

Renal Glucose Transporters Play a Role in Removal of Cadmium from Kidney Cells Mediated by GMDTC – A Novel Metal Chelator

Xiaojiang Tang, Xushen Chen, Bo Xiao, Qile Zhao, Wei Hu, Amber McKenery, Zhiyong Zhong, Xuefeng Ren

Supplementary Material

Supplementary Table 1. Four highest possible off-target sites as analyzed by the Cas-OFFinder.

Supplementary Table 2. Impact of high dose (433 mg/kg body weight) GMDTC on serum biochemical parameters indicating hepatic and renal function.

Supplementary Fig. 1 Representative HPLC chromatogram of GMDTC.

Supplementary Fig. 2 Generation and validation of *GLUT2*^{-/-} and *SGLT2*^{-/-} KO HK-2 cell lines.

Supplementary Fig. 3 Nucleotide BLAST pairwise alignments of *GLUT2* and *SGLT2* gene knockouts against subject sequences.

Supplementary Fig. 4 Detection of off-target effects using T7E1 assay.

Supplementary Fig. 5 Effect of GMDTC and cadmium on HK-2 cell viability.

Supplementary Fig. 6 Cell viability analysis of *GLUT2*^{-/-} and *SGLT2*^{-/-} cells with GMDTC rescue for 24 hours.

Supplementary Fig. 7 Full and uncropped western blots of blotting images shown in Supplementary Fig. 2.

Supplementary Table 1. Four highest possible off-target sites as analyzed by the Cas-OFFinder.

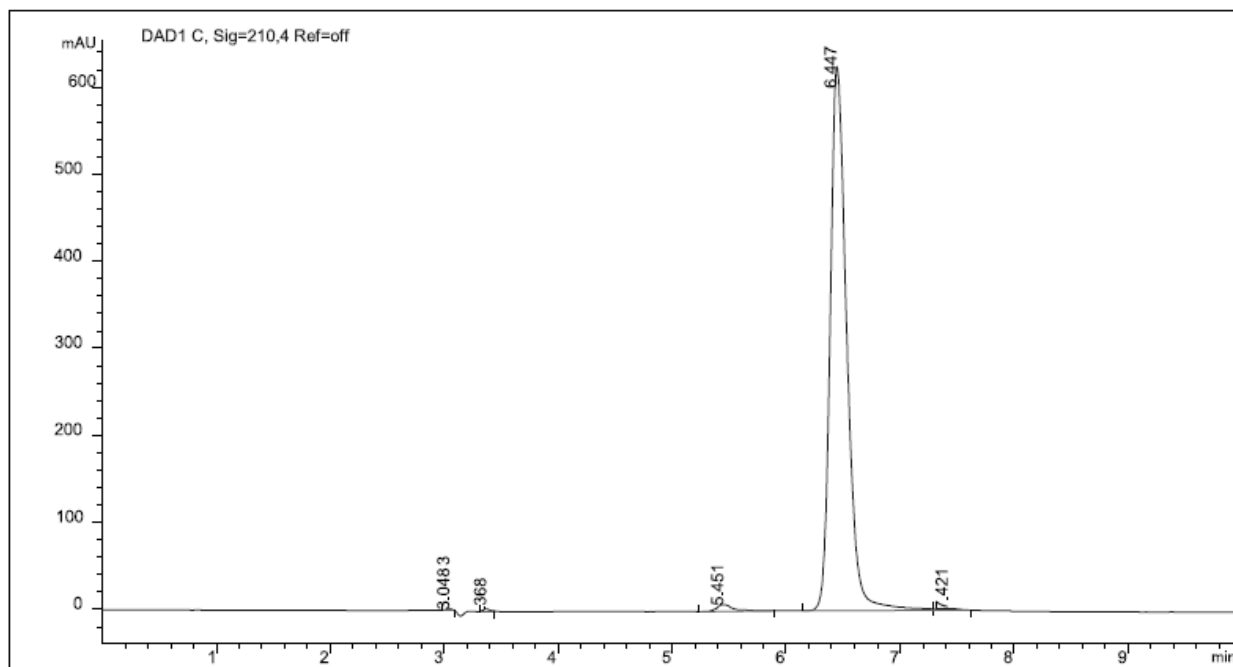
Name	Chr	Sequence	Mismatch	Strand
GLUT2-off target	5	CTCCCAACAGCCTATTCTTG*	3	+
SGLT2-off target	3	AAGAGCCTGGGCAGGTACTG	3	-
	14	AAGCGCATGTGCTGGTACTG	3	-
	X	AAGGGCTTGCTCAGGTACT T	3	+

* The bold words represent mismatch sites.

Supplementary Table 2. Impact of high dose (433 mg/kg body weight) GMDTC on serum biochemical parameters indicating hepatic and renal function. (mean $\pm$ SD, n=6)

Group	ALT (U/L)	AST (U/L)	CRE (μ mol/L)	BUN (mmol/L)	TBIL (μ mol/L)	UA (μ mol/L)
Control	32.9 $\pm$ 3.0	99.4 $\pm$ 9.5	13.4 $\pm$ 1.4	6.1 $\pm$ 1.0	1.0 $\pm$ 0.1	33.8 $\pm$ 3.8
GMDTC (433 mg/kg body weight)	31.7 $\pm$ 3.5	101.8 $\pm$ 7.0	13.8 $\pm$ 0.5	5.0 $\pm$ 0.6	1.2 $\pm$ 0.2	32.7 $\pm$ 3.1

Note: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRE, creatinine; BUN, blood urea nitrogen; TBIL, total bilirubin; UA, uric acid. Compared to control group, no significant changes were observed in high dose GMDTC group.



