Supplementary Materials for

Silencing of solute carrier family 13 member 5 disrupts energy homeostasis and inhibits proliferation of human hepatocarcinoma cells

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Figure S1

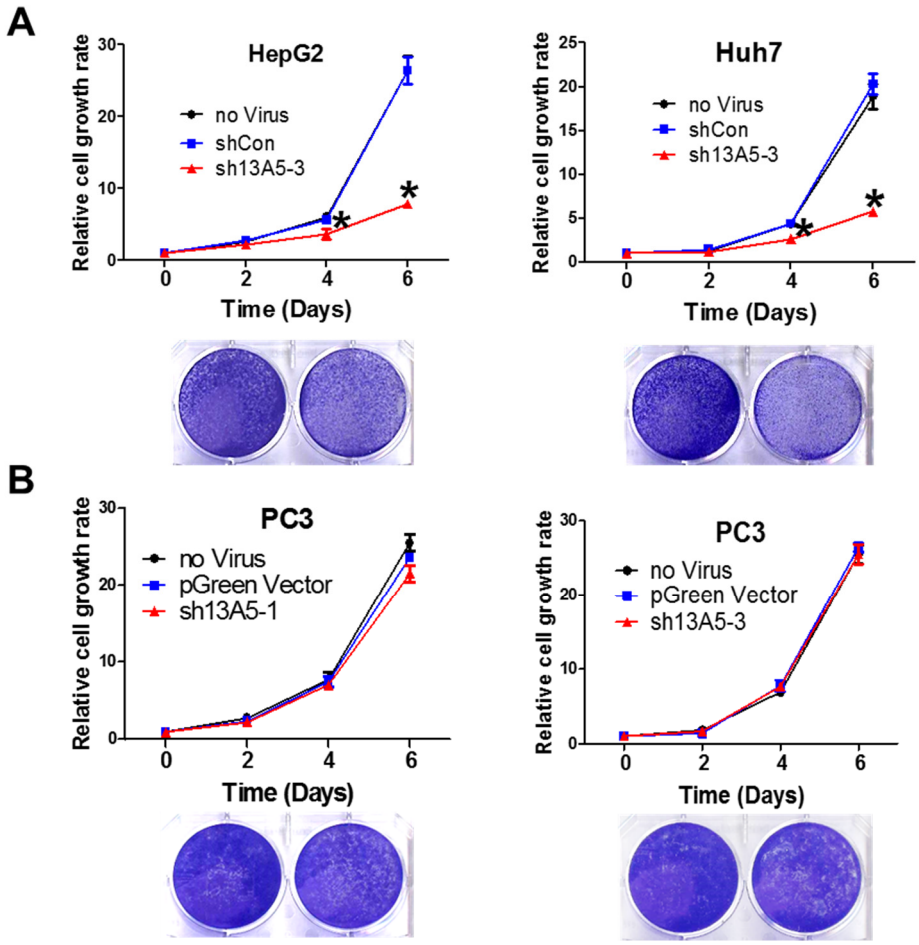

Figure S1. Knockdown of SLC13A5 suppresses the growth of HepG2 and Huh7 but not PC3 cells. Cultured HepG2, Huh7 or PC3 cells were transduced with lentivirus shRNA targeting different regions of SLCsh13A5-1 (GATCCGAGATCAACGTGCTGATCTGCTTCTCGAG-AAGCAGATCAGCACGTTGATCTCTTTTTG), sh13A5-3 (GATCCGGAGGGTGAGACA-AAGTATGTCTCGAGACATACTTTGTCTCACCCTCCTTTTTG) or shCon. Relative cell growth rates were assayed using CCK-8 (Enzo Life Sciences, Inc.) as detailed in Materials and Methods at the indicated time points from *A*, HepG2 and Huh7, and *B*, PC3 cells. Results are expressed as mean ± SD from three independent experiments. * $p < 0.05$.

