## **Supplementary information**

## 27-Hydroxycholesterol regulates human SLC22A12 gene expression through estrogen receptor action

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## Figure legends

**Figure S1. Information on amino acid sequences.** (A) Alignment made with MUSCLE. Conserved regions are highlighted in dark red, light red, and light red text, in the order of conservation levels from high to low. Dark red boxes showed conserved sequences constituted by over two residues between URAT1/*SLC22A12*, OAT4/*SLC22A11*, and OAT10/*SLC22A13*. (B) Accession numbers of each protein.

**Figure S2. Co-expression analysis of three uric acid reabsorption transporters.** (A) Distinct cell types identified by unsupervised clustering of single-nucleus RNA-sequencing (snRNA-seq) of an adult human kidney. The cluster names are explained in Materials and Methods. PT was further separated into three clusters by genetic character. These clusters, such as S1, S2, and S3 segments, are not anatomically separated. PT clusters are highlighted by the red box. (B) List of marker genes of each cluster used for the cell clustering. (C) Schematic diagram of nephron and connecting duct. Each part shares the same color code as (A). (D-F) Scatter plots of co-expression among three uric acid reabsorption transporters. Each dot represents a single PT cell. Axes show the gene expression levels.

Figure S3. Information on gene promoter sequences. (A) The alignment of three reabsorption transporter

gene promoter regions was made with Clustal. Red shades show the complete conserved sequences. Blue boxes show the three-quarters match. (B-J) Positions and scores of potential ERE sequences in uric acid transporter gene promoter regions; (B) URAT1/*SLC22A12*, (C) OAT4/*SLC22A11*, (D) OAT10/*SLC22A13*, (E) NPT1/*SLC17A1*, (F) NPT4/*SLC17A3*, (G) ABCG2/*ABCG2*, (H) GLUT9/*SLC2A9*, (I) OAT1/*SLC22A6*, (J) OAT3/*SLC22A8*.

**Figure S4.** *ESR1* (ERα) and *ESR2* (ERβ) expression levels. (A) Expression of the *ESR1* and *ESR2* gene in various tissues; the expression is relatively low in the kidney (shown red column) from GTEx RNA-seq normal tissues database (<u>https://www.proteinatlas.org/ENSG00000091831-ESR1/tissue</u>). Y-axis showed transcripts per million. (B) There is no sex difference in *ESR1* and *ESR2* expression in the kidney from GTEx RNA-seq data (<u>https://www.proteinatlas.org/ENSG0000091831-ESR1/tissue/kidney</u>). Dots showed each human kidney sample data. Y-axis showed protein-transcripts per million (pTPM).

**Figure S5. Image analysis of mouse kidneys.** (A) The scheme of the analysis. We inverted the images to grayscale and selected 230-240 proximal tubular cells (*e.g.*, red box in the left panel). The right panel shows the points of analysis. Since URAT1 is localized in the apical membrane of proximal tubular cells, we measured the signal intensity indicated the apical membrane shown as brown color. Blue lines show measurement sites. Max signal intensity on each red line is defined as URAT1 expression. (B) Each signal intensities by per images. Dots show signal intensity, and red borders show the mean.

**Figure S6. Information on human** *SLC22A12* and mouse *Slc22a12* gene promoter sequences. (A)The alignment of human *SLC22A12* and mouse *SLC22A12* gene promoter regions were made with Clustal. Red shades show the complete conserved sequences. (B) Positions and scores of potential ERE sequences in mouse *SLC22A12* gene promoter regions.