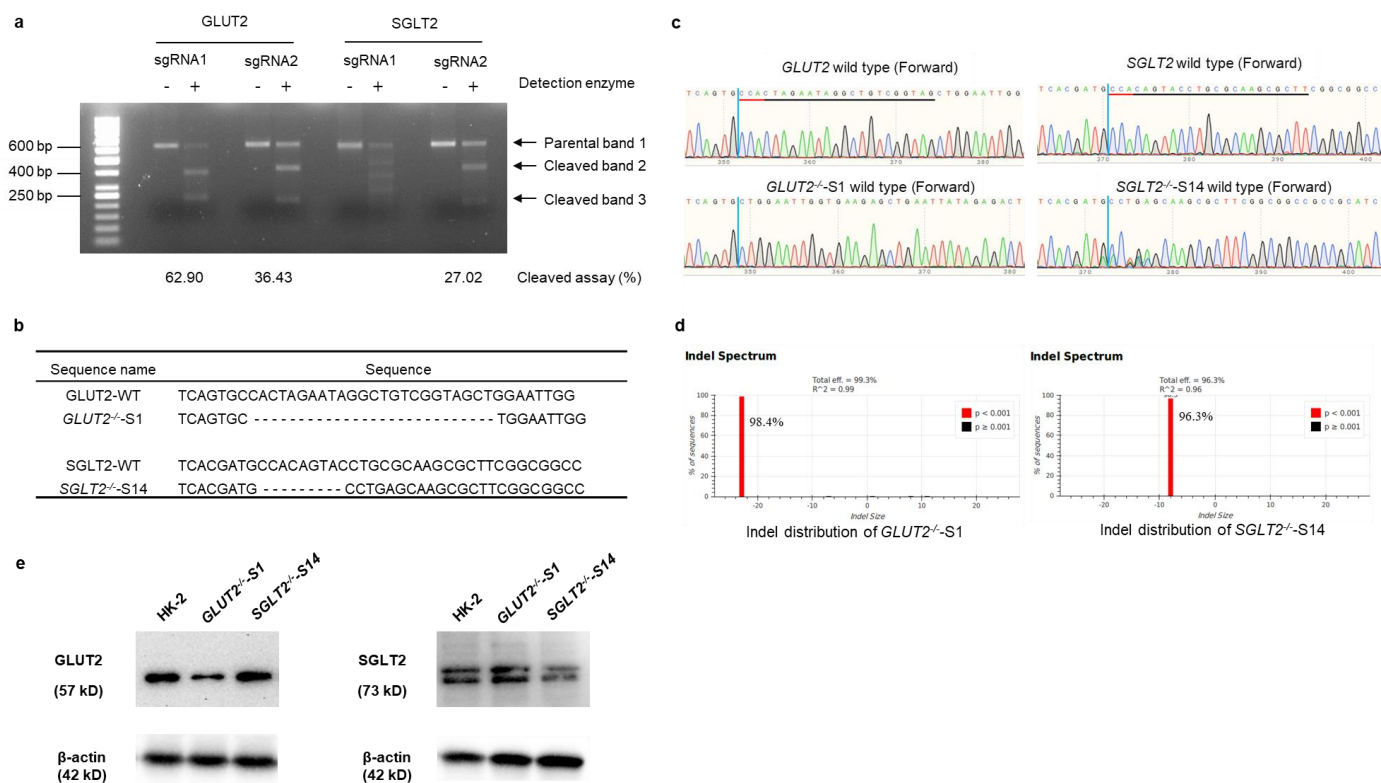
=====
Area Percent Report
=====

Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 C, Sig=210,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.048	MM	0.0619	5.92963	1.59766	0.0920
2	3.368	MM	0.0653	9.58025	2.44435	0.1486
3	5.451	BB	0.1498	81.60532	8.08452	1.2661
4	6.447	MF	0.1687	6322.00000	624.54358	98.0826
5	7.421	FM	0.2341	26.47194	1.88467	0.4107

Supplementary Fig. 1 Representative HPLC chromatogram of GMDTC.



Supplementary Fig. 2 Generation and validation of *GLUT2*^{-/-} and *SGLT2*^{-/-} KO HK-2 cell lines. **(A)** T7E1 assay. HK-2 cells were transfected with GLUT2 sgRNAs and SGLT2 sgRNAs, and genomic PCR products were analyzed by T7E1 assay. Transfected cells were selected to isolate single clones: *GLUT2*^{-/-}-S1 and *SGLT2*^{-/-}-S14. **(B)** CRISPR/Cas9-induced indel mutations in the target sites. Dashes, deleted bases. **(C)** DNA sequence analysis showed the presence of *GLUT2*^{-/-} and *SGLT2*^{-/-} mutations in clones. The horizontal, black line signifies the guide sequence; the horizontal, red line signifies the PAM sequence; the vertical, blue line represents the cut-site. **(D)** TIDE assay. Distribution of indels within the targeted GLUT2 and SGLT2 gene locus were calculated using the TIDE algorithm. **(E)** Detection of protein expression levels of GLUT2 and SGLT2 using western blotting assay. (Full and uncropped western blots are shown in **Supplementary Fig. 7**)

