

## 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

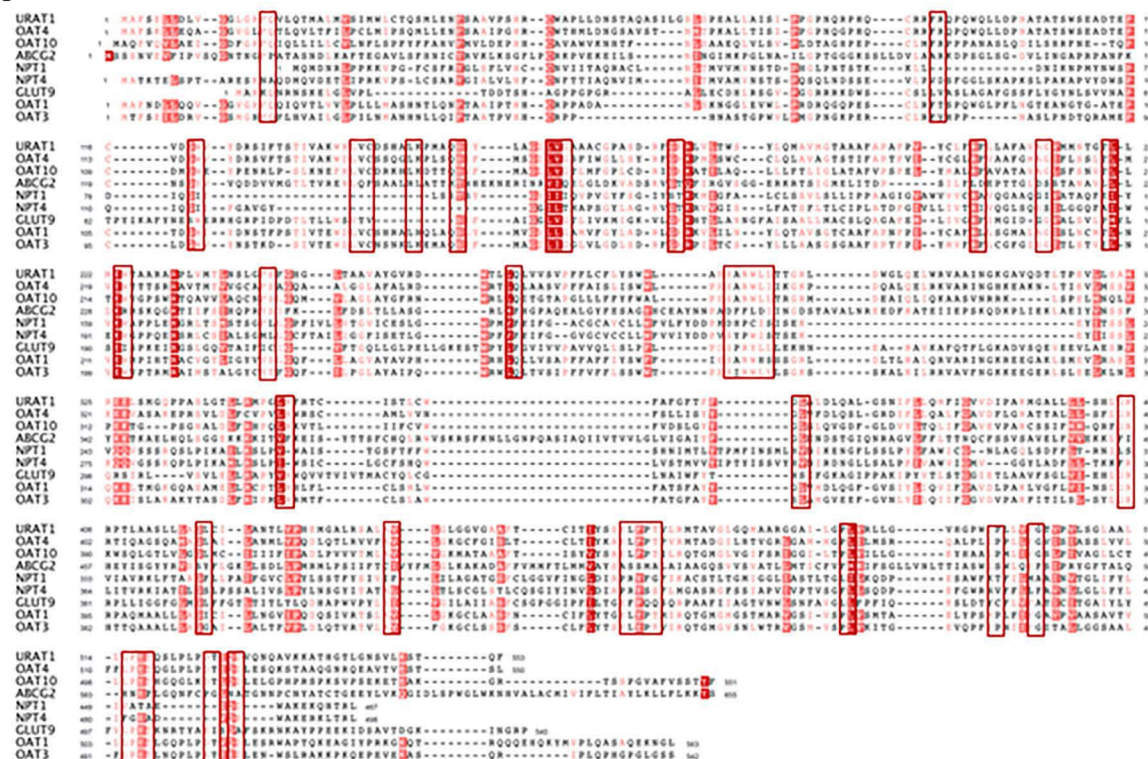
gene promoter regions was made with Clustal. Red shades show the complete conserved sequences. Blue boxes show the three-quarters match. (B-J) Positions and scores of potential ERE sequences in uric acid transporter gene promoter regions; (B) URAT1/*SLC22A12*, (C) OAT4/*SLC22A11*, (D) OAT10/*SLC22A13*,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

**Figure S4. *ESR1* (ER $\alpha$ ) and *ESR2* (ER $\beta$ ) 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 (<https://www.proteinatlas.org/ENSG00000091831-ESR1/tissue>). 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 (<https://www.proteinatlas.org/ENSG00000091831-ESR1/tissue/kidney>). 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.