Figure S2

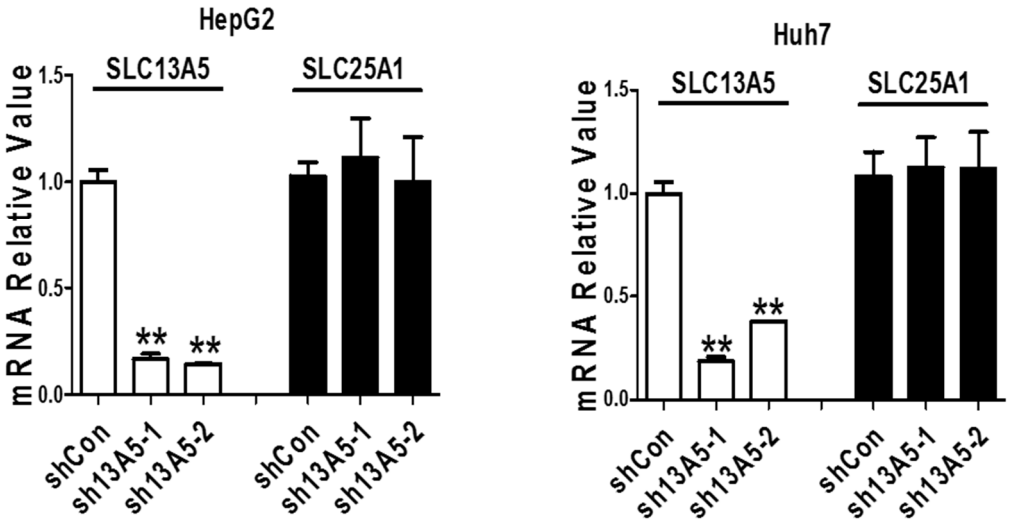

Figure S2. Knockdown of SLC13A5 does not influence the expression of SLC25A1 in HepG2 and Huh7 cells. Cultured HepG2 and Huh7 cells were transduced with lentivirus sh13A5-1, 13A5-2, or shCon as described in *Materials and Methods*. Relative mRNA expression of SLC13A5 and SLC25A1 in these cell lines was measured using real-time PCR. Results are expressed as mean ± SD from three independent experiments. ** $p < 0.01$.

Figure S3

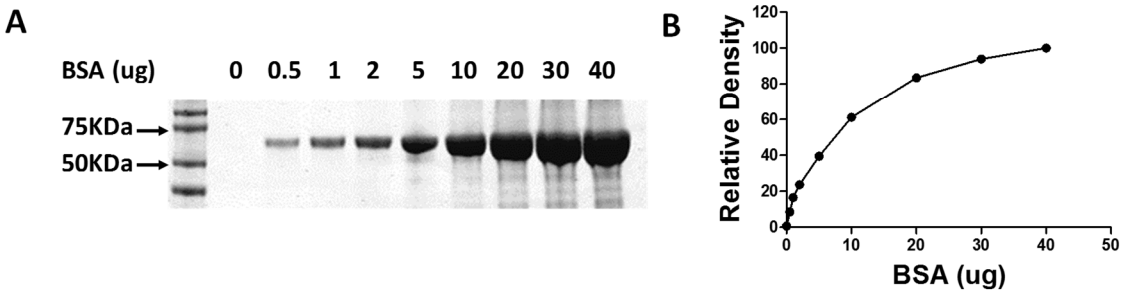

Figure S3. Bovine serum albumin (BSA) Coomassie blue staining curve. *A,* Serial dilutions of BSA on SDS-PAGE stained with coomassie blue. *B,* Densitometry curve of the BSA blot. Densitometry was analyzed using NIH ImageJ software.

Figure S4

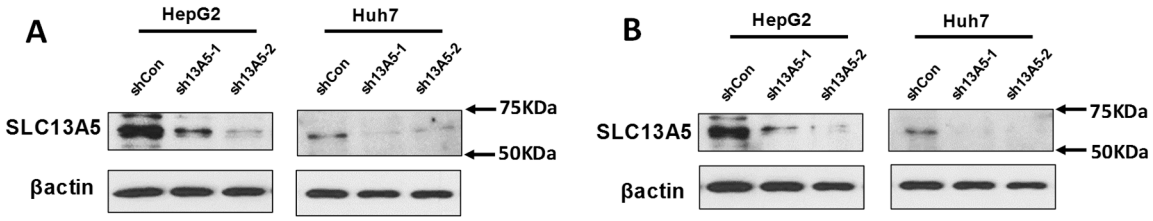

Figure S4. Knockdown of SLC13A5 expression. *A* and *B*, Expression of protein of SLC13A5 in HepG2 and Huh7 cells 72 h after SLC13A5 knockdown via lentivirus shRNA targeting different regions of SLC13A5 (sh13A5-1 and sh13A5-2). *A* and *B* are western bloting results from two independent experiment as replicates of Fig. 1B.