A

Homo sapiens solute carrier family 2 member 2 (SLC2A2), RefSeqGene on chromosome 3

Sequence ID: [NG_008108.1](#) Length: 37632 Number of Matches: 1

Range 1: 26414 to 26982 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
902 bits(488)	0.0	546/569(96%)	23/569(4%)	Plus/Minus
Query 1	TTATTCTCCTTTCCCTCCTTGATGTATTAAGATTTTGCCTTTGCCTTCACCTTAAT	60		
Sbjct 26982	TTATTCTCCTTTCCCTCCTTGATGTATTAAGATTTTGCCTTTGCCTTCACCTTAAT	26923		
Query 61	AGATATATAGTACCATTGACTGTTGACACTTTAAAAAATTGTTAAGTAATTTAGAAAAAC	120		
Sbjct 26922	AGATATATAGTACCATTGACTGTTGACACTTTAAAAAATTGTTAAGTAATTTAGAAAAAC	26863		
Query 121	TTTTGAAGCAAAATGGAAATTAATGCTTACTCAATGTACGTTTGTATAGCAAGACCCAT	180		
Sbjct 26862	TTTTGAAGCAAAATGGAAATTAATGCTTACTCAATGTACGTTTGTATAGCAAGACCCAT	26803		
Query 181	GATGCCAATACCTTAAATATCTTTAGCTATTTAAACTTGTACCCCTTGCCTTCCCTAT	240		
Sbjct 26802	GATGCCAATACCTTAAATATCTTTAGCTATTTAAACTTGTACCCCTTGCCTTCCCTAT	26743		
Query 241	TTTAATTCACGTGCTCTTAAGAGTTAGTGGGTGTTCTTAAACTTACGCCATTGATTCCG	300		
Sbjct 26742	TTTAATTCACGTGCTCTTAAGAGTTAGTGGGTGTTCTTAAACTTACGCCATTGATTCCG	26683		
Query 301	GAAAATTGCTGAGCCACATGCAGCATCAGTGC-----TGGAA	337		
Sbjct 26682	GAAAATTGCTGAGCCACATGCAGCATCAGTGCCTAGCAATAGGCTGTCGGTAGCTGGAA	26623		
Query 338	TTGGTGAAGAGCTGAATTATAGAGACTTTCTGCTCACTCGATGCTTCTCTCTTTCTTTT	397		
Sbjct 26622	TTGGTGAAGAGCTGAATTATAGAGACTTTCTGCTCACTCGATGCTTCTCTCTTTCTTTT	26563		
Query 398	CTCATTTTCAATATCTTTGGTGACATCATCATATCCTCTGAGTCTTTTCAAGCCTGTC	457		
Sbjct 26562	CTCATTTTCAATATCTTTGGTGACATCATCATATCCTCTGAGTCTTTTCAAGCCTGTC	26503		
Query 458	CAAGAAAATGATCAGGTTGAAACAACCTCAGGCCAAAACAAAAAGAAATTTATTTTATGT	517		
Sbjct 26502	CAAGAAAATGATCAGGTTGAAACAACCTCAGGCCAAAACAAAAAGAAATTTATTTTATGT	26443		
Query 518	TAATGAAAGAGCTACATTTCTGCATGCCC	546		
Sbjct 26442	TAATGAAAGAGCTACATTTCTGCATGCCC	26414		

B

Homo sapiens solute carrier family 5 member 2 (SLC5A2), RefSeqGene on chromosome 16

Sequence ID: [NG_012892.1](#) Length: 14653 Number of Matches: 1Range 1: 7325 to 7891 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
979 bits(530)	0.0	556/567(98%)	8/567(1%)	Plus/Plus
Query 1	GGTCTATGCTGGGGTTTCATATCTAGATGATCATAGGGACAACATTGGGAAAAACGGGCCA	60		
Sbjct 7325	GGTCTATGCTGGGGTTTCATATCTAGATGATCATAGGGACAACATTGGGAAAAACGGGCCA	7384		
Query 61	GAAGTGATAGCTGGGGAGACAAGGAGGGCTCCAGTTAAGAGCCCAGGAGGGTGTGAGCT	120		
Sbjct 7385	GAAGTGATAGCTGGGGAGACAAGGAGGGCTCCAGTTAAGAGCCCAGGAGGGTGTGAGCT	7444		
Query 121	CTGTTCTTGGTGCCAGGGCCCCGGGGCCATCACCTGCAGTTGTCTCTGGGCCCCAGA	180		
Sbjct 7445	CTGTTCTTGGTGCCAGGGCCCCGGGGCCATCACCTGCAGTTGTCTCTGGGCCCCAGA	7504		
Query 181	TGTGGCCCTTCCCAGGGCCACTTGTCTGGAGTAGCACCTTCACGAAGAGGTGAGATCCTC	240		
Sbjct 7505	TGTGGCCCTTCCCAGGGCCACTTGTCTGGAGTAGCACCTTCACGAAGAGGTGAGATCCTC	7564		
Query 241	AGGGATGAGGGCAAAGCCACCCTCAGCGGCAGTACTGCCCCCGTAGGCGCTCTTCGTGG	300		
Sbjct 7565	AGGGATGAGGGCAAAGCCACCCTCAGCGGCAGTACTGCCCCCGTAGGCGCTCTTCGTGG	7624		
Query 301	TGCTGCTACTGGGCTGGCTGTTTGACCCGTGTACCTGACAGCGGGGGTCATCACGATG-	359		
Sbjct 7625	TGCTGCTACTGGGCTGGCTGTTTGACCCGTGTACCTGACAGCGGGGGTCATCACGATGC	7684		
Query 360	-----CCTGCGCAAGCGCTTCGGCGGCCGCCGCATCCGCCTCTACCTGTCTGTGCTCT	412		
Sbjct 7685	CACAGTACCTGCGCAAGCGCTTCGGCGGCCGCCGCATCCGCCTCTACCTGTCTGTGCTCT	7744		
Query 413	CCCTTTTCTGTACATCTTCACCAAGATCTCAGTGAGTGCCTGTGGCAAATGCGATTGGG	472		
Sbjct 7745	CCCTTTTCTGTACATCTTCACCAAGATCTCAGTGAGTGCCTGTGGCAGATGCGATTGGG	7804		
Query 473	CCCTAGAAGGGTGGGGCTCAAGTGGGGTCTCTAGAGAACCCTAGAGGGCGTGAGACCTAA	532		
Sbjct 7805	CCCTAGAAGGGTGGGGCTCAAGTGGGGTCTCTAGAGAACCCTAGAGGGCGTGAGACCTAG	7864		
Query 533	GAGAAACCACTGCGAGGATTATGATGA	559		
Sbjct 7865	GAGAAACCACTGCGAGGATTATGATGA	7891		

