

## SUPPLEMENTARY INFORMATION

**Inhibitory effect of *Citrus* flavonoids on the *in vitro* transport activity of human urate transporter 1 (URAT1/SLC22A12), a renal re-absorber of urate**

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**Supplementary Table 1**

**Supplementary Figures 1–3**

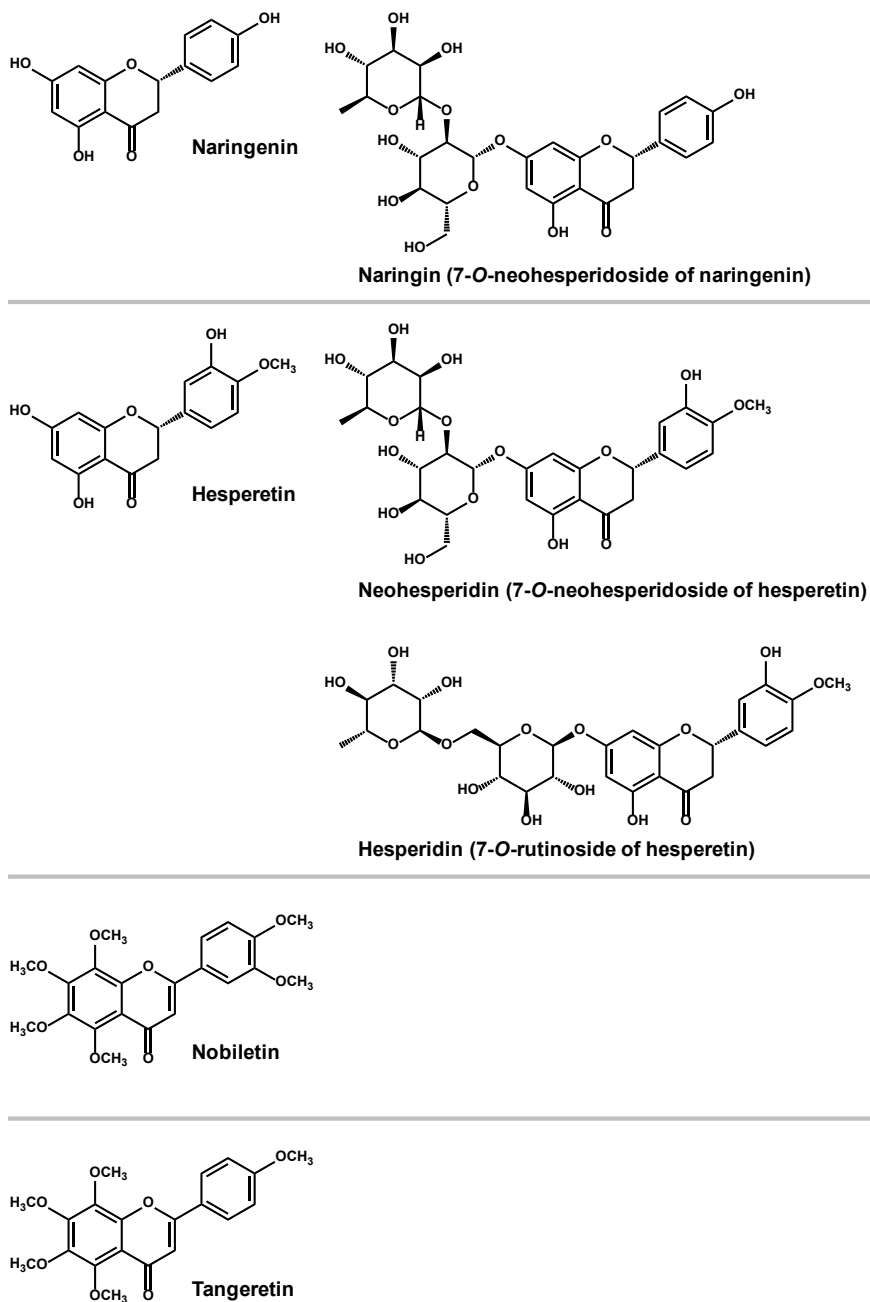
**Supplementary References**

**Supplementary Table 1** Key resources.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-EGFP	Life Technologies	Cat# A11122; RRID: AB_221569
Rabbit polyclonal anti- $\alpha$ -tubulin	Abcam	Cat# ab15246; RRID: AB_301787
Donkey anti-rabbit IgG-horseradish peroxidase (HRP)-conjugate	GE Healthcare	Cat# NA934V; RRID: AB_772206
Chemicals		
[8- <sup>14</sup> C]-uric acid (53 mCi/mmol)	American Radiolabeled Chemicals	Cat# ARC0513
Benzbromarone	FUJIFILM Wako Pure Chemical Corporation	Cat# 028-1585; CAS: 3562-84-3; Purity: >98%
Clear-sol II	Nacalai Tesque	Cat# 09136-83
Dimethyl Sulfoxide	Nacalai Tesque	Cat# 13445-74; CAS: 67-68-5
Hesperetin	FUJIFILM Wako Pure Chemical Corporation	Cat# 320-93841; CAS: 520-33-2; Purity: >96%
Hesperidin	FUJIFILM Wako Pure Chemical Corporation	Cat# 088-07341; CAS: 520-26-3; Purity: >95%
Naringenin	Combi-Blocks	Cat# QA-3717; CAS: 480-41-1; Purity: >98%