Figure S5

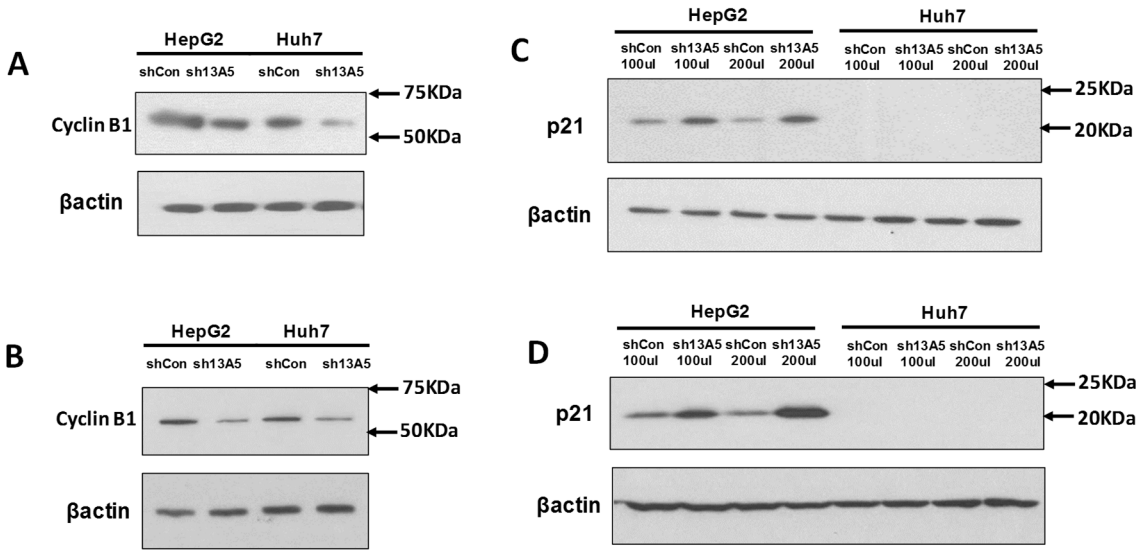

Figure S5. Knockdown of SLC13A5 inhibits cell cycle progression in HepG2 and Huh7 cells. *A* and *B*,Western blotting was carried out to measure the protein levels of Cyclin B1 in HepG2 and Huh7 cells infected with sh13A5 or shCon. *C* and *D*, Expression of p21 protein was measured in HepG2 and Huh7 cells infected with sh13A5 or shCon. *A* and *B* are western bloting results from two independent experiment as replicates of Fig. 2D; *C* and *D* are western bloting results from two independent experiment as replicates of Fig. 2F. The Cyclin B1 immunoblot shown in A is from the same samples shown in Fig. 4C and the β-actin immunoblot is duplicated to show equal loading.