C

Homo sapiens chromosome 5 clone RP11-205J9, complete sequenceSequence ID: [AC106757.2](#) Length: 122504 Number of Matches: 1Range 1: 118749 to 119116 [GenBank](#) [Graphics](#)[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
664 bits(359)	0.0	366/369(99%)	2/369(0%)	Plus/Minus
Query 4	GCT-ATATATTTAAGGCTTTTCTCTAAGAGTGACATTCATGATTTTCTCTTTGCAACTGC	62		
Sbjct 119116	GCTAATATATTTAA-GCTTTTCTCTAAGAGTGACATTCATGATTTTCTCTTTGCAACTGC	119058		
Query 63	TTGCCAATCGATACCAGCCGAGATGAACTCAAAGTCATGAATAAGGTGATTATGTGGCTT	122		
Sbjct 119057	TTGCCAATCGATACCAGCCGAGATGAACTCAAAGTCATGAATAAGGTGATTATGTGGCTT	118998		
Query 123	TTGTGCTCGCCTGCTTATGCCGCTCCCTCCAGGGAAAACCTGACATGCCTGAGGTTCTT	182		
Sbjct 118997	TTGTGCTCGCCTGCTTATGCCGCTCCCTCCAGGGAAAACCTGACATGCCTGAGGTTCTT	118938		
Query 183	ACATTCTTTGCCACATCGTATCCCTGAGTTTTGATTTGGCTGATTTACTACTGAATGAAA	242		
Sbjct 118937	ACATTCTTTGCCACATCGTATCCCTGAGTTTTGATTTGGCTGATTTACTACTGAATGAAA	118878		
Query 243	ATCACAGAGACCTCTCTGCTTCCTCCCAACAGCCTATTCTTGTGGTAAGTCAGTTTAGCA	302		
Sbjct 118877	ATCACAGAGACCTCTCTGCTTCCTCCCAACAGCCTATTCTTGTGGTAAGTCAGTTTAGCA	118818		
Query 303	GAATATGACATCTGTTGACAAGCTTTCTCTGCTTAGAGGCCCTAGTCAGCCAGTTTGG	362		
Sbjct 118817	GAATGTGACATCTGTTGACAAGCTTTCTCTGCTTAGAGGCCCTAGTCAGCCAGTTTGG	118758		
Query 363	AAGCCAGTC	371		
Sbjct 118757	AAGCCAGTC	118749		

D1

Homo sapiens 3 BAC RP11-385J1 (Roswell Park Cancer Institute Human BAC Library) complete sequence

Sequence ID: [AC117457.11](#) Length: 204899 Number of Matches: 1

Range 1: 144036 to 144467 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
798 bits(432)	0.0	432/432(100%)	0/432(0%)	Plus/Plus
Query 9	AGAGTTTGTGTTGCCCTACAGGAATATTGTAAGTGTCAACAGCCAGCTGTCCTGAAATTCT	68		
Sbjct 144036	AGAGTTTGTGTTGCCCTACAGGAATATTGTAAGTGTCAACAGCCAGCTGTCCTGAAATTCT	144095		
Query 69	GAAGGCTTTATTAGAGCAAGCGAAGTGAATATATTCTTTACACCTTCTATTTACTCTCA	128		
Sbjct 144096	GAAGGCTTTATTAGAGCAAGCGAAGTGAATATATTCTTTACACCTTCTATTTACTCTCA	144155		
Query 129	GCAAATAATTTAATATATTTTGCTACTTTTTATGAACAAAGGAATTAACATTTAAGAAGC	188		
Sbjct 144156	GCAAATAATTTAATATATTTTGCTACTTTTTATGAACAAAGGAATTAACATTTAAGAAGC	144215		
Query 189	AATTACTGTGTACTGTAGGTTTTAATTGTGTTCTCTTAAGATAGATATTATTATGCTGGC	248		
Sbjct 144216	AATTACTGTGTACTGTAGGTTTTAATTGTGTTCTCTTAAGATAGATATTATTATGCTGGC	144275		
Query 249	TTTACAGATGAGGAAACTGAGGCTTTTTAAATAACTGAGTGATTTTCAATAACTTCCAAA	308		
Sbjct 144276	TTTACAGATGAGGAAACTGAGGCTTTTTAAATAACTGAGTGATTTTCAATAACTTCCAAA	144335		
Query 309	GGCCCAACAAC TAGCCAGTGATGGAATCAGGATTTGAACCCAGCTGATGCTCTCTCCCTG	368		
Sbjct 144336	GGCCCAACAAC TAGCCAGTGATGGAATCAGGATTTGAACCCAGCTGATGCTCTCTCCCTG	144395		
Query 369	TACCCAGTACCTGCCAGGCTCTTGTCAAACTTGAAATATTTGGAGTGATAGTGAACAG	428		
Sbjct 144396	TACCCAGTACCTGCCAGGCTCTTGTCAAACTTGAAATATTTGGAGTGATAGTGAACAG	144455		
Query 429	CCAAGCCACAA 440			
Sbjct 144456	CCAAGCCACAA 144467			

