Supplemental Information

Mitochondrial reprogramming via ATP5H loss promotes multi-modal cancer therapy resistance

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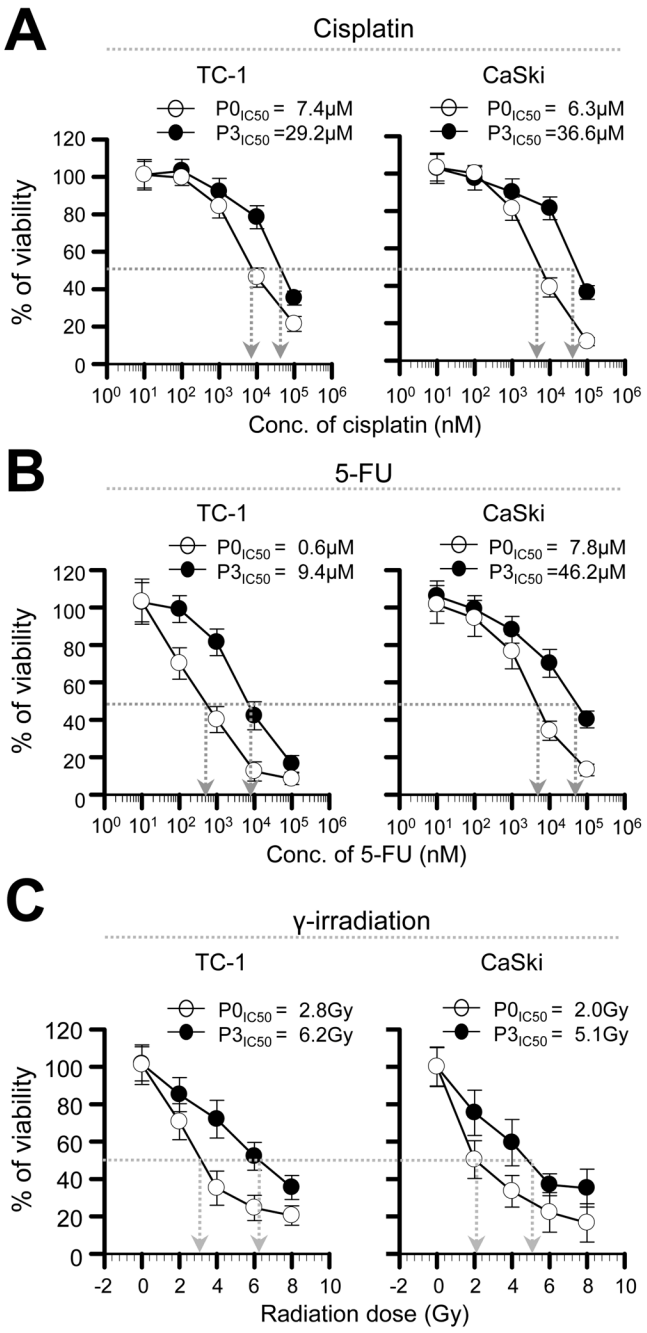

Figure S1. Tumor cells subjected to immune editing acquire broad-spectrum resistance to conventional therapeutic agents. TC-1 or CaSki cells without (P0) or with (P3) immune editing were treated with the pharmacologic agents cisplatin **(A)** or 5-FU **(B)** or with γ-irradiation **(C)**. The percentage of viable cells was determined by trypan blue exclusion assay either 24 hr after drug challenge or 48 hr after irradiation. IC_{50} was determined as the dose of drug or radiation necessary to kill 50% of tumor cells. All experiments were performed in triplicate (*n*=3). Error bars represent mean ± SD.

