated cells with a PARP inhibitor, Olaparib, or a cell-permeable NAD^+ precursor, nicotinamide mononucleotide (NMN), prior to vitamin C treatment. Cell viability following vitamin C treatment was partially rescued by inhibiting PARP or supplementing with NMN (Fig. 4D). Taken together, these results indicate that in *KRAS* and *BRAF* mutant cells vitamin C-induced endogenous ROS inhibits GAPDH by both post-translational modifications and NAD^+ depletion ultimately leading to an energetic crisis and cell death (Fig. 4E).

High-dose vitamin C cancer therapy has a controversial history. While some early clinical studies indicated that vitamin C had anti-tumor activity (*26*, *27*), others have shown little effect (*28*, *29*). Recent studies suggest that the contradictory clinical data may be explained, at least in part, by differences in administration route; the millimolar vitamin C plasma concentrations cytotoxic to cancer cells are only achievable via intravenous administration, not via oral administration (*30*, *31*). Given these findings, a growing number of phase I/II clinical trials are reevaluating intravenous infusion of vitamin C to treat various cancers (*12*, *13*, *32*, *33*). However, despite the previous studies demonstrating that high-dose vitamin C is cytotoxic to cancer cells in vitro (*34–36*) and delays tumor growth in xenograft models (*37*, *38*), the mechanism by which vitamin C kills cancer cells while sparing normal cells has been unclear. Our findings address this fundamental question, suggesting that the oxidized form of vitamin C, DHA, is the pharmaceutically active agent, and that the selective toxicity of vitamin C to tumor cells stems from high GLUT1 expression combined with *KRAS* or *BRAF* oncogene-induced glycolytic addiction. Although it is unclear whether the results we have observed in our cell culture and mouse studies will translate to human tumors, our findings on the mechanism of action of vitamin C may warrant future investigation in clinical trials.