D2

Human chromosome 14 DNA sequence BAC R-102G14 of library RPCI-11 from chromosome 14 of Homo sapiens (Human), complete sequence

Sequence ID: [AL122125.4](#) Length: 188849 Number of Matches: 1

Range 1: 174796 to 175218 [GenBank](#) [Graphics](#)

[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
763 bits(413)	0.0	420/423(99%)	1/423(0%)	Plus/Minus
Query 2	CTAAGGCA-GAACAAAATACCTTCTCTGCCCTGCCCTCTCCCAAAACCTGGGAATGAA	60		
Sbjct 175218	CTAAGGCAGGGACCAAAATACCTTCTCTGCCCTGCCCTCTCCCAAAACCTGGGAATGAA	175159		
Query 61	GTTGAATGGGGAGTGGGGTGGGTGGAGCCCTTCCCTTTGGAAGACTCTTCCCTGGGTG	120		
Sbjct 175158	GTTGAATGGGGAGTGGGGTGGGTGGAGCCCTTCCCTTTGGAAGACTCTTCCCTGGGTG	175099		
Query 121	TCTCCAAGGGAAGCTTCTTTTCTCTTTTGATCGTTCTGAATGCCCTAGGCCCTCAACAA	180		
Sbjct 175098	TCTCCAAGGGAAGCTTCTTTTCTCTTTTGATCGTTCTGAATGCCCTAGGCCCTCAACAA	175039		
Query 181	ACATTCTTGAACTTCTTTCATGTTAGCATGGGGCCCTACCTGCCCTAAGAGGCTAACT	240		
Sbjct 175038	ACATTCTTGAACTTCTTTCATGTTAGCATGGGGCCCTACCTGCCCTAAGAGGCTAACT	174979		
Query 241	CCTTAGCTGGACTGAGGCAGGCCACAGGCAAGGTTGATAGATGTAAGGTCACCTCAGT	300		
Sbjct 174978	CCTTAGCTGGACTGAGGCAGGCCACAGGCAAGGTTGATAGATGTAAGGTCACCTCAGT	174919		
Query 301	ACCAGCACATGCGCTTCTTTTCTGCTTCTTACAGCTGCTTCTTCTTGGTTGCTA	360		
Sbjct 174918	ACCAGCACATGCGCTTCTTTTCTGCTTCTTACAGCTGCTTCTTCTTGGTTGCTA	174859		
Query 361	TAAATAACATCCTTCCCACTCTGGAAAATGCTGAGTTTTTCATTGATGGTCCCCAGC	420		
Sbjct 174858	TAAATAACATCCTTCCCACTCTGGAAAATGCTGAGTTTTTCATTGATGGTCCCCAGC	174799		
Query 421	TCC 423			
Sbjct 174798	TCC 174796			

Related Information

[Genome Data Viewer](#) - aligned genomic context

D3

Homo sapiens ALG13 UDP-N-acetylglucosaminyltransferase subunit (ALG13), RefSeqGene on chromosome X

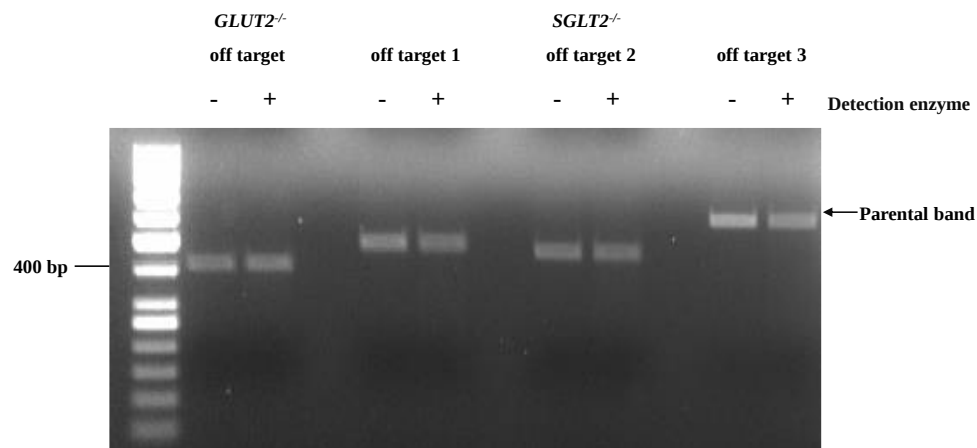
Sequence ID: [NG_016238.1](#) Length: 86532 Number of Matches: 1