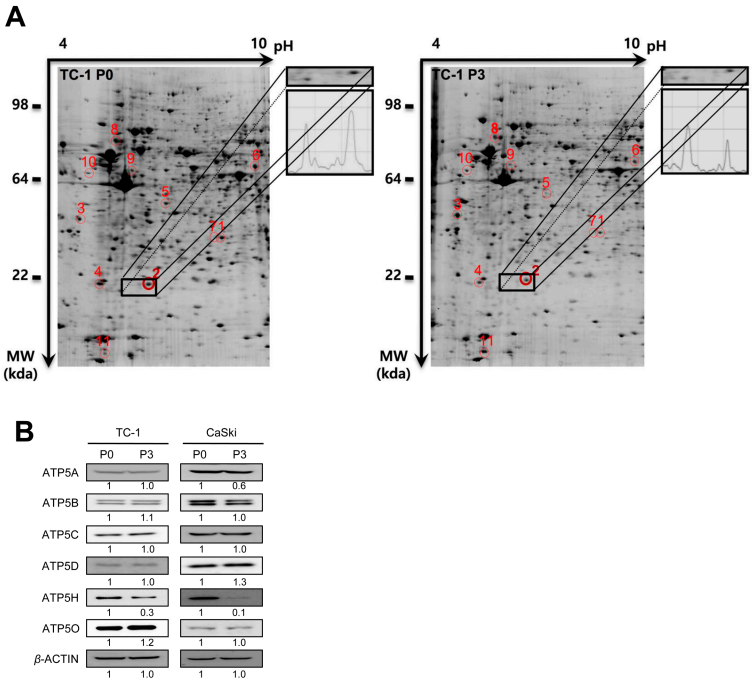

Figure S2. Mass spectrometry analysis of proteins differentially expressed in tumor cells before and after immune editing. (A) 2-D Coomassie Brilliant Blue G-250 gel electrophoresis of total proteins from tumor cells before (P0) or after (P3) immune editing. 11 spots are differentially expressed between P0 and P3 cells. **(B)** Level of the various individual protein components of the ATP synthase complex in TC-1 or CaSki P0 and P3 tumor cells was probed by Western blot. All experiments were repeated independently at least three times.

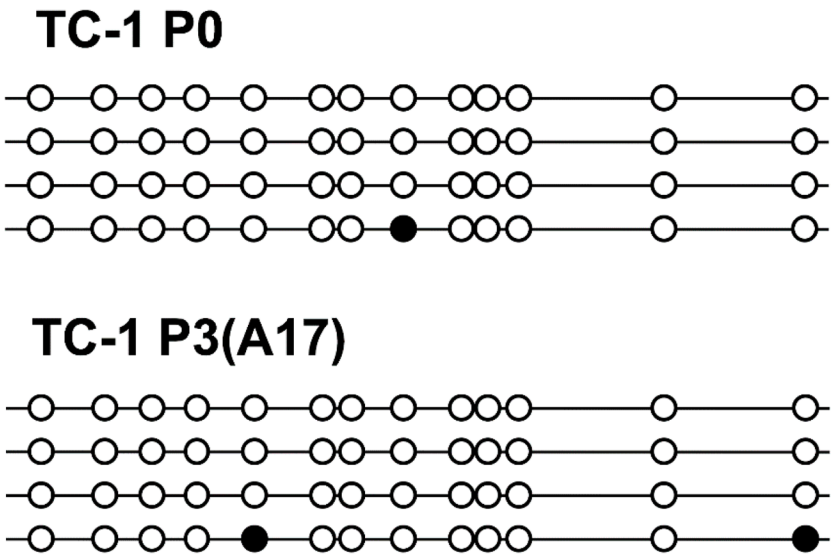

Figure S3. DNA methylation analysis of the *Atp5h* promoter locus. Promoter methylation analysis of the *Atp5h* locus by bisulfite sequencing. Circles indicate CpG sites (open circles: hypomethylated region, closed circles: hypermethylated region). All experiments were repeated independently at least three times.