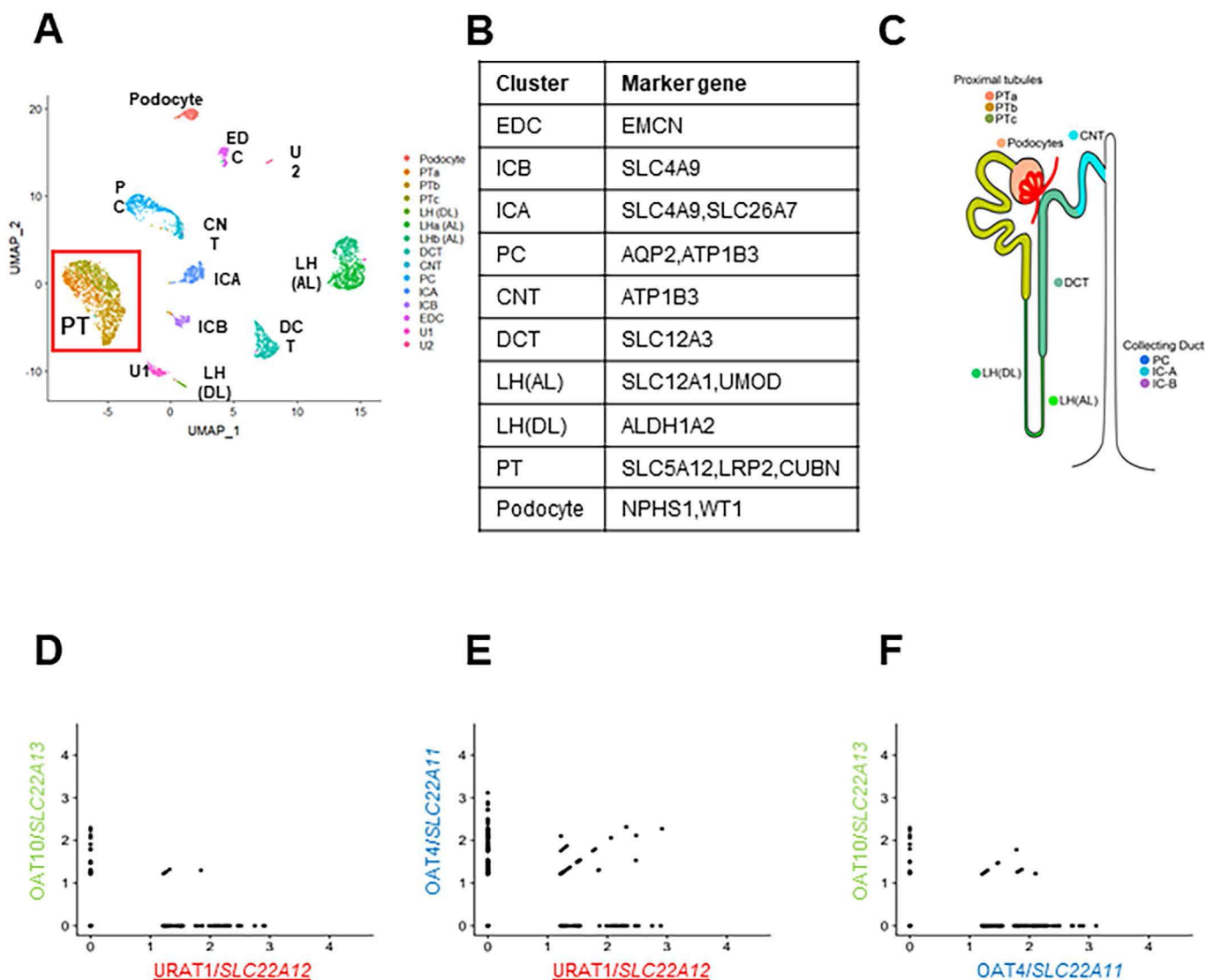
**Figure S6. Information on human *SLC22A12* and mouse *Slc22a12* gene promoter sequences.** (A) The alignment of human *SLC22A12* and mouse *SLC22A12* gene promoter regions were made with Clustal. Red shades show the complete conserved sequences. (B) Positions and scores of potential ERE sequences in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