Range 1: 54470 to 55023 [GenBank](#) [Graphics](#)

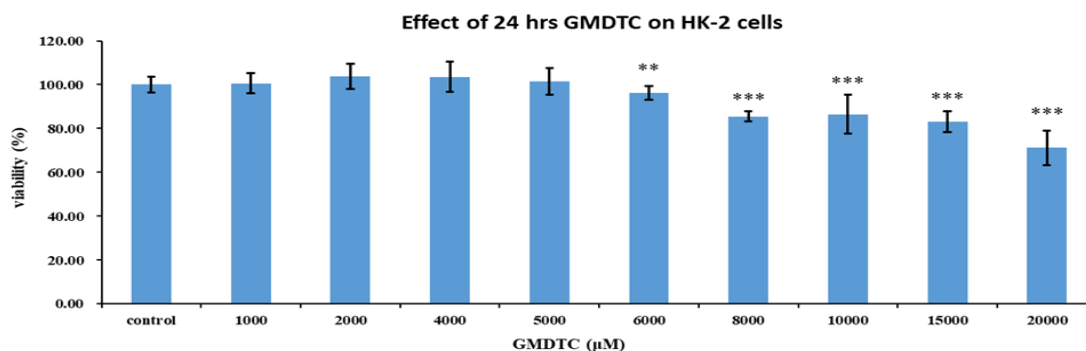
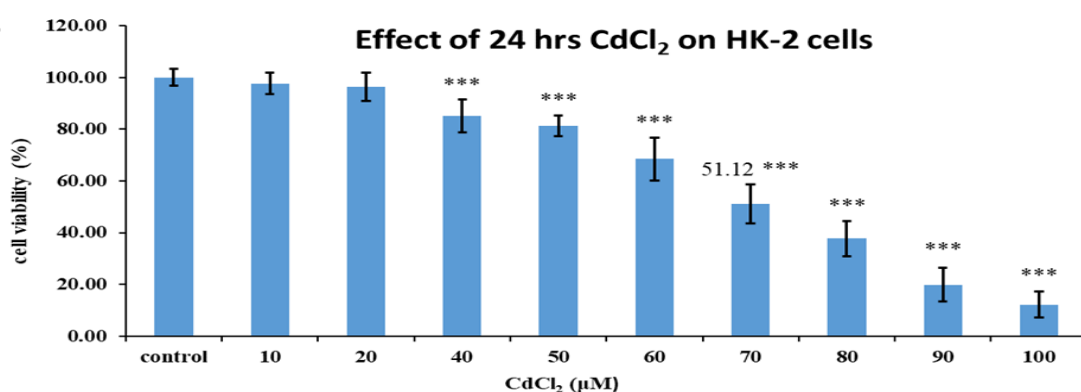
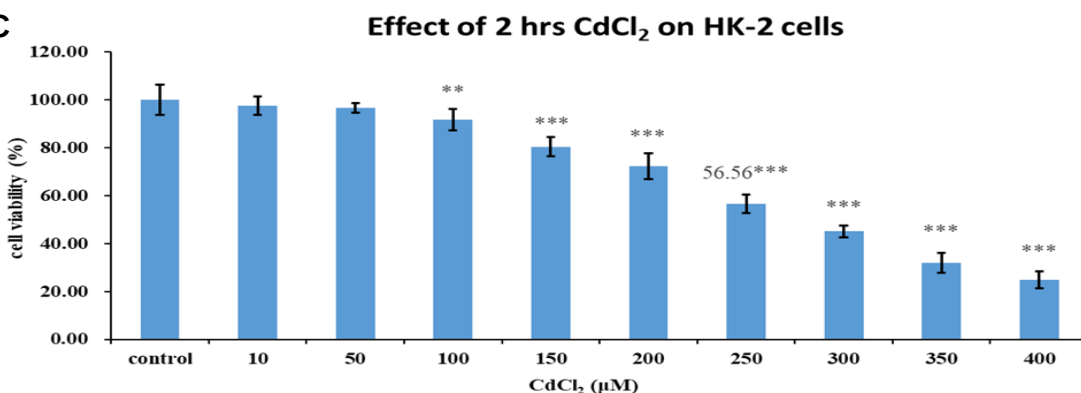
[▼ Next Match](#) [▲ Previous Match](#)