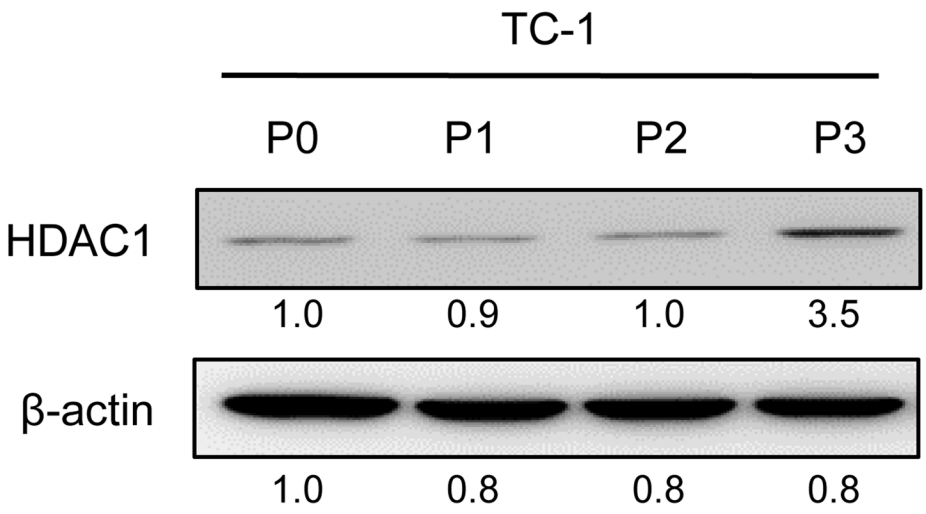

Figure S4. HDAC1 upregulation upon immune editing of TC-1 tumor cells. HDAC1 protein level in TC-1 tumor cells at various stages of immune editing was determined by Western blot. *β*-ACTIN was included as an internal loading control. Numbers below blots indicate expression as measured by fold change. Representative blots of three repeated experiments (*n*=3) are displayed.

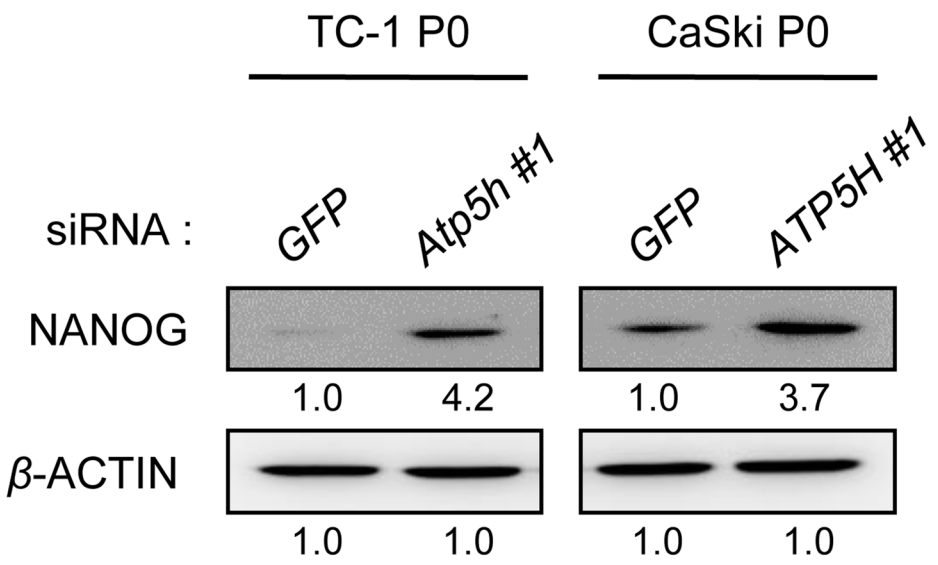

Figure S5. ATP5H loss upregulates stemness factor NANOG. TC-1 or CaSki P0 cells were transfected with indicated siRNAs. Protein level of the stem-like factor NANOG in these cells was determined by Western blot. *β*-ACTIN was included as an internal loading control. Numbers below blots indicate expression as measured by fold change. Representative blots of three repeated experiments (*n*=3) are displayed.

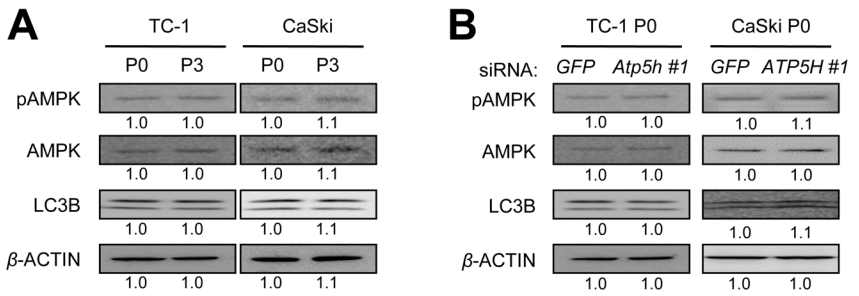

Figure S6. Immune editing-induced ATP5H loss does not alter AMPK signaling and induce autophagy. **(A)** Levels of pAMPK, AMPK and LC3B in TC-1 or CaSki P0 and P3 tumor cells were determined by Western blot. **(B)** TC-1 or CaSki P0 cells were transfected with indicated siRNAs. Levels of pAMPK, AMPK and LC3B in these cells were determined by Western blot. *β*-ACTIN was included as an internal loading control. Numbers below blots indicate expression as measured by fold change. Representative blots of three repeated experiments (*n*=3) are displayed.