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Molecular name	Accession number
URAT1/SLC22A12	BAB96750.1
OAT4/SLC22A11	AAK68155.1
OAT10/SLC22A13	NP_004247.2
ABCG2/ABCG2	Q9UNQ0.3
NPT1/SLC17A1	NP_005065.2
NPT4/SLC17A3	NP_001091956.1
GLUT9/SLC2A9	NP_064425.2
OAT1/SLC22A6	Q4U2R8.1
OAT3/SLC22A8	NP_001171661.1

С



E	3	
	Cluster	Markergene
	EDC	EMCN
Cyfe	ICB	SLC4A9
L)	ICA	SLC4A9,SLC26A7
AL)	PC	AQP2,ATP1B3
	CNT	ATP1B3
	DCT	SLC12A3
	LH(AL)	SLC12A1,UMOD
	LH(DL)	ALDH1A2
	PT	SLC5A12,LRP2,CUBN
	Podocyte	NPHS1,WT1







# В

### URAT1/SLC22A12

	start	stop	score
ERE1	-849	-831	0.748099
ERE2	- 805	-787	0.827709
ERE3	- 603	-585	0.859078
ERE4	-448	-430	0.748337
ERE5	- 371	-353	0.743346
ERE6	-337	-319	0.854563
ERE7	-252	-234	0.735029
ERE8	-71	-53	0.748099
ERE9	-19	-1	0.780181

#### OAT4/SLC22A11

	start	stop	score
ERE1	- 998	-980	0.865019
ERE2	- 620	-602	0.789686
ERE3	-472	-454	0.734791
ERE4	-405	-387	0.737167
ERE5	-279	-261	0.855513
ERE6	-89	-71	0.747861
ERE7	-37	-19	0.730989

## D

Γ

DATIONSL	CZZAT	3	
	start	stop	score

	Jeen C	200	2004		
ERE1	- 662	-644	0.743108		

## Ε

#### NPT1/SLC17A1

	start	start stop sco	
ERE1	-742	-724	0.773289
ERE2	-429	-411	0.749287
ERE3	-104	-86	0.75499

## F

С

### NPT4/SLC17A3

	start	stop score	
ERE1	-763	-745	0.78826
ERE2	-648	-630	0.764496
ERE3	- 524	-506	0.740732
ERE4	-493	-475	0.743584
ERE5	- 189	-171	0.743108
ERE6	-171	-153	0.768536

# G

#### ABCG2/ABCG2

	start	stop	score
ERE1	-970	-952	0.762595
ERE2	- 576	-558	0.823907
ERE3	-344	-326	0.757605
ERE4	-93	-75	0.749287

# Η

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	start	stop	score
ERE1	-801	-783	0.792538
ERE2	-759	-741	0.737405
ERE3	-635	-617	0.7 59743
ERE4	-495	-477	0.814639
ERE5	-436	-418	0.748574
ERE6	- 300	-282	0.748574

OAT1/S	LC22A6			
	start	stop	score	
ERE1	-977	-959	0.777329	
ERE2	- 956	-938	0.749287	
ERE3	-837	-819	0.744297	
ERE4	-675	-657	0.792063	
ERE5	- 395	-377	0.750713	
ERE6	-291	-273	0.751188	
ERE7	-261	-243	0.760456	
ERE8	-208	-190	0.780656	