Figure S6

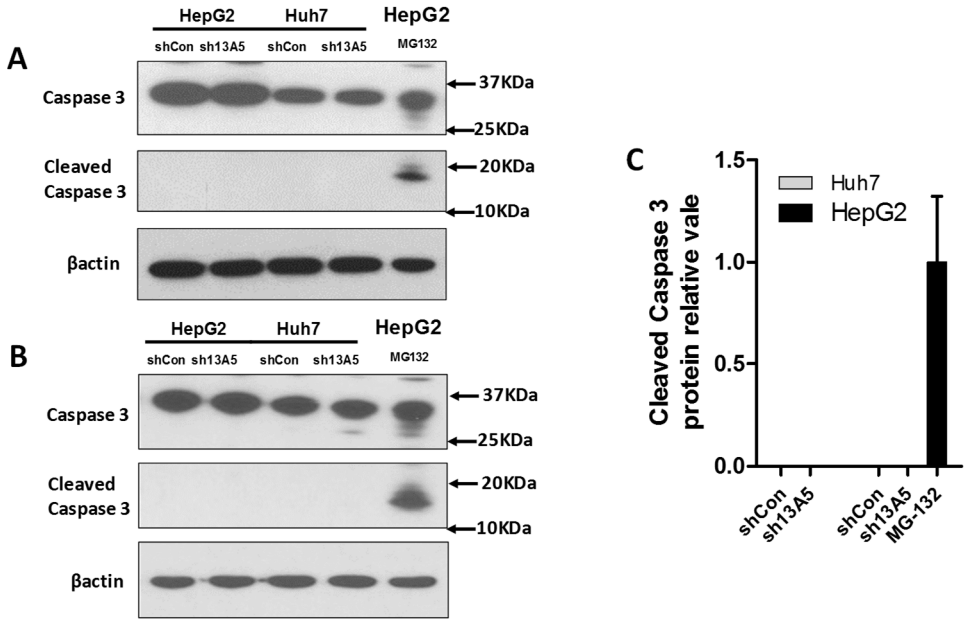

Figure S6. *A* and *B*, Caspase 3 activity was analyzed with Western blotting to detect the large fragment (17/19 kDa) of cleaved caspase 3 in HepG2 and Huh7 cells with or without SLC13A5 knockdown. β actin was used to normalize protein loading. *A* and *B* are western bloting results from two independent experiment as replicates of Fig. 3C. *C*, Densitometry analysis of the cleaved caspase 3 western blots of Fig. 3C, S6A, and S6B. Densitometry was analyzed using NIH ImageJ software.

Figure S7

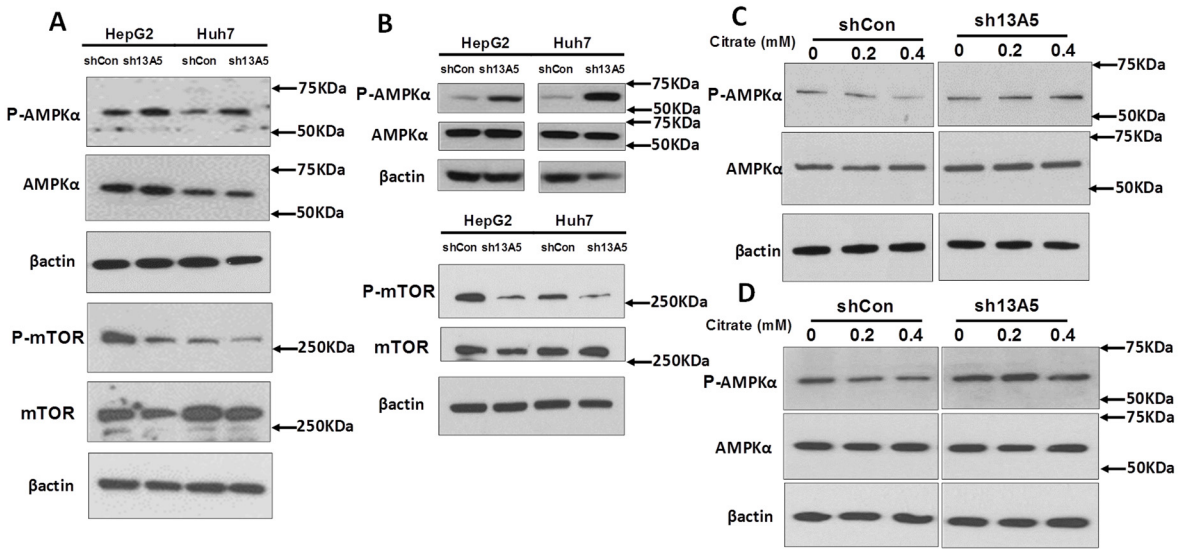

Figure S7. HepG2 and Huh7 cells were infected with lentivirus encoding the sh13A5 or shCon for 72 h. *A* and *B*, The protein levels of phospho-AMPKα, total AMPKα, phospho-mTOR, total mTOR, and β actin were analyzed by immunoblotting. *A* and *B* are western bloting results from two independent experiment as replicates of Fig. 4C. In separate experiments, Huh7 cells infected with sh13A5 or shCon for 72 h were treated with citrate at 0, 0.2, and 0.4 (mM) for another 24 h. *C* and *D*, the protein levels of phospho-AMPKα, total AMPKα, and β actin were subjected to immunoblotting analysis. *C* and *D* are western bloting results from two independent experiment as replicates of Fig. 4D.