Score	Expect	Identities	Gaps	Strand
1003 bits(543)	0.0	551/554(99%)	3/554(0%)	Plus/Plus
Query 5	ATTT-AT-ATAGC-AAGAGTTATAGCTCCTACTATGTACTAGGGCACTGTTAAGAGCTAT	61		
Sbjct 54470	ATTTAATAATAGCAAGAGTTATAGCTCCTACTATGTACTAGGGCACTGTTAAGAGCTAT	54529		
Query 62	ACATATAAATGTATTTAAATCAGAACAACCTGTGAGGCTTAGTATCATTCTCATTTTGA	121		
Sbjct 54530	ACATATAAATGTATTTAAATCAGAACAACCTGTGAGGCTTAGTATCATTCTCATTTTGA	54589		
Query 122	AGATGAAATTGAGACAGAAATTAACTCGTGTTCTGGAACATAATATTAGAGATATTCT	181		
Sbjct 54590	AGATGAAATTGAGACAGAAATTAACTCGTGTTCTGGAACATAATATTAGAGATATTCT	54649		
Query 182	AACATAGGCAGTAATGATCTTTTAGAAGGTTTATTATGAttttttATTAGAATGGATG	241		
Sbjct 54650	AACATAGGCAGTAATGATCTTTTAGAAGGTTTATTATGATTTTTATTAGAATGGATG	54709		
Query 242	ATACATCTCACTCAATATAACAAAAGTTTGCTTCTCCTTTATGCTACATGTCTAGTAGAG	301		
Sbjct 54710	ATACATCTCACTCAATATAACAAAAGTTTGCTTCTCCTTTATGCTACATGTCTAGTAGAG	54769		
Query 302	GTCTGTAGAAGGGCTTGCTCAGGTACTTAGGCTGATGGAAATTCATCCACAATTGCCAAG	361		
Sbjct 54770	GTCTGTAGAAGGGCTTGCTCAGGTACTTAGGCTGATGGAAATTCATCCACAATTGCCAAG	54829		
Query 362	GCAGGAAATAAAATTCGGTAAATGGTGCCTGAGGCTTTCCACTAGAGGTGATA	421		
Sbjct 54830	GCAGGAAATAAAATTCGGTAAATGGTGCCTGAGGCTTTCCACTAGAGGTGATA	54889		
Query 422	CATGTCACTTTCTTTTCCATTTCCTGAGGCTGAGGCTCCACTGAAGAGTCTTAACCT	481		
Sbjct 54890	CATGTCACTTTCTTTTCCATTTCCTGAGGCTGAGGCTCCACTGAAGAGTCTTAACCT	54949		
Query 482	TTCTGTGCTGGAAGGCAAAAAGAAACATGTTAACAGCGCTAAGAACTATATCATTCAACT	541		
Sbjct 54950	TTCTGTGCTGGAAGGCAAAAAGAAACATGTTAACAGCGCTAAGAACTATATCATTCAACT	55009		
Query 542	TGCCCTCCCTCTCA	555		
Sbjct 55010	TGCCCTCCCTCTCA	55023		

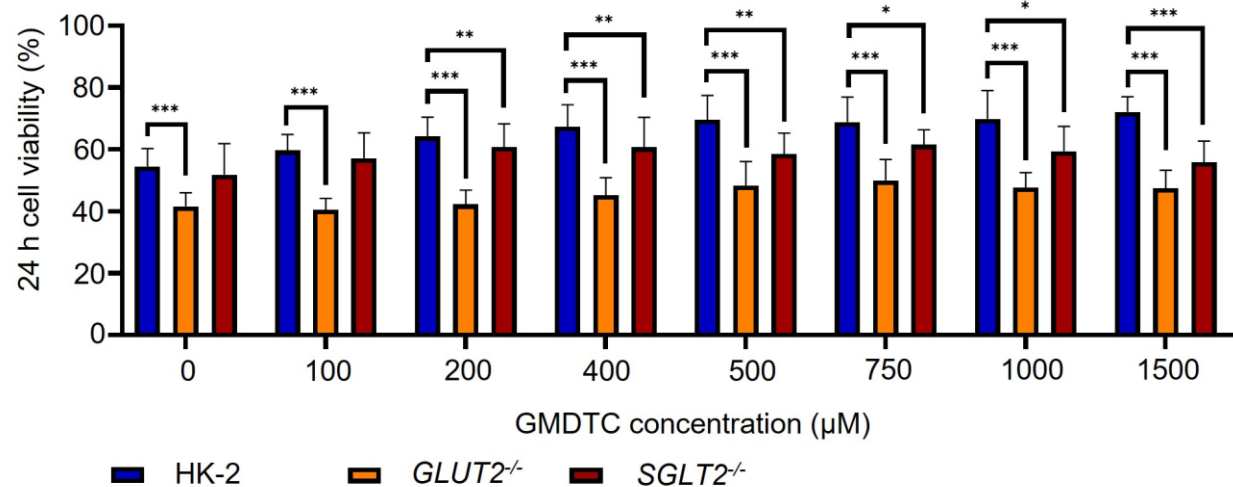
Supplementary Fig. 3 Nucleotide BLAST pairwise alignments of *GLUT2* and *SGLT2* gene knockouts against subject sequences. PCR products containing on and off target loci were submitted for Sanger sequencing. Then, nucleotide BLAST pairwise alignments were applied for comparison of nucleotide sequences with a database of sequences (subject sequences). **(A)** A pairwise alignment display of a 546 bp-long Query (*GLUT2*^{-/-}) against the NG_008108.1 (*SLC2A2*, Subject) sequence with its total length of 37632 bp. Query alignment starts with its first base (T) and it matches Subject location 26982; the end of Query alignment matches Subject location 26414. Query has 23 bases deletion compared with subject and the rest are identical. **(B)** A pairwise alignment display of a 559 bp-long Query (*SGLT2*^{-/-}) against the NG_012892.1 (*SLC5A2*, Subject) sequence with its total length of 14653 bp. Query alignment starts with its first base (G) and it matches Subject location 7325; the end of Query alignment matches Subject location 7891. Query has 8 bases deletion compared with subject and the rest are identical. **(C, D1-3)** Alignments display of off-target sites of *GLUT2*^{-/-} and *SGLT2*^{-/-} with human genome showed no mismatches, indicating no off-target effects occurred during the editing process.