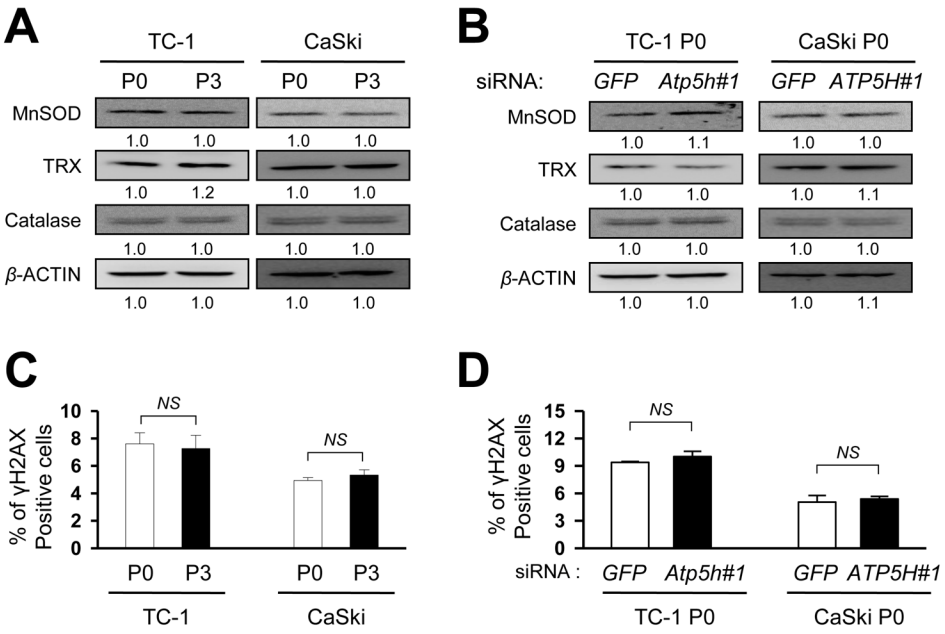

Figure S7. Immune editing-induced ATP5H loss does not affect levels of endogenous antioxidants and DNA damage. (A) Levels of endogenous mitochondrial antioxidants in TC-1 or CaSki P0 and P3 tumor cells were determined by Western blot. **(B)** TC-1 or CaSki P0 cells were transfected with indicated siRNAs. Levels of endogenous mitochondrial antioxidants in these cells were determined by Western blot. *β*-ACTIN was included as an internal loading control. Numbers below blots indicate expression as measured by fold change. **(C)** DNA damage in P0 and P3 cells was determined by γH2AX staining, followed by flow cytometry. **(D)** TC-1 or CaSki P0 cells were transfected with indicated siRNAs. The frequency of γH2AX positive cells was determined by flow cytometry analysis. All experiments were performed in triplicate (*n*=3). *p*-values were calculated by 2-tailed Student's *t* test. NS = not significant ($p>0.05$). Error bars represent mean ± SD.

