

Hiroaki Takesue, Takeshi Hirota, Mami Tachimura, Ayane Tokashiki, Ichiro Ieiri

Nucleosome positioning and gene regulation of the *SGLT2* gene in the human kidney

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### Supplemental Table 1

Primer sequences for the cloning of *SGLT2* reporter plasmids

Primer		Sequence (5' to 3') <sup>a</sup>	Annealing (°C)	Restriction enzymes
Cloning of the <i>SGLT2</i> 5'-flanking region				
-3185	Forward	TGGCCTAACTGGCCGGTACCTTCCCGACCGCCT	60.0	-
+18	Reverse	AGTACCGGATTGCCACTCCCCAGGATCTGCCCC		
In-Fusion cloning and site-directed mutagenesis				
Del-(-51/-37)	Forward	GGCTCAGTGCCCCTGCTTCCCCTGGGGGAATCC	60.0	-
	Reverse	CAGGGGCACTGAGCCGACAAGTCCCCCAGGTCT		
-2320	Forward	GTTTGTAAATGAAGGAAGGT <u>ACCAGGAAGGAAGAAAGA</u>	60.0	Kpn□
	Reverse	TCTTTCCTTCCTTCCTGGT <u>TACCTTCCTTCATTAACAAAC</u>		
-1587	Forward	CCAAGTCTCTTTGTGGT <u>TACCTGACAAATGACACAC</u>	60.0	Kpn□
	Reverse	GTGTGTCATTTGTCAGGGT <u>ACCACAAAGAGCAGTTGG</u>		
-485	Forward	CAAAAATCTGGGCTGGT <u>TACCTTAAAGGAGTGGGAAAGGA</u>	60.0	Kpn□
	Reverse	TCCTTTCCTTCCTTTAAGGT <u>ACCAGCCCAGATTTTTG</u>		
-154	Forward	TGGAAGGGCCCAGGT <u>ACCCAAGACCAGCC</u>	60.0	Kpn□
	Reverse	GGCTGGTCTTGGT <u>TACCTGGGCCCTTCCA</u>		
-44	Forward	GGCTCAGTGCCCCTGAGGT <u>ACCCATTAATCCTTC</u>	60.0	Kpn□
	Reverse	GAAGGATTAATGGT <u>TACCTCAGGGGCACTGAGCC</u>		

<sup>a</sup>The restriction site is underlined, and nucleotide changes are marked in bold letters.

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### Supplemental Table 2

Direct sequence oligonucleotides for the cloning of *SGLT2* reporter plasmids

Location		Sequence (5' to 3')
SGLT2	Antisense	TGAGAGAAATCCAGTGCCAAGT
	Antisense	CCTGAGATGAGAATTTGTGTGC
	Sense	GCTTTGTTGGTTTTTCTCCTTGTT
	Sense	CCACACCCAGCCAGTCCTAC
	Sense	GGAAGGATGAGCGGGAATTG
pGL4.10	Sense	GCAGGTGCCAGAACATTTCT

### Supplemental Table 3

Primer sequences and oligonucleotides for the cloning of the HNF1 $\alpha$  expression plasmid

Primer		Sequence (5' to 3')
Cloning of the HNF1 $\alpha$ coding region		
	Forward	CAGTGTGGTGAATTATGGTTTCTAAACTGAGCCA
	Reverse	GCCACTGTGCTGGATTTACTGGGAGGAAGAGGC
Direct sequencing		
HNF1 $\alpha$	Sense	AGCAGTTCACCCATGCAGG
T7	Sense	TTGTAATACGACTCACTATAG
BGH	Antisense	TAGAAGGCACAGTCGAGG

### Supplemental Table 4

Primer sequences for quantitative PCR

Gene		Sequence (5' to 3')	Position
SGLT2	Forward	TTCAGTCTCCGGCATAGCAA	1700 to 1719
	Reverse	CATCTCCATGGCACTCTCTGG	1807 to 1787
HNF1 $\alpha$	Forward	CCCTGGGTCCTACGTTCA	1463 to 1480
	Reverse	GGGTCACATGGCTCTGCA	1657 to 1640
RPL13	Forward	GAGACAGTTCTGCTGAAGAACTGAA	486 to 510
	Reverse	TCCGGACGGGCATGAC	551 to 536