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/cgi/content/full/science.aaa5004/DC1
Materials and Methods
Figs. S1 to S19
References (*39*, *40*)

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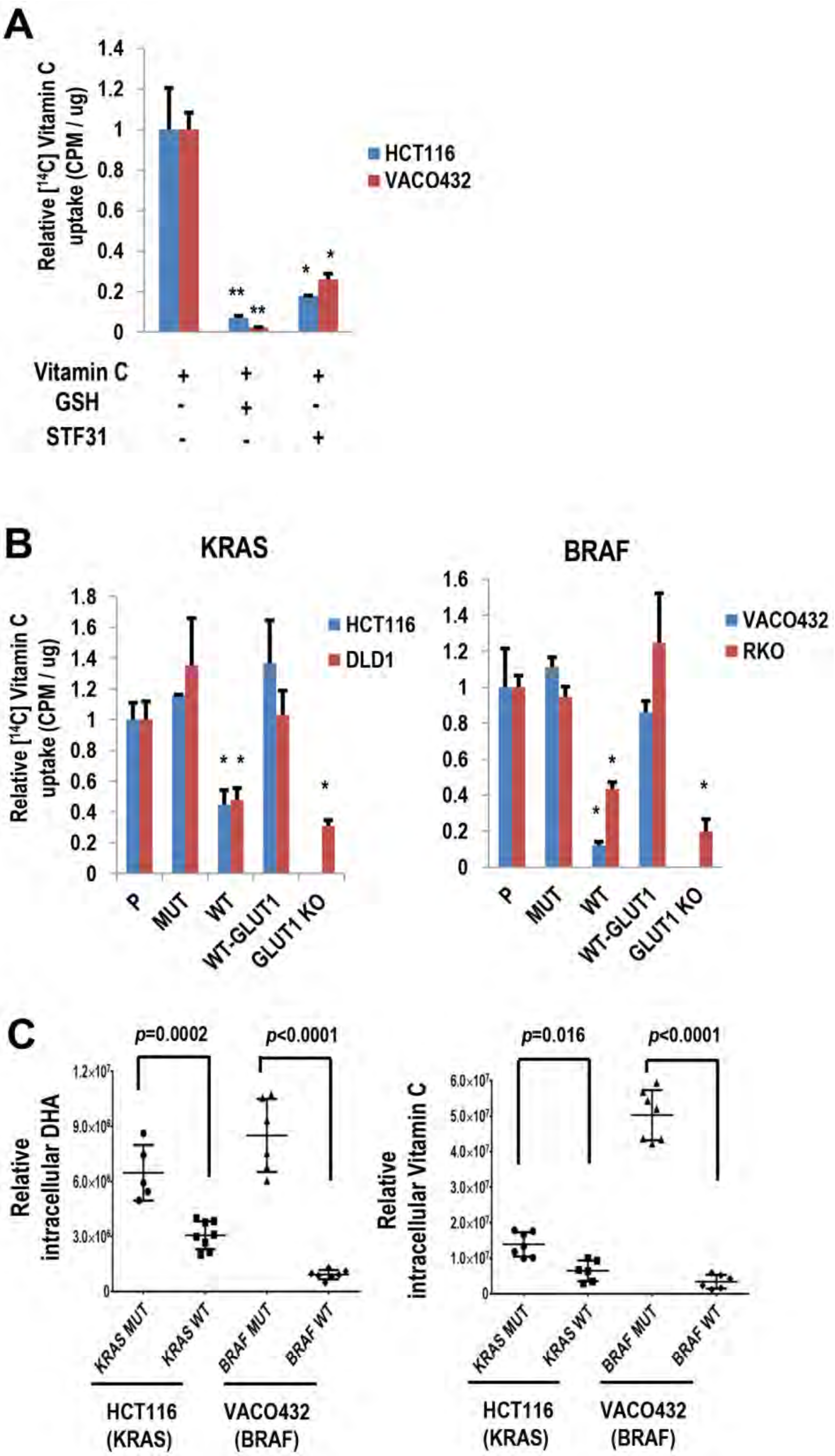


Fig. 1. *KRAS* and *BRAF* mutant cells predominantly take up DHA, the oxidized form of vitamin C, via GLUT1. (**A**) DHA, but not vitamin C, is transported into colorectal cancer cells (CRC) via GLUT1. [^{14}C]-vitamin C was added to the culture media (2 mM glucose) for 30 min. [^{14}C] Scintillation count per microgram of protein input was measured. Treating cells with GSH or STF31 (GLUT1 inhibitor) significantly reduced vitamin C uptake in all cases when compared to no GSH or STF31 treatment. One-way ANOVA followed by Dunnett's post-test for multiple comparisons. $*p < 0.01$, $**p < 0.001$, n = 3. (**B**) [^{14}C]-vitamin C uptake was monitored in 2 mM glucose and signal normalized to total protein. P; Parental cells, WT-GLUT1; Exogenously expressed *GLUT1* in WT cells, GLUT1 KO; *GLUT1* knockout cells. Asterisks indicate significant decreases in vitamin C uptake of WT or GLUT1 KO cells relative to the parental lines, MUT, and WT-GLUT1. One-way ANOVA followed by Dunnett's post-test. $*p < 0.01$, n = 3. (**C**) LC/MS analysis of intracellular vitamin C and DHA in *KRAS* or *BRAF* isogenic cell lines derived from HCT116 and VACO432, respectively. Cells were treated with 1 mM (HCT116) or 2 mM (VAOC432) vitamin C for one hour before extracting vitamin C and DHA (Student's *t* test, n = 6). All data represent means ± s.d.