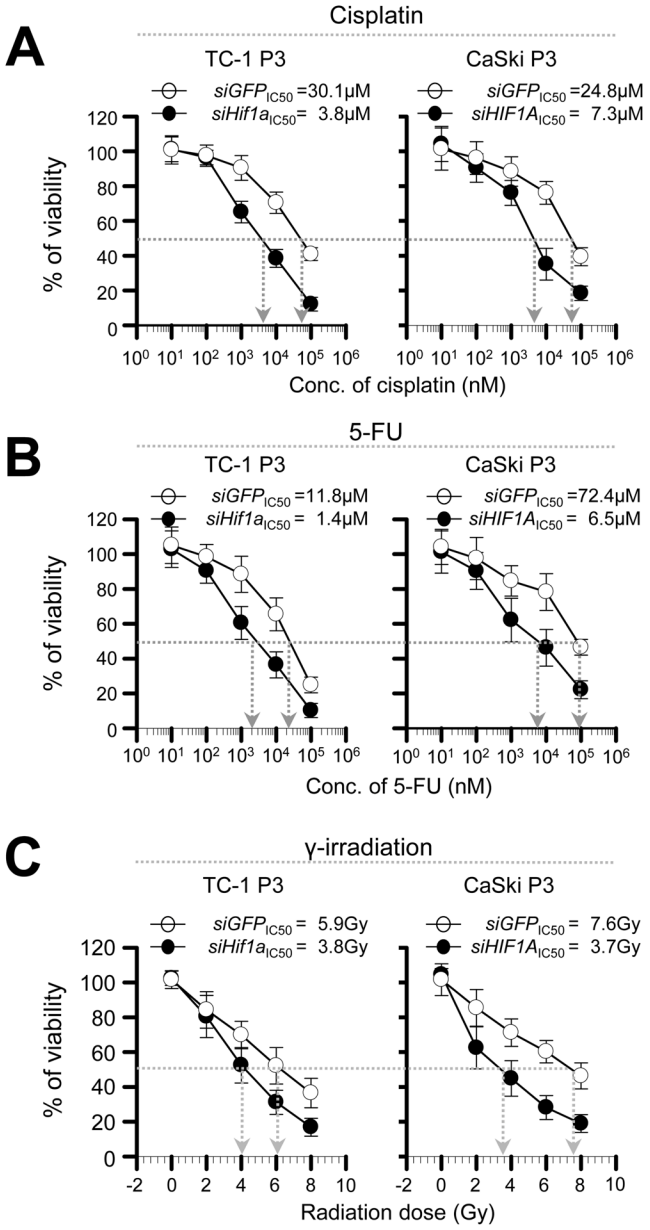

Figure S8. Tumor cells subjected to immune editing acquire broad-spectrum resistance to conventional therapeutic agents through a HIF-1α-dependent manner. TC-1 or CaSki cells with immune editing (P3) were treated with indicated siRNAs and then treated with cisplatin **(D)**, 5-FU **(E)**, or irradiation **(F)**. The percentage of viable cells was determined by trypan blue exclusion assay either 24 hr after drug challenge or 48 hr after irradiation. IC_{50} was determined as the dose of drug or radiation necessary to kill 50% of tumor cells. All experiments were performed in triplicate (*n*=3) under normoxic conditions. Error bars represent mean ± SD.

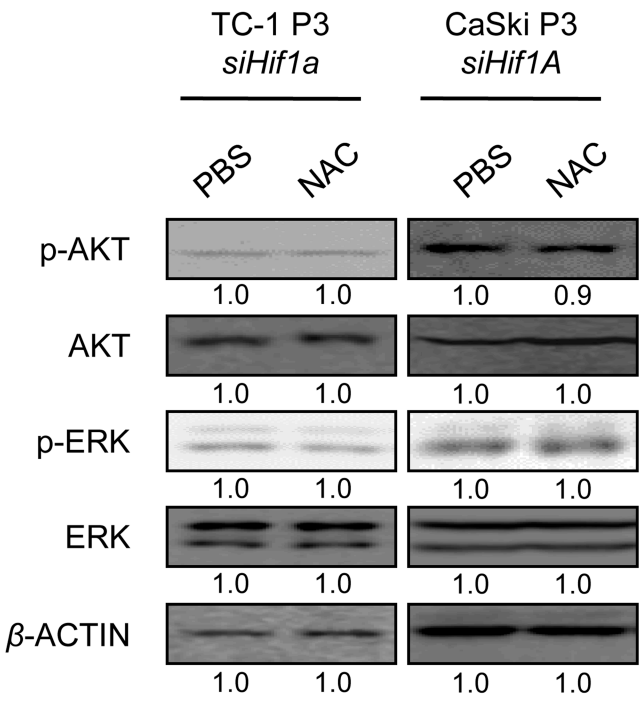

Figure S9. NAC treatment in *Hif1a* or *HIF1A* silenced P3 cells does not affect AKT/ERK signaling pathway. *siHif1a* or *siHIF1A*-transfected TC-1 or CaSki P3 cells were treated with PBS or NAC under normoxic conditions. Levels of HIF-1α, as well as phosphorylated or total AKT/ERK were determined by Western blot. *β*-ACTIN was included as an internal loading control. Numbers below blots indicate expression as measured by fold change. Representative blots of three repeated experiments (*n*=3) are displayed.

