

Supplementary Figures and Table

Vacuolar H⁺-ATPase subunit V0C regulates aerobic glycolysis of esophageal cancer cells via PKM2 signaling

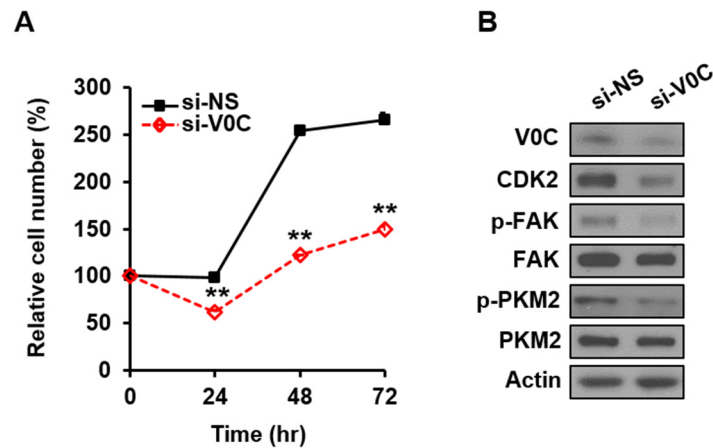
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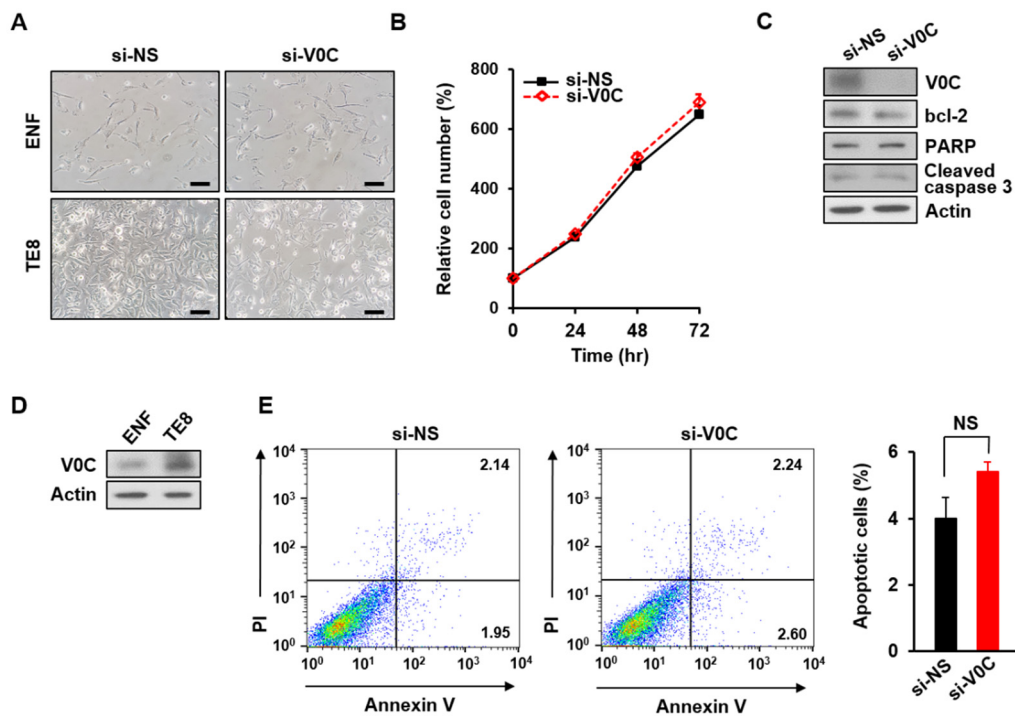
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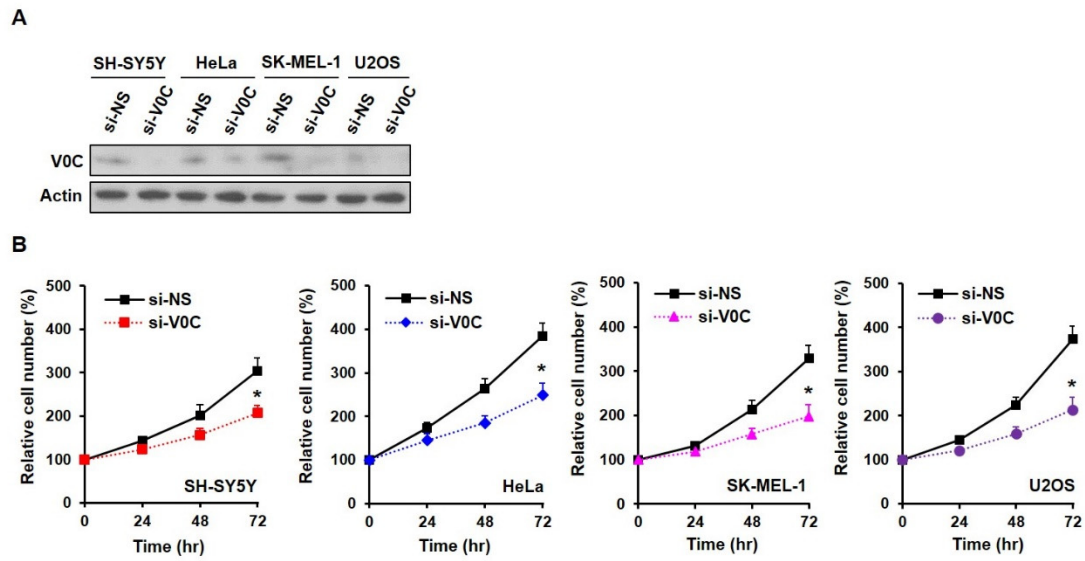
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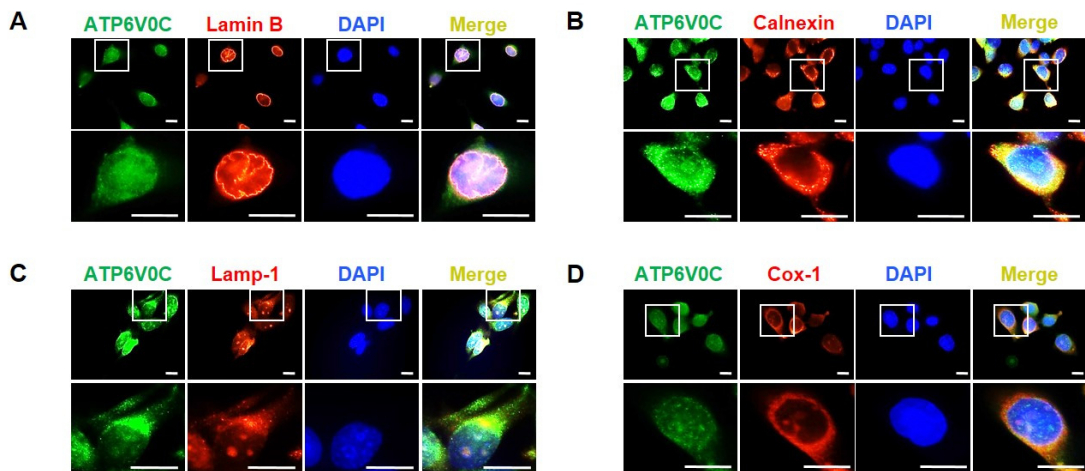
Supplementary Figure S1. ATP6V0C is crucial for ECC proliferation. TE1 cells were transfected with non-silencing siRNA (NS) or si-V0C for 72 h. (A) Cell viability was analyzed using a trypan blue assay. Each data point is the average of three independent measurements. (B) Western blot showing the expression of the indicated proteins in ATP6V0C-depleted TE1 cells. Values are mean $\pm$ SEM. (Student's *t*-test; ***P* < 0.01).



