Supplemental Table and Figure Legend

Table S1. The differential expressed proteins identified by MALDI-TOF mass spectrometry.

Figure S1 Distribution of respiratory chain complexes in A549 and CL1-0 cells. Cells were allowed to attach onto the coverslips for 16 h, fixed by 4% paraformaldehyde, permeabilized with 0.25% Triton X-100 and blocked with 10% goat normal serum. The respiratory chain complexes were probed by NUDUFB4, SDHA, UQCRC2, COX5A, and ATP synthase antibodies for complex I to V, respectively, followed by Alexa 488 conjugated anti-mouse IgG secondary antibody and then counterstained with fluorescent DAPI for DNA. The lower left squares show the enlarged portion of each panel.

Figure S2 Biotinylated purification of plasma membrane proteins. (A) By this approach, cell surface proteins were covalently labeled with biotin on available extracellular lysine residues using a membrane impermeant, disulfide-coupled biotinylation reagent (sulfo-NHS-SS-biotin). After cell lysis, biotinylated plasma membrane proteins were isolated by streptavidin affinity chromatography and the proteins of interest were detected by immunoblotting. After the streptavidin chromatography, the flow-through (FT) and the elution (E) fraction were collected and analyzed by immunoblotting. (B) Surface receptor protein Neu was used to determine the purification efficiency of plasma membrane proteins. Endoplasmic reticulum protein Calnexin, mitochondrial outer membrane protein Porin isoform 1 and 2, and cytosolic protein Actin were used to determine the contamination of elution portion.

Figure S3 Protein expression profiles of citreoviridin-treated cells. The protein expression profiles of citreoviridin- (Citreo, lower) treated CL1-0 cells were compared to DMSO- (upper) treated controls on a time-course from 12 to 72 h. Proteins were separated by isoelectric point from pH 4 to 7 on a 10-18% SDS-PAGE gel. Information of protein identification in each spot was provided in Table S1.

Figure S4 Citreoviridin does not induce apoptosis in CL1-0 or A549 cells. Cells were treated with 5, 10, or 20 μM citreoviridin. DMSO was used as the vehicle control. After 48 h treatment, cells were detached and stained with propidium iodide (PI) and annexin V(AV)-conjugated FITC for detecting apoptosis and analyzed by cytometry. The X-axis is AV, and the Y-axis is PI. The AV positive/PI negative and AV/PI double positive populations represent the early apoptosis and secondary necrotic cells, respectively.

Figure S5 Citreoviridin does not inhibit the cell proliferation of p53 null cell H1299. Cell proliferation was monitored by xCELLigence RTCA system. The cell index was normalized to the time when the drug was added. Growth was measured for 48 h. The average IC_{50} after 48 h was calculated by the RTCA system to be more than 8 μM for H1299 cells. The upper left shows the expression of ectopic ATP synthase in H1299 analyzed by flow cytometry. The isotype control, ATP β, and ATP synthase complex antibodies are represented in black, red, and blue, respectively.

Figure S6 Summary of the plausible mechanisms induced by ATP synthase inhibitor citreoviridin. The ectopic ATP synthase is located on the surface of lung cancer cells with ATP

synthesis activity. Inhibition of ectopic ATP synthase by citreoviridin induces UPR and inhibits cell proliferation by arresting cell cycle at G_0/G_1 phase further suppresses cell proliferation and anchorage-independent growth (AIG). The citreoviridin caused cell proliferation inhibition is also mediated by extracellular ATP signaling, and ROS accumulation via p53 dependent pathway. The role of ectopic ATP synthase in cellular metabolic homeostasis might be complex and versatile. Synergic/addictive therapy applied with citreoviridin could be a promising way to treat heterogeneous lung cancer.

Figure S7 Addictive effects of citreoviridin (Citreo) and 26S proteosome inhibitor. CL1-0 and A549 cells (5000 cells/well) were incubated with 10 nM bortezomib (BioVision, Mountain View, CA, USA) in the presence or absence of 1 μM citreoviridin. Cell viability was accessed by MTS assay after 48 h. *, $P < 0.05$, compared with DMSO group; #, $P < 0.05$, compared with bortezomib alone.