Naringin	ChromaDex	Cat# ASB-00014222-001; CAS: 10236-47-2; Purity: not available
Neohesperidin	Combi-Blocks	Cat# QV-2102; CAS: 13241-33-3; Purity: >98%
Nobiletin	FUJIFILM Wako Pure Chemical Corporation	Cat# 149-09341; CAS: 478-01-3; Purity: >98%
Polyethelenimine "MAX" (PEI-MAX)	Polysciences	Cat# 24765; CAS: 49553-93-7
Tangeretin	FUJIFILM Wako Pure Chemical Corporation	Cat# 208-15671; CAS: 481-53-8; Purity: >95%
Critical Commercial Assays		
Pierce <sup>TM</sup> BCA Protein Assay Reagent A	Thermo Fisher Scientific	Cat# 23223
Pierce <sup>TM</sup> BCA Protein Assay Reagent B	Thermo Fisher Scientific	Cat# 23224
PureLink <sup>TM</sup> HiPure Plasmid Filter Midiprep Kit	Thermo Fisher Scientific	Cat# K210015
Recombinant DNA		
The complete human URAT1 cDNA	Miyata et al, 2016 <sup>1</sup>	NCBI Reference Sequence: NM_144585.3
Experimental Models: Cell Lines		
293A	Invitrogen	R70507
Software and Algorithms		
Excel 2013	Microsoft	<a href="https://products.office.com/ja-jp/microsoft-excel-2013">https://products.office.com/ja-jp/microsoft-excel-2013</a>
Statcel3 add-in software	OMS Publishing	<a href="http://www.oms-publ.co.jp/">http://www.oms-publ.co.jp/</a>

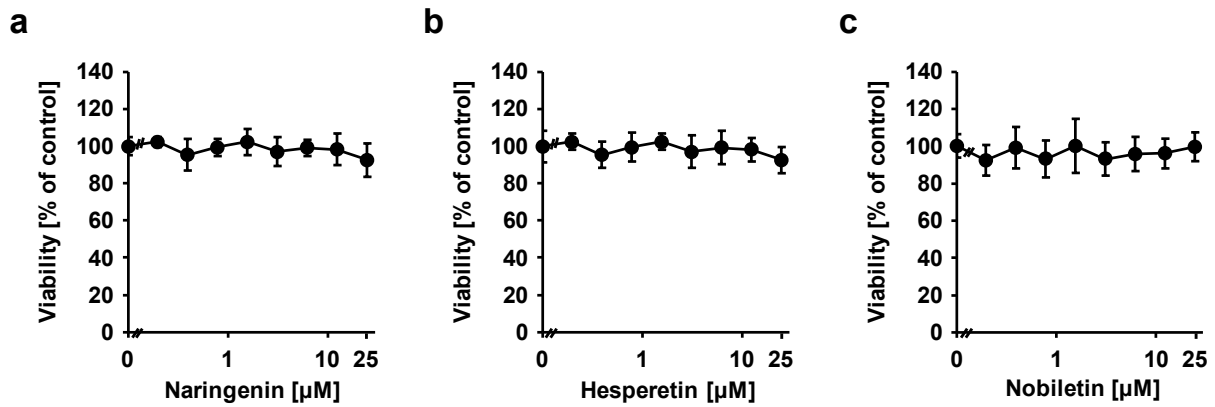
Information on purity was from data sheets of each chemical provided by suppliers.