Supplementary Figure S2. The effect of ATP6V0C siRNA on the viability of Esophageal Normal Fibroblasts (ENFs). Cells were transfected with 50 nM of non-silencing siRNA (NS) or si-V0C for 72 h. (A) Representative images of ENFs and esophageal cancer cell line (TE8). Scale bars = 100 μ m. (B) Cell viability was analyzed using a trypan blue assay. (C) Cell lysates were analyzed by immunoblotting using the indicated antibodies. (D) ENF lysates were analyzed by immunoblotting with the indicated antibodies. (E) Representative flow cytometry plots of Annexin V staining in ENFs (percentage of apoptosis cells stained with Annexin V). Values are mean $\pm$ SEM. (Student's *t*-test; non-significant (NS)).



Supplementary Figure S3. ATP6V0C depletion attenuates proliferation of various cancer cell lines. SH-SY5Y, HeLa, SK-MEL-1, and U2OS cells were transfected with non-silencing siRNA (NS) or si-V0C for 72 h. (A) ATP6V0C and Actin expression were analyzed using Western blotting. (B) Cell viability was analyzed using a trypan blue assay. Each data point is the average of three independent measurements. Values are mean $\pm$ SEM. (Student's *t*-test; **P* < 0.05).



Supplementary Figure S4. The sub-cellular localization of ATP6V0C in TE8 cells. Co-immunostaining analysis was performed using specific antibodies for (A) ATP6V0C (green), Lamin B (red), (B) Calnexin (red), (C) Lamp-1 (red) (D) Cox-1 (red) in TE8 cells. Cell nuclei were counterstained with DAPI (blue). Merged images (Merge) are shown. Scale bars = 20 μ m.

Supplementary Table S1: Primers used for quantitative RT-PCR

Primer name	sequence
hV-ATPase V0C-F	5'-AGC AGA TCA TGA AGT CCA TC-3'
hV-ATPase V0C-R	5'-GAC GAT GAG ACC GTA GAG G -3'
hHIF-1a-F	5'-CGT TCC TTC GAT CAG TTG TC-3'
hHIF-1a-R	5'-TCA GTG GTG GCA GTG GTA GT-3'
hGlut1-F	5'-CGG GCC AAG AGT GTG CTA AA-3'
hGlut1-R	5'-TGA CGA TAC CGG AGC CAA TG-3'
hHK1-F	5'-TGG AGT CCG AGG TTT ATG-3'
hHK1-R	5'-TTT GGA TTG TTG GCA AGG-3'
hHK2-F	5'-CAA AGT GAC AGT GGG TGT GG-3'
hHK2-R	5'-GCC AGG TCC TTC ACT GTC TC-3'
hPFK-F	5'-CAT GAC CCA TGA AGA GCA CC-3'
hPFK-R	5'-CCA ACT CGA ACC ACA GCC CTG-3'
hENO1-F	5'-CGT ACC GCT TCC TTA GAA C-3'
hENO1-R	5'-CAA TGA CTT GGG CCA ATT AC-3'
hPKM2-F	5'-GCC TGC TGT GTC GGA GAA G-3'
hPKM2-R	5'-CTC TCC CAG GAC CTT CCT AA-3'
hLDHA-F	5'-ATC TTG ACC TAC GTG GCT TGG A-3'
hLDHA-R	5'-CCA TAC AGG CAC ACT GGA ATC TC-3'
hPDK1-F	5'-ACC AGG ACA GCC AAT ACA AG -3'
hPDK1-R	5'-CCT CGG TCA CTC ATC TTC AC -3'
hLDHA-HRE-F	5'-TTG GAG GGC AGC ACC TTA CTT AGA-3'
hLDHA-HRE-R	5'-GCC TTA AGT GGA ACA GCT ATG CTG AC-3'
hRPL13A-non-HRE-F	5'-GAG GCG AGG GTG ATA GAG-3'
hRPL13A-non-HRE-R	5'-ACA CAC AAG GGT CCA ATT C-3'
h18s rRNA F	5'-CGG CGA CGA CCC ATT CGA AC-3'
h18s rRNA R	5'-GAA TCG AAC CCT GAT TCC CCG TC-3'