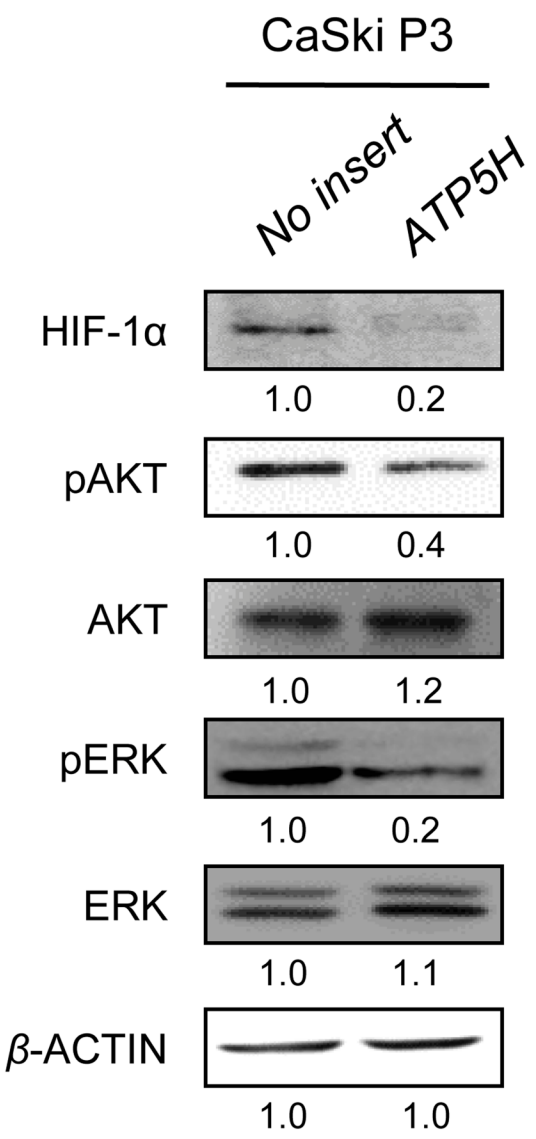

Figure S10. Restoring ATP5H reverses activation of the HIF-1α/AKT/ERK signaling pathway in CaSki P3 cells. Levels of HIF-1α, as well as phosphorylated or total AKT/ERK in CaSki P3-*no insert* and –*ATP5H* cells under normoxic conditions were determined by Western blot. *β*-ACTIN was included as an internal loading control. Numbers below blots indicate expression as measured by fold change. Representative blots of three repeated experiments (*n*=3) are displayed.