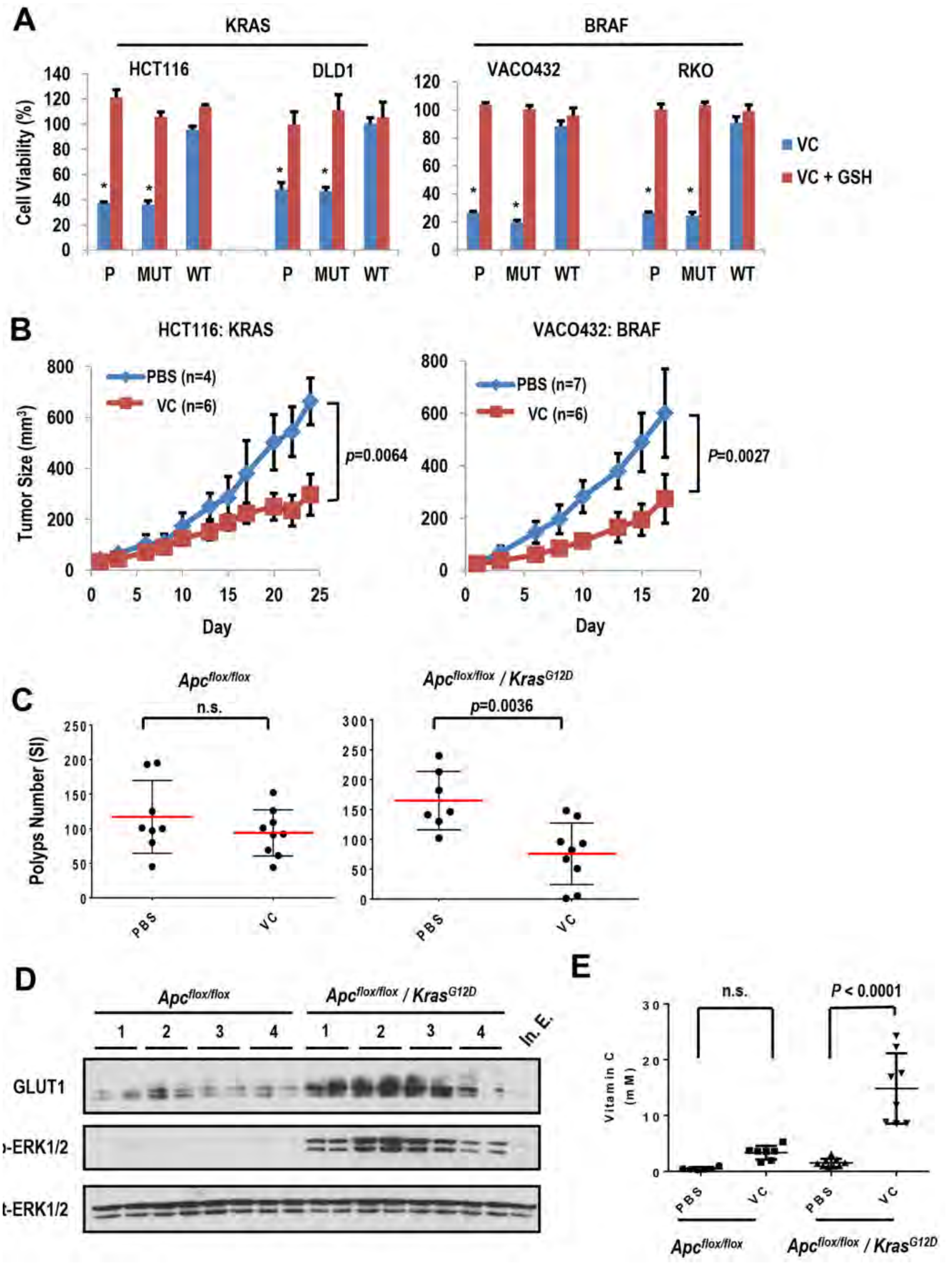


Fig. 2. See caption, next page.