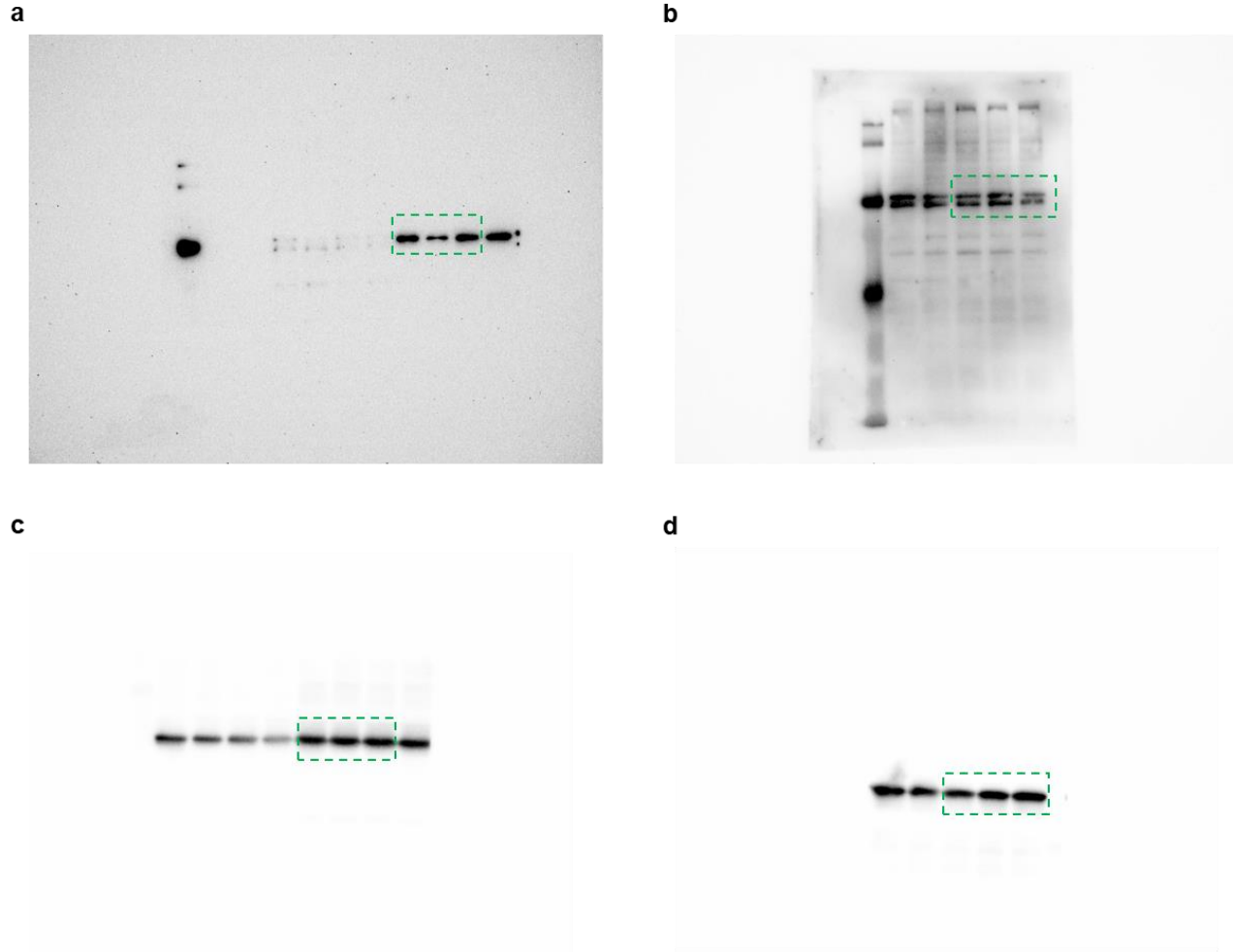
Supplementary Fig. 4 Detection of off-target effects using T7E1 assay. The genomic sequences containing off-target sites were PCR amplified using the primers listed in the table 2. Then, purified PCR products were digested with T7E1 and quantified using a 1.5% agarose gel electrophoresis. No cleaved band occurred during the on-target editing, indicating sgRNAs are highly specific for intended target sequences.

A**B****C**

Supplementary Fig. 5 Effect of GMDTC and cadmium on HK-2 cell viability. **(A)** Effect of GMDTC on cell viability in HK-2 cells treated with various concentrations of GMDTC for 24 h. **(B)** Effect of cadmium on cell viability in HK-2 cells treated with different concentrations of CdCl₂ for 24 h. **(C)** Effect of cadmium on cell viability in HK-2 cells pretreated with different concentrations of CdCl₂ for 2 h, washed twice with PBS, replaced with fresh medium, and then incubated for 24 h. Cell viability was measured using alarmBlue assay and was relative to control. Data was obtained from 3 independent experiments. ***P*<0.01, ****P*<0.001 compared to control.



Supplementary Fig. 6 Cell viability analysis of *GLUT2*^{-/-} and *SGLT2*^{-/-} cells with GMDTC rescue for 24 hours. Data was obtained from 3 independent experiments. ***P*<0.01; ****P*<0.001 compared to CdCl₂ treated alone shown as “0” concentration.



Supplementary Fig. 7 Full and uncropped western blots of blotting images shown in Supplementary Fig. 2, detecting expression of GLUT2 (A), SGLT2 (B), β -actin for GLUT2 (C), and β -actin for SGLT2 (D). Green dotted boxes indicate cropped areas.