A



B

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





**B** 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

**C** 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** OAT10/SLC22A13

	start	stop	score
ERE1	-662	-644	0.743108

**E** NPT1/SLC17A1

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

**F** 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

**H** GLUT9/SLC2A9

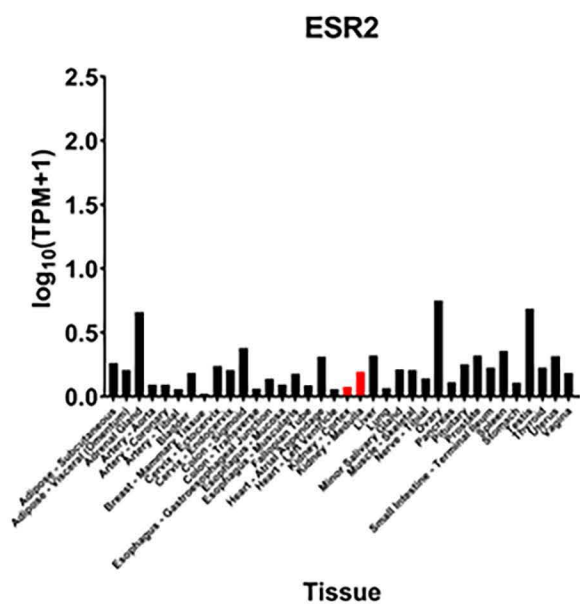
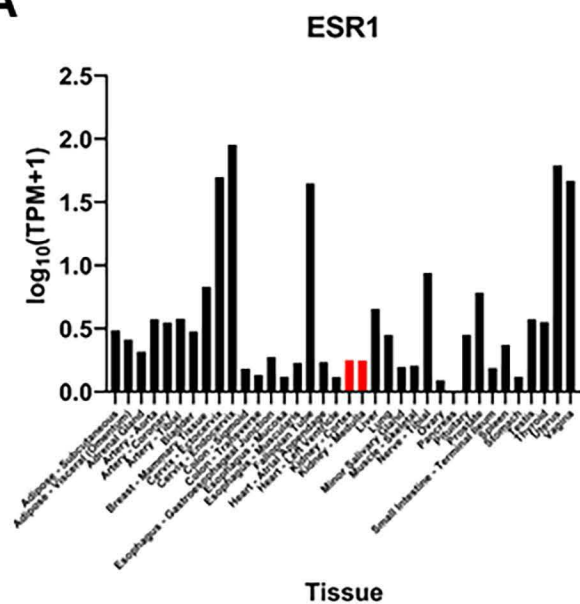
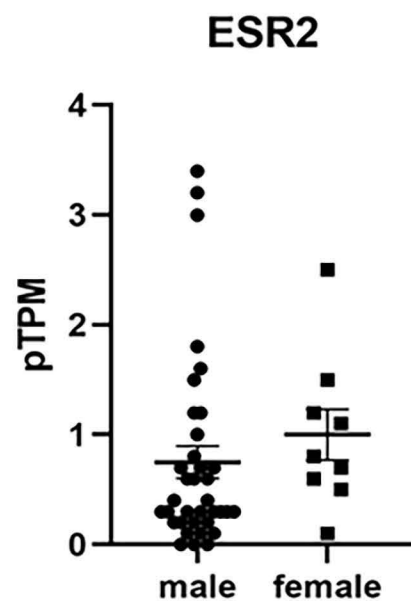
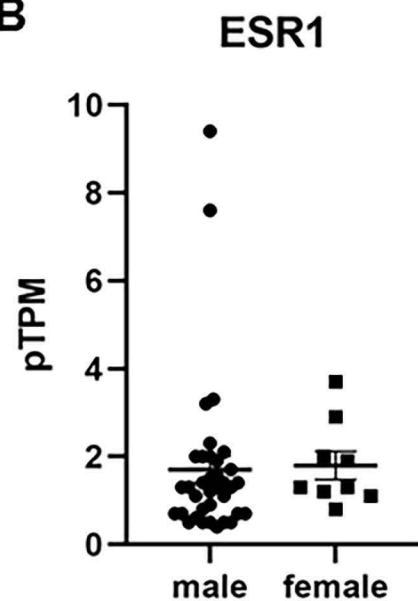
	start	stop	score
ERE1	-801	-783	0.792538
ERE2	-759	-741	0.737405
ERE3	-635	-617	0.759743
ERE4	-495	-477	0.814639
ERE5	-436	-418	0.748574
ERE6	-300	-282	0.748574