Fig. 2 (preceding page). Vitamin C is selectively toxic to cells with mutant *KRAS* or *BRAF* alleles. (**A**) Cell viability assay in 2 mM glucose or 2 mM glucose plus GSH in the presence of vitamin C (VC) for 48 hours (HCT116, DLD1, RKO: 0.125 mM; VACO432: 0.375 mM) after cells were plated at a low density. Values were normalized to vehicle control. Parental (P) and MUT cells were significantly more sensitive than WT cells in the presence of vitamin C. One-way ANOVA ($p < 0.0001$) with Dunnett's post-test. *$p < 0.0001$, n = 3. (**B**) HCT116 (*KRAS*: G13D/+) or VACO432 (*BRAF*: V600E/+) cells were injected subcutaneously into the flank of 6 to 8-week-old female athymic nude mice. After 7-10 days, mice were randomly divided into two groups. One group was treated with freshly prepared vitamin C in 400 ul PBS (4 g/kg) twice a day via IP injection (HCT116: n = 6, VACO432: n = 6). Control group mice were treated with PBS with the same dosing schedule (HCT116: n = 4, VACO432: n = 7). Tumor sizes were measured 2-3 times per week in an unblinded manner. Experiments were repeated twice independently. (**C**) At 7 weeks of age, *Apc*$^{flox/flox}$ mice and *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} mice were treated with a single intraperitoneal injection (IP) of low dose tamoxifen (20 mg/kg) to activate the stem-cell-specific *Cre* and facilitate loss of *Apc* and activation of the *Kras* G12D allele. 3 weeks after tamoxifen injection, *Apc*$^{flox/flox}$ mice (male = 8 and female = 9 mice) and *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} mice (male = 7 and female = 9) were divided into two groups (vitamin C at 4 g/kg or PBS) and treated daily with IP injections (5-6 times per week). Based on weight loss and Hemoccult score, all *Apc*$^{flox/flox}$ mice were sacrificed at 6 weeks of treatment. *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} male mice were sacrificed at 5 weeks after treatment and *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} female