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### Supplemental Table 5

Primer sequences for NOME-Seq

Primer		Sequence (5' to 3')	Position
Bisulfite PCR			
SGLT2	Forward	TGGGAAAGGATTTTTGATTTTTTT	-477 to -454
	Reverse	CCCCTAAATCCCCCAAAAA	-14 to -33
GAPDH	Forward	GGGTTTTTGTTTTGATTTTTTAGTGTTT	-220 to -192
	Reverse	CAATCCCAGCCCAAATCTTAAA	+25 to +3
Colony PCR			
T7	Forward	TTCAGTCTCCGGCATAGCAA	
SP6	Reverse	CATCTCCATGGCACTCTCTGG	

**Supplemental Table 6**

## Primer sequences for NuSA

Position		Sequence (5' to 3')	Midpoint
-547 to -522	Forward	TTTGGTGGGGATAAAATATCTGGTCA	-462
-377 to -400	Reverse	TCTTCAGCCTGATTTCCAATCCTG	
-508 to -487	Forward	GCAAAAATCTGGGCTGGGTAGG	-445
-383 to -405	Reverse	GCCTGATTTCCAATCCTGGTCAT	
-454 to -430	Forward	CTAGATTTGGTTTGGAGAAGCAGGG	-381
-309 to -334	Reverse	TTTCAAATCCAAGTCTGACAGGGTC	
-419 to -402	Forward	GCGGGAATTGGGGCATGA	-353
-287 to -318	Reverse	TTTAACTAATCCAGAGGAATCATTTTCAAATC	
-365 to -342	Forward	GAGCTATGGAGGGTTCCTGAGGAG	-289
-214 to -237	Reverse	TGCTCCAGGCTCAAATCACTCTT	
-316 to -287	Forward	TTTGAAAATGATTCCTCTGGATTAGTTAAA	-258
-200 to -217	Reverse	CGCCCTCTCCCCTGTGCT	
-316 to -287	Forward	TTTGAAAATGATTCCTCTGGATTAGTTAAA	-236
-157 to -179	Reverse	GCCCTTCCAAGTTCAAGAGCACT	
-237 to -214	Forward	AAGAGTGATTTTGAGCCTGGAGCA	-170
-104 to -131	Reverse	TGTTTAGCTGAATCAGGTCATATCAAGG	
-181 to -158	Forward	AGAGTGCTCTTGAACCTGGAAGGG	-123
-65 to -84	Reverse	CCGACAAGTCCCCCAGGTCT	
-144 to -120	Forward	GACCAGCCTTCAGCCTTGATATGAC	-69
+6 to -13	Reverse	CCCCATCCAGGAACCAGCC	
-91 to -71	Forward	GGGAATGAGACCTGGGGGACT	-25
+41 to +20	Reverse	CTGCCTCTGTGTGCTCCTCCAT	
-17 to +1	Forward	GGGGCTGGTTCCTGGATG	+51
+119 to +95	Reverse	GGAAATATGCAGCAATGACTAGGAT	
+3 to +23	Forward	GGCAGATCCTGGGGAGAATGG	+74
+144 to +124	Reverse	CACAAGCCAACGCCAATGACC	