## Supplementary Figure 1



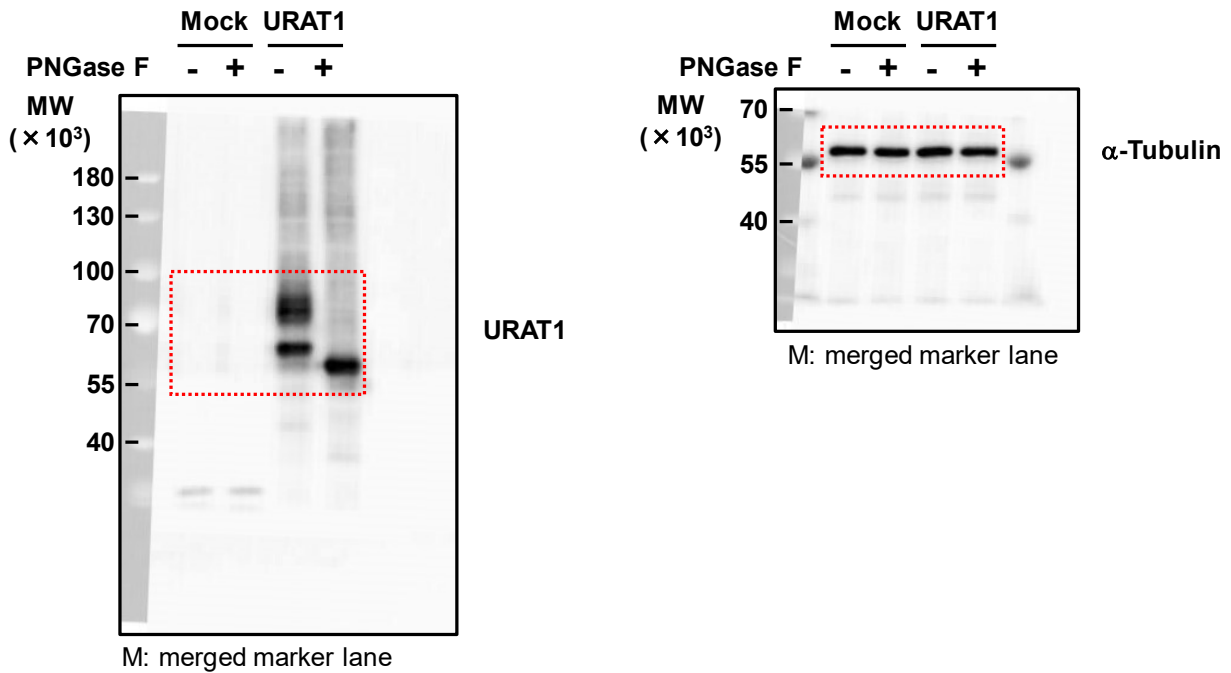
**Supplementary Figure 1 Chemical structures of seven *Citrus* flavonoids evaluated in the present study.** According to a previous study,<sup>2</sup> the values of the half maximal inhibitory concentration against xanthine oxidase of each *Citrus* flavonoid are 16.5  $\mu\text{M}$  (hesperetin), 107.5  $\mu\text{M}$  (nobiletin), about 200  $\mu\text{M}$  (naringenin) and >200  $\mu\text{M}$  (naringin, neohesperidin, hesperidin, and tangeretin), respectively.

## Supplementary Figure 2



**Supplementary Figure 2 Effect of *Citrus* flavonoids on the viability of 293A cells in long term culture.** 293A cells were incubated with each flavonoid at indicated concentrations for 48 h. The cell viability (vs. vehicle control) was measured by WST-8 assay. Under these experimental conditions, all tested flavonoids had little effect on cytotoxicity, suggesting that these flavonoids could be safe for 293A cells at concentrations of under 25  $\mu\text{M}$  (maximum concentration in this assay). Data are expressed as mean  $\pm$  SD.  $n = 8$ .

### Supplementary Figure 3



### Supplementary Figure 3. Uncropped images of Figure 1a.

Full images of immunoblotting of EGFP-tagged URAT1 (*left*) and  $\alpha$ -tubulin (*right*: membrane was sliced before detection based on the molecular size of interested proteins). Blots in each cropped area indicated by red dashed box derived from the same experiment.

### Supplementary References

1. Miyata, H. *et al.* Identification of Febuxostat as a New Strong ABCG2 Inhibitor: Potential Applications and Risks in Clinical Situations. *Front. Pharmacol.* **7**, 518 (2016).
2. Liu, K. *et al.* Chemical Evidence for Potent Xanthine Oxidase Inhibitory Activity of Ethyl Acetate Extract of *Citrus aurantium* L. Dried Immature Fruits. *Molecules* **21**, 302 (2016).