mice were sacrificed at 7 weeks after treatment; average polyp numbers in the PBS group for female and male mice were similar. *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} mice experiments were repeated twice. Polyp number and volume was determined in whole mount tissue following methylene blue staining using a dissecting microscope in an unblinded manner. (**D**) Immunoblots of GLUT1 protein, phospo-ERK1/2, and total-ERK in tumors from *Apc*$^{flox/flox}$ mice (n = 4) and *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} mice (n = 4). In. E.: normal intestinal epithelial cells. (**E**) Absolute amounts of intracellular vitamin C (VC) were measured in tumors derived from *Apc*$^{flox/flox}$ mice and *Apc*$^{flox/flox}$/*LSL-Kras*^{G12D} mice treated with either vitamin C (4 g/kg) or PBS. Samples were harvested one hour post treatment. Two-way ANOVA ($p = 0.0002$) followed by tukey's test for multiple comparisons. All data represent means ±s.d.

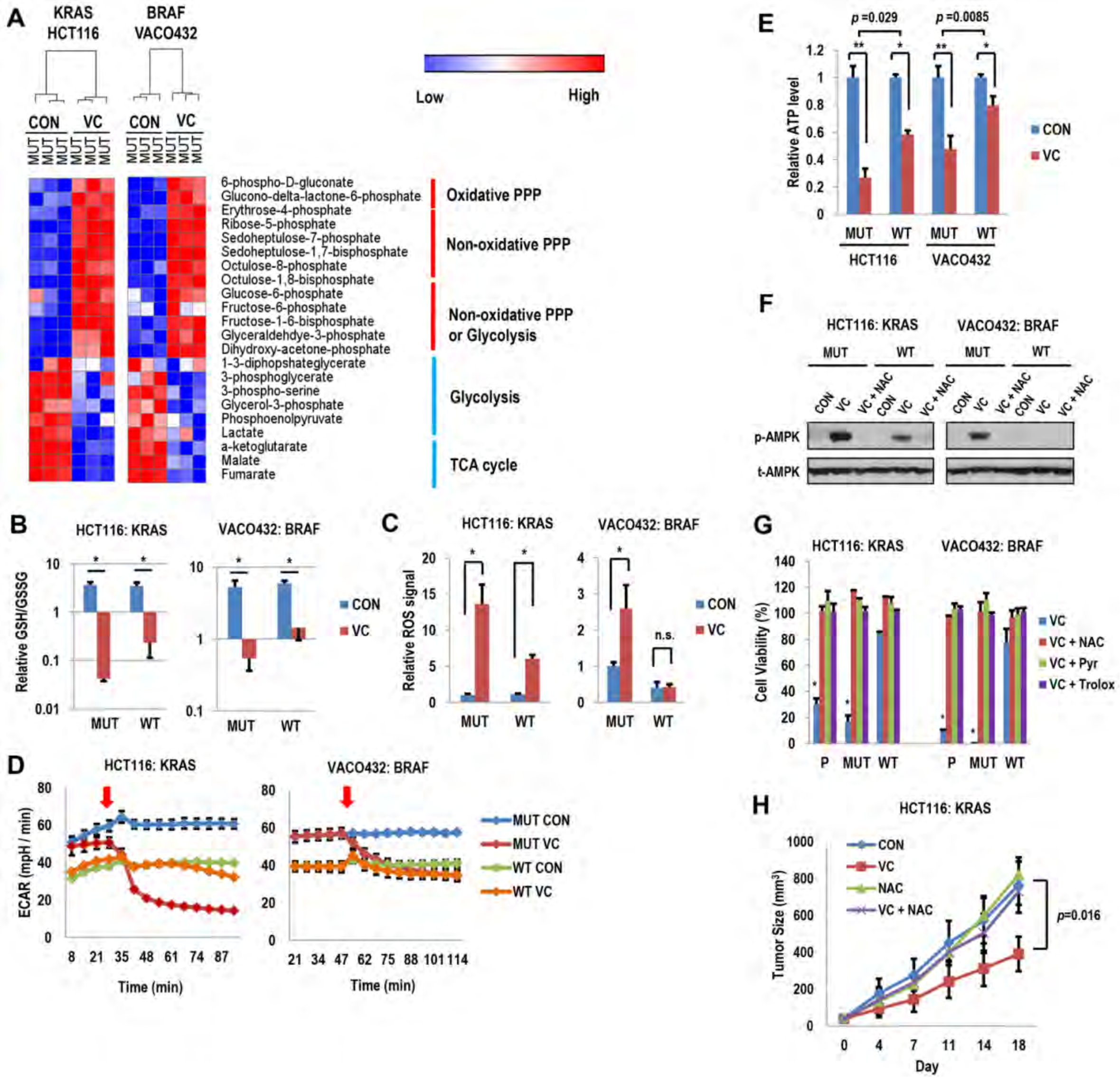
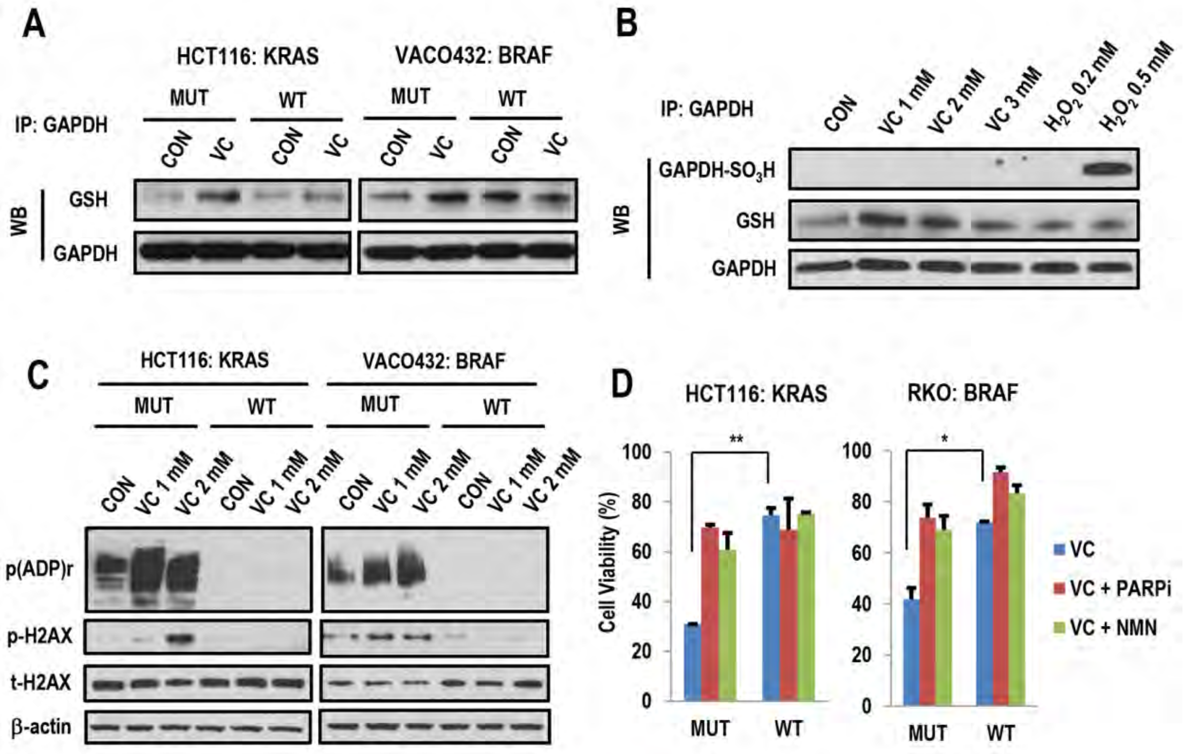


Fig. 3. Vitamin C inhibits glycolysis thereby depleting ATP and selectively killing *KRAS* and *BRAF* mutant cells. (**A**) Heatmap depicting significantly changed glycolytic and PPP metabolite levels in mutant cells after a one-hour vitamin C or vehicle treatment as analyzed by LC-MS/MS. Red: increase; blue: decrease. PPP; Pentose phosphate pathway, TCA; tricarboxylic acid cycle. (**B**) Relative ratios of reduced to oxidized glutathione (GSH/GSSG) in *KRAS* and *BRAF* isogenic cell lines determined by LC-MS/MS as in (A). The ratio was significantly decreased following vitamin C in both MUT and WT cells (Student's *t* test, $^*p < 0.002$, $n = 3$) but the extent was greater in the MUT cells than in the WT cells. (**C**) Following a one-hour vitamin C (VC) treatment, cells were incubated with the ROS-sensitive fluorescent dye, DCF-DA, for 30 min and fluorescence measured by flow cytometry. Asterisks indicate significant