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### Supplemental Table 7

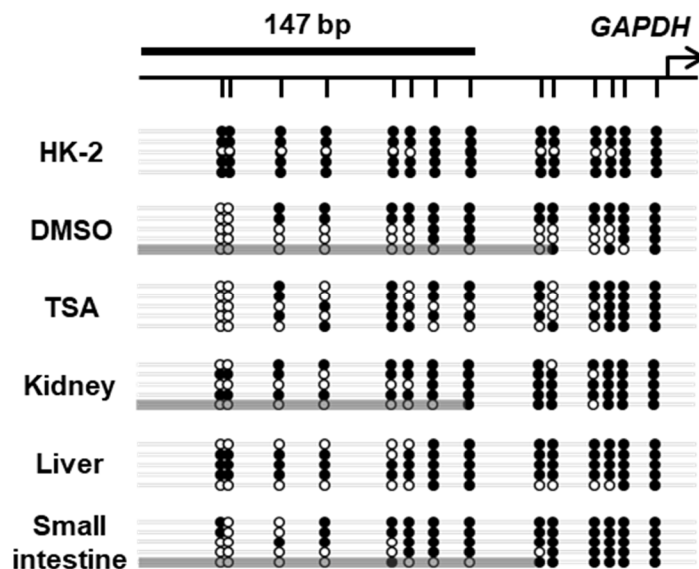
Primer sequences for the ChIP assay

Name		Sequence (5' to 3')	Position	Annealing (°C)
Semi-quantitative PCR				
Distal	Forward	TTTGGTGGGGATAAAATATCTGGTCAA	-547 to -521	60.0
	Reverse	TGTGCTCCAGGCTCAAATCACTC	-212 to -235	
Proximal	Forward	AGACCAGCCTTCAGCCTTGATATGA	-145 to -121	60.0
	Reverse	ACGCCAATGACCAGCAGGAAATA	+135 to +113	
Quantitative PCR				
	Forward	GACCAGCCTTCAGCCTTGATATGACC	-144 to -119	
	Reverse	CCTCCATTCTCCCCAGGATCTGC	+26 to +4	