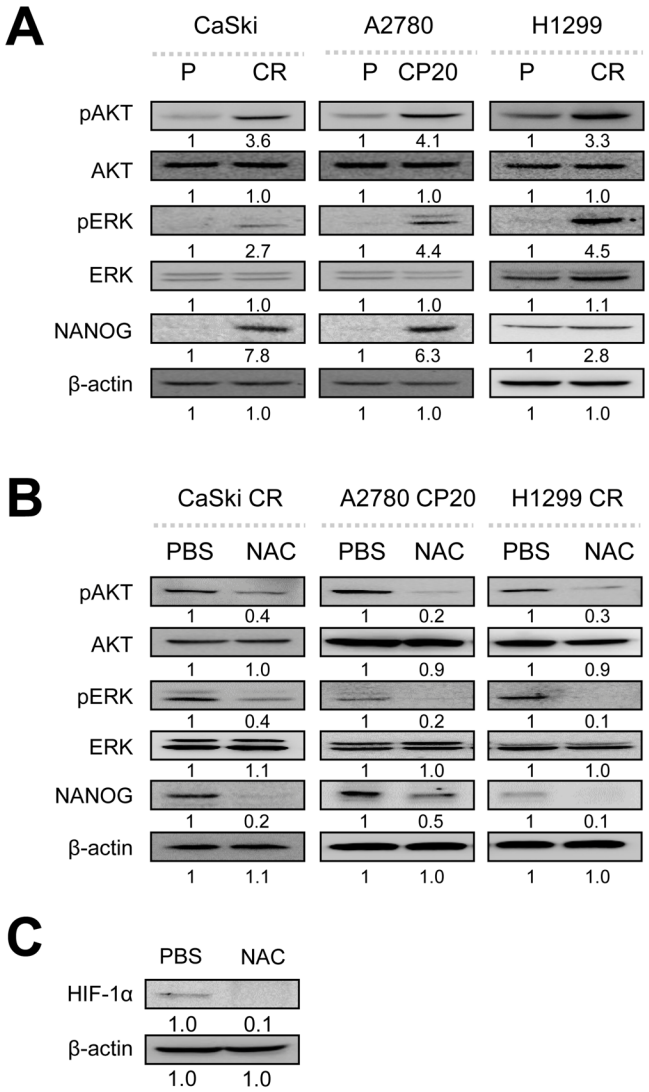

Figure S11. AKT/ERK activation in drug refractory human cancer cells was revered by addition of antioxidants. (A) Drug sensitive (P) or refractory (CR) human cancer cells (CaSki, A2780, H1299) were probed for total or phosphorylated AKT/ERK and stem-like factor NANOG by Western blot. **(B)** Drug refractory (CR) human cancer cells (CaSki, A2780, H1299) were treated with PBS or antioxidants (NAC). Cells were probed for total or phosphorylated AKT/ERK and NANOG. **(C)** HIF-1α levels under normoxic conditions were probed by Western blot in excised tumor tissue from mice bearing CaSki CR tumor cells 7 days after tumor challenge. The mice were administered either with subcutaneous NAC via chitosan hydrogel or with PBS control. Note: CP20 is a refractory version of A2780 cells. Representative blots of three repeated experiments (*n*=3) are displayed.

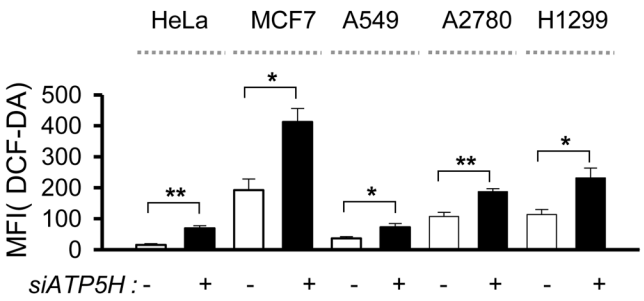

Figure S12. Loss of ATP5H in human cancer cells triggers accumulation of reactive oxygen species. Various types of human tumor cells were transfected with siRNA against *GFP* or *ATP5H*. Abundance of reactive oxygen species (H_2O_2) was determined by DCFH-DA staining, followed by flow cytometry. All experiments were performed in triplicate (n=3). p-values were calculated by 2-tailed t tests, * = p<0.05; ** = p<0.01. Error bars represent mean ± SD.

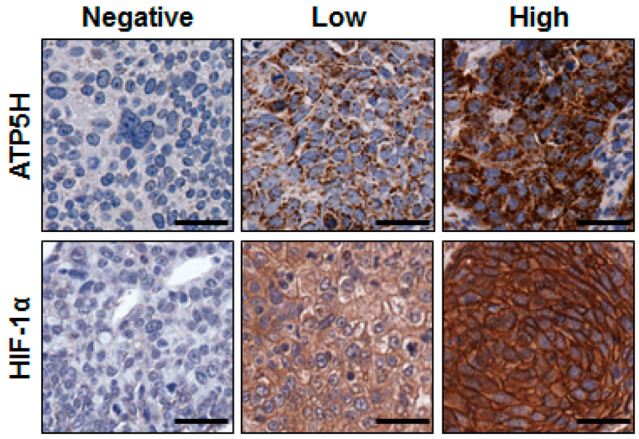

Figure S13. Characterization of ATP5H and HIF-1α expression in cervical carcinoma patients by immunohistochemistry. Representative Immunohistochemistry data of three repeated experiments (*n*=3) depicting various expression levels of ATP5H (top) or HIF-1α (bottom) in tumor tissue sections from cervical carcinoma patients (scale bar: 200 μm).

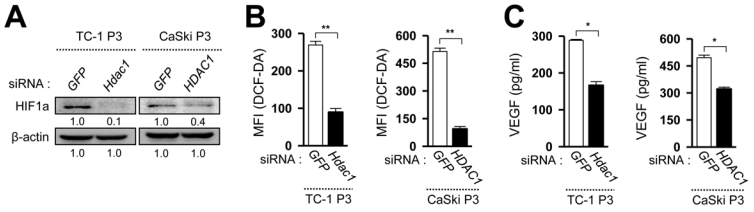

Figure S14. Silencing of HDAC1 in immune edited tumor cells leads to reduction of ROS as well as decrease of HIF-1α and VEGF secretion. TC-1 or CaSki P3 cells were transfected with indicated siRNAs. **(A)** Protein level of HIF-1α under normoxic condition was determined by Western blot, and **(B)** abundance of H_2O_2 in these cell was determined by DCFH-DA staining. **(C)** VEGF levels in supernatant of these cells were determined by ELISA. All experiments were performed in triplicate (n=3). p-values were calculated by 2-tailed t tests, * = p<0.05; ** = p<0.01. Error bars represent mean ± SD.