increases in ROS following vitamin C treatment (Student's *t* test, $^*p < 0.01$, $n = 3$). (**D**) The extracellular acidification rate (ECAR) was monitored in *KRAS* and *BRAF* isogenic cell lines. Red arrows indicate the time of vitamin C (VC) or vehicle (CON) addition ($n = 6$). (**E**) ATP levels were determined in *KRAS* and *BRAF* isogenic cell lines after a one-hour vitamin C (VC) treatment. Although ATP levels were significantly decreased in all cells (Student's *t* test, $^*p < 0.05$, $^{**}p < 0.002$, $n = 3$), the decrease was much more pronounced in MUT cells (two way ANOVA). (**F**) Cells were treated with vitamin C (VC) or vitamin C combined with N-acetyl cysteine (NAC) for one hour before immunoblotting for Thr172 phosphorylation (p-AMPK) or total AMPK (t-AMPK). (**G**) Cells were treated with vitamin C alone (VC) or vitamin C plus NAC, pyruvate (Pyr), or Trolox for 48 hours and viability measured with a CellTiter-Glo assay. Cell viability in parental (P) and MUT cells compared to WT cells was significantly decreased in vitamin C alone but not vitamin C combination treatments. One-way ANOVA ($p < 0.0001$, VC group) with Dunnett's post-test. $^*p < 0.0001$, $n = 3$. (**H**) 8-week-old female athymic nude mice with subcutaneous tumors from parental HCT116 cells were treated with vitamin C (VC) alone (4 g/kg), NAC alone (30 mM in drinking water), VC plus NAC, or PBS twice a day via IP injection. Tumor sizes were measured once per week in an unblinded manner. Experiments were repeated twice independently. Vitamin C treatment alone significantly decreased tumor growth compared to PBS ($p = 0.016$) but adding NAC to the vitamin C treatment abolished this effect ($p = 0.845$). Mixed effect analysis followed by Tukey's test. 1 and 2 mM Vitamin C was used for HCT116 and VACO432 cells, respectively (A-F). For viability assays at low cell densities, 0.125 and 0.375 mM vitamin C was used for HCT116 and VACO432 cells, respectively (G). All data represent means ±s.d.