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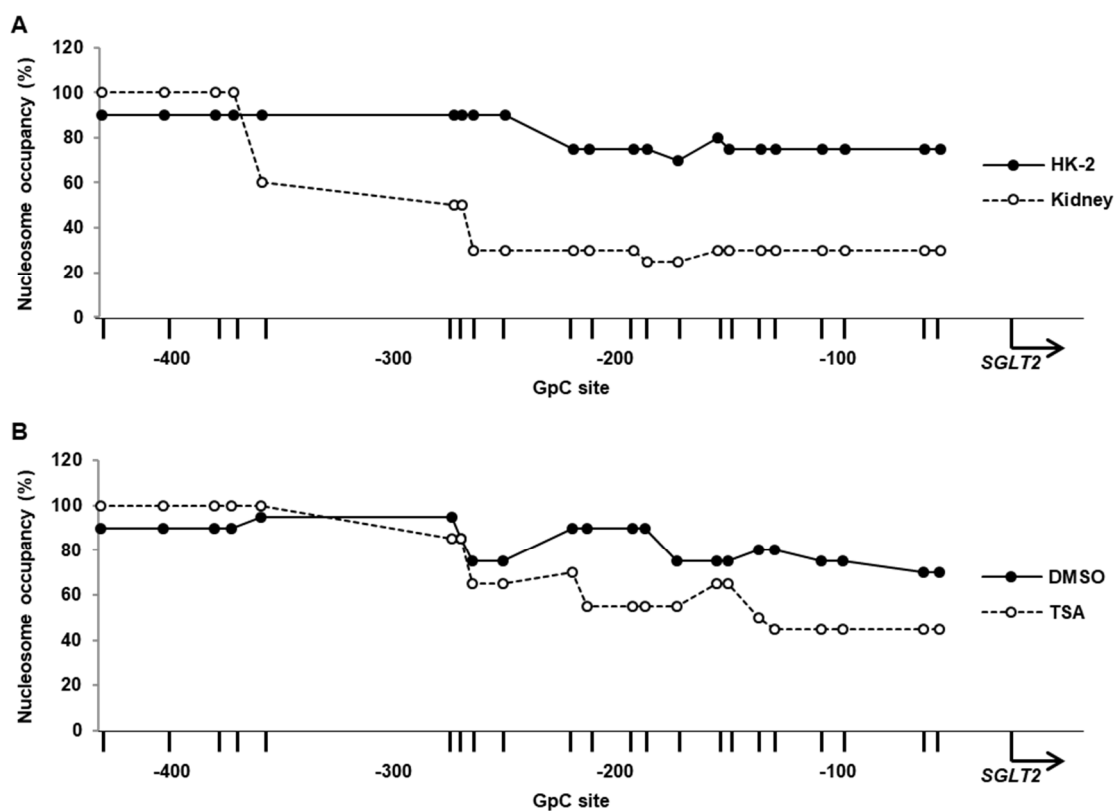
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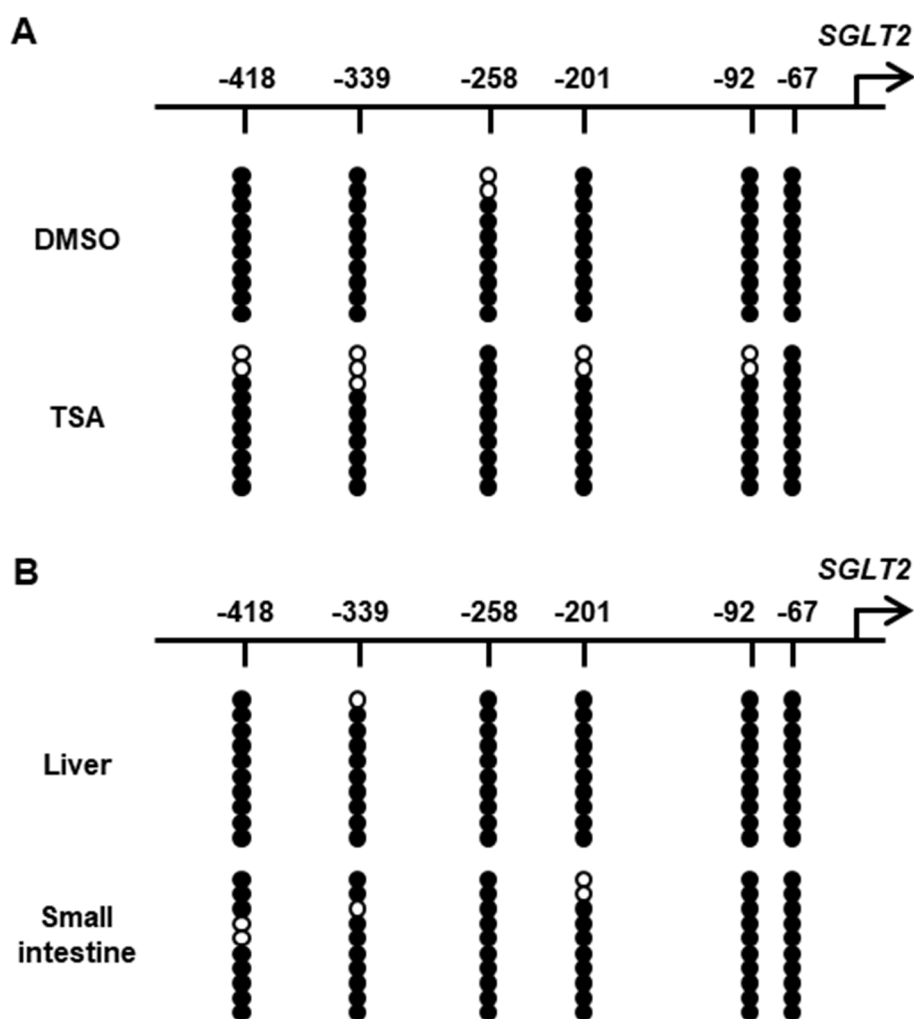
**Supplemental Figure 1 NOME-Seq analysis of nucleosome occupancy in *GAPDH* 5'-FR in HK-2 cells and human tissues.**

The arrow indicates the *GAPDH* TSS. Vertical lines indicate GpC sites. White circles represent unmethylated GpC sites and black circles represent methylated GpC sites. Gray bars represent nucleosome occupancy, which is the region of consecutive unmethylated GpC sites over 147 bp.