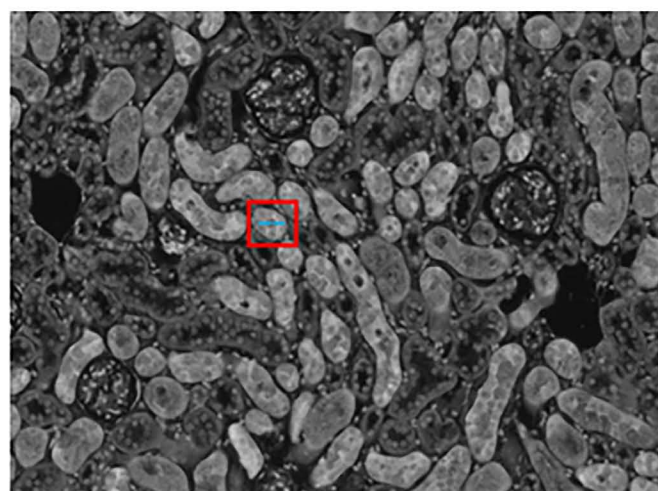
**I** OAT1/SLC22A6

	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

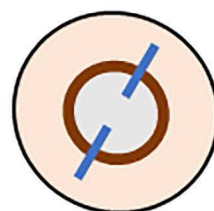
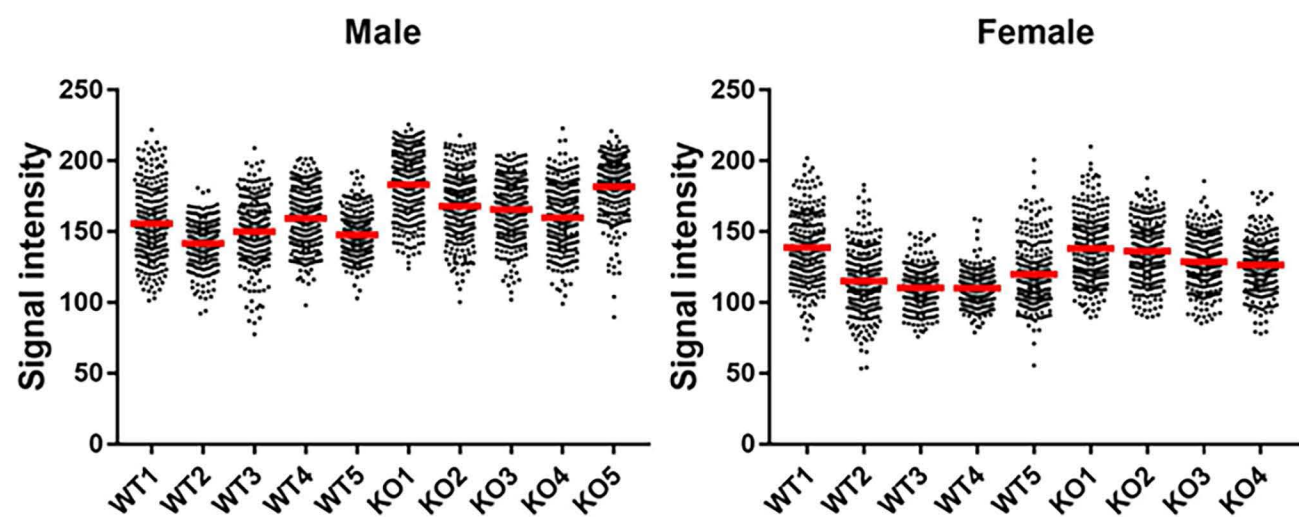
**J** OAT3/SLC22A8

	start	stop	score
ERE1	-599	-581	0.798717
ERE2	-339	-321	0.742871

**A****B**

**A**

Proximal tubular cell

**B**

A



B

Mouse Urat1/Slc22a12

	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