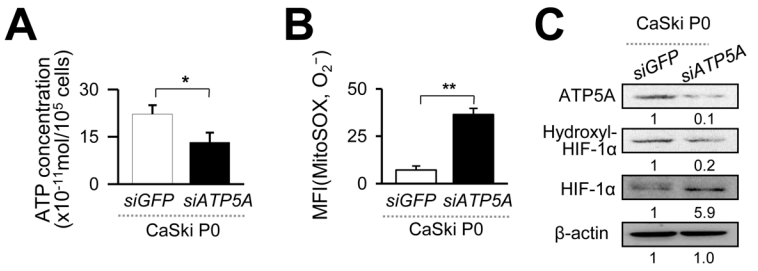

Figure S15. Loss of ATP5A triggers mitochondrial metabolic reprogramming. CaSki P0 cells were transfected with siRNA against *GFP* or *ATP5A*. ATP concentration **(A)** and levels of reactive oxygen species **(B)** were measured in transfected cells. **(C)** HIF-1α expression in transfected cells under normoxic condition was probed by Western blot. All experiments were performed in triplicate (n=3). p-values were calculated by 2-tailed t tests, * = p<0.05; ** = p<0.01. Error bars represent mean ± SD.

Table S1. Mass spectrometry analysis of global protein expression in TC-1 tumor cells without (P0) or with (P3) selection.

No.	Expression ratio of P3 to P0	Protein name	Accession Number	Biological function
1	0.313	NEBU	Q61953	Celluar structure
2	**0.375**	**ATP5H**	**Q9DCX2**	**ATP synthesis**
3	0.410	TPM1	P58771	Protein biosynthesis
4	0.444	GCC1	Q9D4H2	Golgi Structure
5	0.460	IDH3A	Q9D6R2	Metabolism
6	0.511	SEP11	Q8C1B7	Cell cycle
7	0.530	GPATCH4	Q3TFK5	mRNA processing
8	0.750	HNRNPK	P61979	mRNA processing
9	1.432	EIF2B5	Q8CHW4	GTP exchange
10	1.500	VIM	P20152	Celluar structure
11	3.214	LUC7L33	Q5SUF2	mRNA processing

The most highly down-regulated or up-regulated protein candidates are shown. Expression ratio is calculated as the ratio of protein level in P3 cells relative to P0 cells.

Table S2. Clinicopathologic significance of ATP5H and HIF-1α protein expression in human cervical neoplasias

	ATP5H			HIF-1α		
	No.	Mean IHC score (95% CI)	*P value*	No.	Mean IHC score (95% CI)	*P value*
Age			*0.455*			*0.671*
≤ 50	100	5.51 (4.90-6.12)		104	6.23 (5.72-6.74)	
> 50	72	5.89 (5.06-6.71)		78	6.06 (5.48-6.65)	
FIGO stage			***<0.001***			***0.001***
I - IIA	127	6.39 (5.82-6.97)		137	5.79 (5.35-6.23)	
IIB - IV	45	3.62 (2.97-4.28)		45	7.29 (6.61-7.97)	
Cell type			*0.519*			***<0.001***
SCC	143	5.74 (5.19-6.30)		150	6.50 (6.10-6.90)	
Others	29	5.31 (4.23-6.39)		32	4.56 (3.65-5.47)	
Tumor grade			***0.032***			*0.726*
Well + Moderate	108	6.00 (5.36-6.64)		113	6.10 (5.59-6.61)	
Poor	53	4.81 (3.97-5.65)		56	6.25 (5.59-6.91)	
Tumor size			***<0.001***			*0.266*
< 4 cm	120	6.28 (5.67-6.88)		130	6.02 (5.58-6.47)	
≥ 4 cm	52	4.27 (3.53-5.01)		52	6.50 (5.57-7.25)	
LN metastasis			***0.009***			*0.789*
Negative	108	6.46 (5.82-7.11)		116	6.02 (5.52-6.51)	
Positive	30	4.63 (3.45-5.81)		31	5.87 (4.87-6.87)	
Chemoradiation response			*0.234*			***0.001***
Good	48	5.52 (4.58-6.46)		51	5.14 (4.43-5.84)	
Bad	15	4.40 (2.81-5.99)		15	7.60 (6.28-8.92)	

SCC, squamous cell carcinoma; FIGO, International Federation of Gynecology and Obstetrics; LN metastasis, Lymph node metastasis. Protein expression was determined through analysis of an immunohistochemically stained tissue array, as described in the materials and methods section.