Figure S8

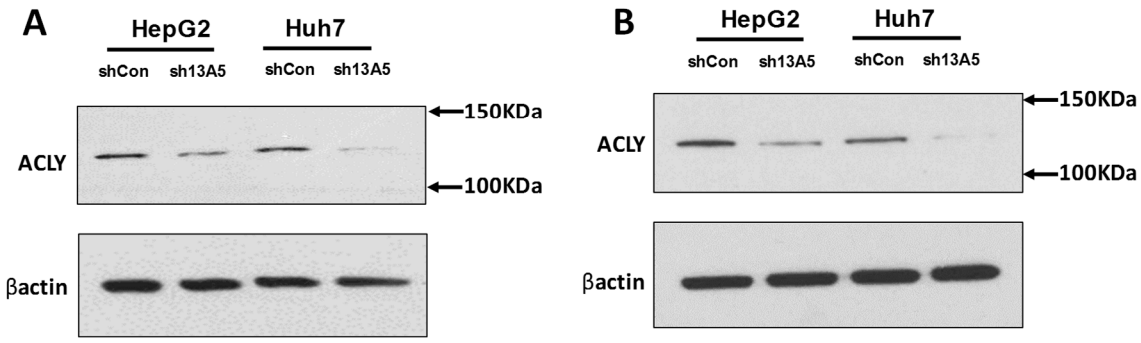

Figure S8. HepG2 and Huh7 cells were infected with lentivirus encoding the sh13A5 or shCon for 72 h. *A* and *B*, The protein levels of ACLY and β actin were measured using immunoblotting. *A* and *B* are western bloting results from two independent experiment as replicates of Fig. 5B.

Figure S9

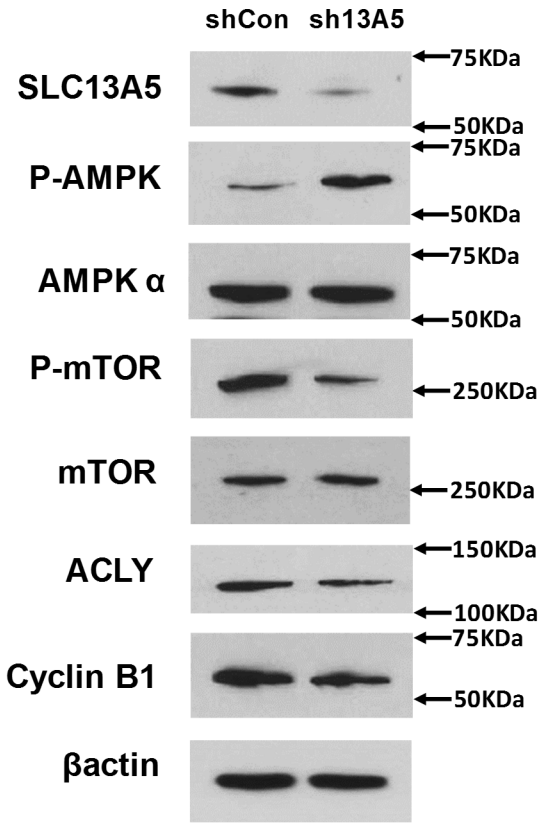

Figure S9. HepG2 cells were infected with lentivirus encoding sh13A5 or shCon. Four days after culture, equal number of HepG2-sh13A5 and HepG2-shCon cells were injected into the nude mice for xenograft formation as detailed in *Materials and Methods*. Western blotting was used to measure the protein expression of SLC13A5, p-AMPKα, total AMPKα, p-mTOR, total mTOR, ACLY, Cyclin B1 and β-actin.

Figure S10

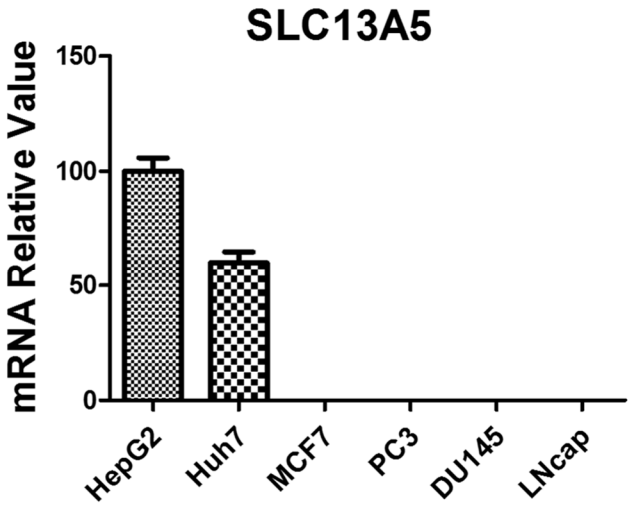

Figure S10. Expression of SLC13A5 in different human cancer cell lines. The relative expression levels of SLC13A5 were quantified using real-time PCR in HepG2, Huh7, MCF7, PC3, DU145, and LNcap cells.