#### OAT3/SLC22A8

	start	stop	score
ERE1	-599	-581	0.7 98717
ERE2	-339	-321	0.7 42871



Α



Proximal tubular cell



В



Α							
			10 2	20 30	40	50	60 70
Ruman mouse	CCTCTGGTGCCCA	CAGACTO TOC	CTTTTTAACIG	TITGIGITCIC CCT.CATTATCG	GCCCTTTCGCCTA TTCTGTCCAGTCA	CAGAATTAGCTA CICCCATGGCT	TCGACACCAAGGGG CCTGGG GACACT
	80	99	100 11		130	140	150
nouse	TTOCCTTTCTCA	CCCTTTCCCCC	CCAGOA GCAGO	ATCT XCANT	AAGTCGAACTGC	TGGAGAGAGAC	T. CCTCGTCAAGC
Ruman mouse	ACCACTAGCTGTT ATTTGCTCTTCTT	GTIGACIGGGAG CTAGAGGGCCAG		AAG.CAGGATTT	ACCCAAGGCCTCG	CAGTGCGTGCTG CAGTGTGGCA	GGTCCAGGGGCAGG GTAGGTGAAAG
	250 26	Q 27Q	280	290	300 310	320	339
Human	GG. GCGGCAGAA	AGACAAG <mark>GG</mark> CAG G <mark>GA</mark> G <mark>A</mark> CA <mark>GG</mark> TCA	GACAGCIGIGO GAGGCGACAGO	GCAGAGCAGAGG	GAGAGAGAAAATCC AAAGGGGGG <mark>AA</mark> GTC	CTCAGAGGCAAA	ATTAGIC GGCTTG
	340 35	ý 360	370	380	390 400	410	420
nouse	GAGAC	ACTAGAGGCAGG	TTGCCAGCCIC	GAACCAAGACGCT GAACTGTGACTC	G TTCCAGGCAG	GACACTGTGACA AGGGCCA <mark>TG</mark> TAA	TTACAGGCTAAAG.
	430 44	Q 45Q	460	470	480 490	500	510
nouse	AAGTGGGGTCCT	AAGACAAGG	GALCCTGGGAAAA	GACAGAGATCTC	AGGGTGGCATTGG	CCALCEGAACC	TGAAGAGTACA.GG
Ruman	520 53		SSQ	560	570 580		
nouse	GGCATATGCTAAT	GICAAAA	ATGAGG . CTC	GTACAGGGAATC	ATAAGG.GAGTGC	AGGAACAGTGGC	AGCITCAAAAGGCT
Ruman	610	620 Bole A CROCERCO	64 Q	65 Q	00000000000000000000000000000000000000	670 670 6	80 690
nouse	AATGAGGCCTAGA	GAC. TGAGTGAC	ACACCATCCTCA	GC AGAGGACGO	CATCACTCA AG	CTAGGAT	CAGGCCAGCTCTGA
	700	710	720 7	730 740	750	760	770 780
Runan	GACTIGCACAGCI GGITGGGAGGACC	AGCCCCAGCCC	TCTCTCCTGGG.	ATCCCCTGGCCCAC	CTTCTCTTGGCTC AGTACAGTCATGA	AGCCACICIGG TGIC.TCCIGGA	AGGIGGGCACACAG
	790	800	810 6	820 830	840	850	860 870
Human mouse	GGGCACCGAAGGG Gagcaccgaagga	AGCAGGCAGCCC	IGGCAICCACAC CAACA.CACTIAC	CCGCCAGGAGAA TG <mark>GC.AG</mark> AGCCCC	AACTT <mark>AG</mark> GC <mark>CT</mark> CC STGTCC <mark>AG</mark> AT <mark>CT</mark> TG	CCAAGACCIGGC CCGCATCCIAAC	ACAGAGCAGGCCGG AGAAACCAGGCCAA
	889	999	900	910 93	20 930	949	950
Runan nouse	CCCTCGCCCCAA TGAACATGGGGGC	GCAGAGGAGGCT GGGGTGGTGGGA	CACTCCC.TC	GCGTCTGTGCCTG TCATCTGCAGTGC	CCTCAACGCGGGT IGTTGIC.CIGGT	TAAACTTTGACC TAAACTTTGA	AAGGAAATGATTGC
	960 970	980	999	1000			
Bunan nouse	TAAACTCGATTCC TAAACCCTCTTCT	AT AAGTGICAC	C.GGTCACACTT?	AATTCC	GTGACTTCTCTCT	CCACCCTCCTTC	ciicic

# В

### Mouse Urat1/SIc22a12

	start	stop	score
ERE1	-922	-904	0.730751
ERE2	-724	-706	0.76212
ERE3	-570	-553	0.758793
ERE4	-490	-478	0.792776
ERE5	-402	-384	0.746673
ERE6	-294	-277	0.802994