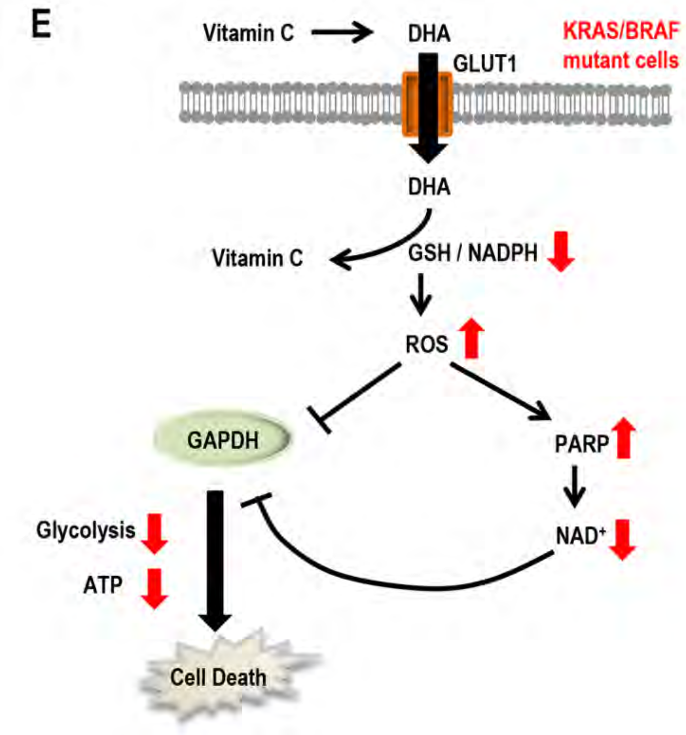


Fig. 4. Vitamin C-induced ROS inhibits GAPDH by cysteine S-glutathionylation and depleting NAD$^+$. (**A**) Cells were incubated with vehicle (CON) or vitamin C (VC) for one hour (HCT116: 1 mM, VACO432: 2 mM). Cell extracts were prepared in the presence of iodoacetic acid (IAA) to prevent S-thiolation during extraction, immunoprecipitated with a GAPDH antibody, and analyzed by non-reducing SDS-PAGE and probed with the indicated antibodies. (**B**) HCT116 cells were incubated with vehicle, vitamin C (VC) or H_2O_2 for one hour. Immunoblots were performed with the indicated antibodies as in (A). (**C**) Immunoblots for p(ADP)r (ADP-ribose polymers), Ser-139-phosphorylated, total H2AX, and β-actin on lysates from cells treated with vehicle (CON) or vitamin C (VC) for one hour. (**D**) Cells were treated with vitamin C alone (VC) (0.125 mM) or vitamin C plus Olaparib (10 uM) (VC + PARPi) or β-nicotinamide mononucleotide (NMN, 1 mM) (VC + NMN). Viability after 48 hours of treatment was measured using a CellTiter-glo assay and normalized to untreated controls. Asterisks indicate significant differences compared to MUT cells treated with vitamin C alone. Two-way ANOVA followed by Tukey's test. $^*p < 0.01$, $^{**}p < 0.001$, MUT groups, n = 3. (**E**) Schematic showing how vitamin C selectively kills cells *KRAS* or *BRAF* mutant cells.