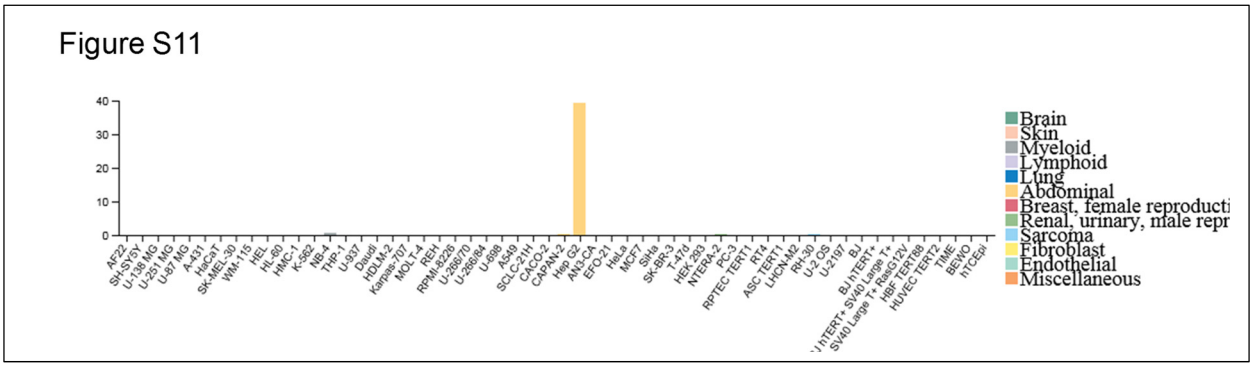

Figure S11. Expression of SLC13A5 in different human cancer cell lines extracted from (http://www.proteinatlas.org/ENSG00000141485-SLC13A5/cell).

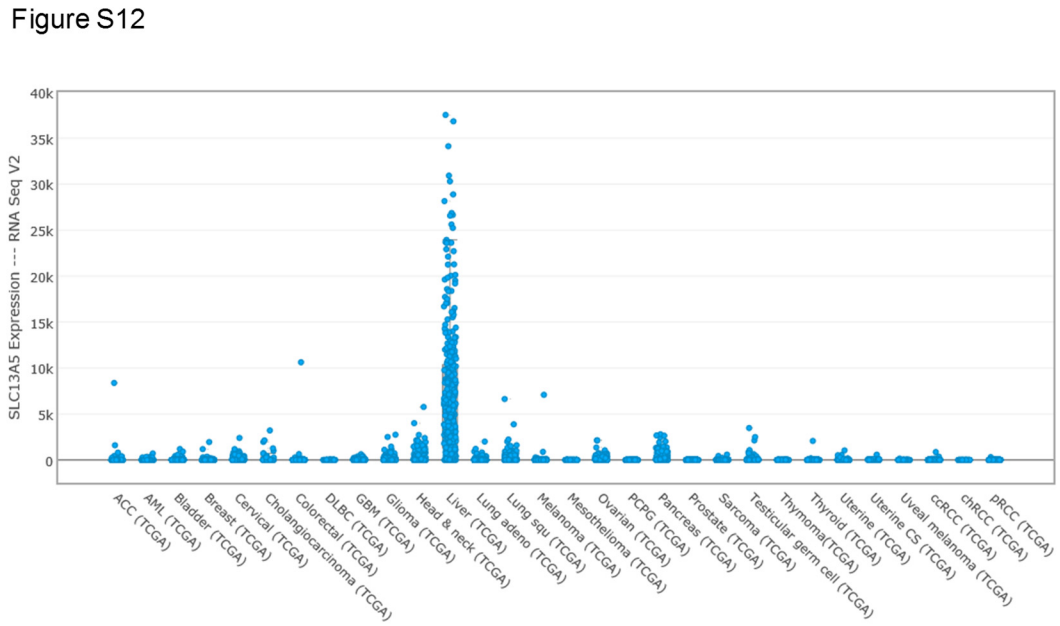

Figure S12. Expression of SLC13A5 in different human cancer tissue extracted from the cBioPortal database (http://www.cbioportal.org/)

Supplemental Table S1: Primer sequences for real-time PCR.

Primer name	Sequence (5'-3')
SLC13A5-F	CTTTGTGGCCACCCTGCTATTC
SLC13A5-R	AGCAAATCCGCCCCCTAGTA
Cyclin B1-F	TACCTATGCTGGTGCCAGTG
Cyclin B1-R	CAGATGTTTCCATTGGGCTT
ACLY-F	TCAGGAGGGCTTACGGGTG
ACLY-R	TCTGTGCCAAAGACATGGATG
P21-F	CTGGAGACTCTCAGGGTCGAAA
P21-R	GATTAGGGCTTCCTCTTGGAGAA
B-actin-F	GCTCGTCGTCGACAACGGCTC
B-actin-R	CAAACATGATCTGGGTCATCT