**Supplemental Figure 2 Analysis of nucleosome occupancy in HK-2 cells and the human kidney**

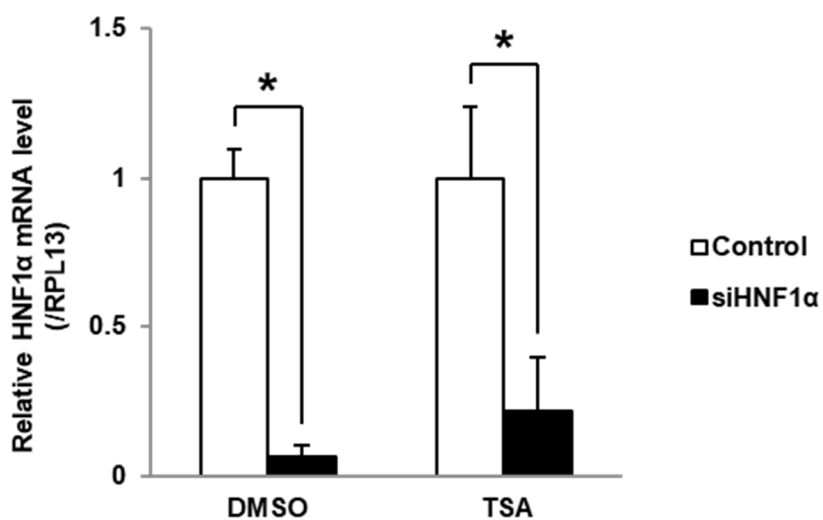
NOME-Seq data of *SGLT2* 5'-FR in (A) HK-2 cells and the human kidney, and (B) DMSO- and TSA-treated HK-2 cells. Graph represents the proportion of nucleosome-occupied GpC sites in 5'-FR in the three tissues. The arrow indicates the *SGLT2* TSS. Vertical lines indicate GpC sites.



**Supplemental Figure 3 Analysis of endogenous DNA methylation in HK-2 cells and human tissues.**

NOMe-Seq data of *SGLT2* 5'-FR in (A) DMSO- or TSA-treated HK-2 cells and (B) human liver and small intestine. The arrow indicates the *SGLT2* TSS. Vertical lines indicate CpG sites. White circles represent unmethylated CpG sites and black circles represent methylated CpG sites.





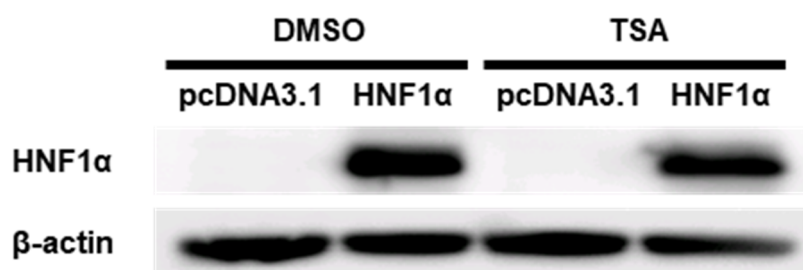
**Supplemental Figure 4 Analysis of HNF1α mRNA expression in siHNF1α-transfected HK-2 cells**

HK-2 cells were reverse-transfected with negative control (white bars) or siHNF1α (black bars) for 24 hours, and then treated with DMSO or TSA for 24 hours. HNF1α mRNA levels were measured by quantitative PCR and normalized to RPL13 mRNA levels. Results are expressed relative to HNF1α mRNA levels in the control group. Results represent the mean ± SD of three independent experiments. \*P < 0.05.

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**Supplemental Figure 5 Analysis of HNF1 $\alpha$  expression in HNF1 $\alpha$ -expressing HK-2 cells**

Western blot analyses representing HNF1 $\alpha$  and  $\beta$ -actin protein expression in HNF1 $\alpha$ -expressing HK-2 cells with DMSO or TSA treatment.