

A

	Ouabain #1	Ouabain #2
THADA probe 1	-9.188	-6.852
THADA probe 2	-47.110	-7.035

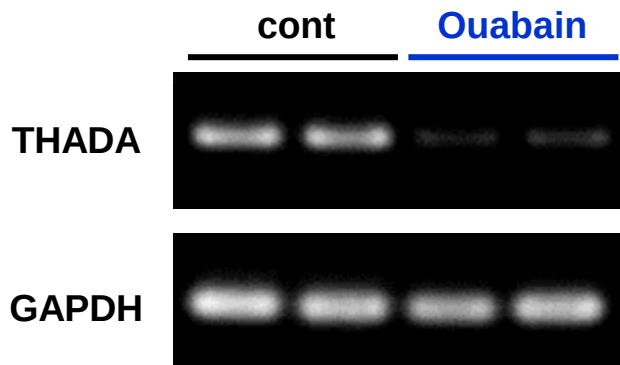
B

Fig. S1 Decrease in expression of THADA mRNA by ouabain. A Change in expression of THADA in HepG2 cells treated with ouabain (1 μ M for 12 h). In microarray gene expression analysis, the expression of THADA was assessed by two probes. The values indicate negative fold change of ouabain-treated samples compared with the ouabain-untreated cells. B RT-PCR images of THADA and GAPDH in HepG2 cells. Cells were cultured in the presence and absence of ouabain (1 μ M) for 24 h.

Figure S2

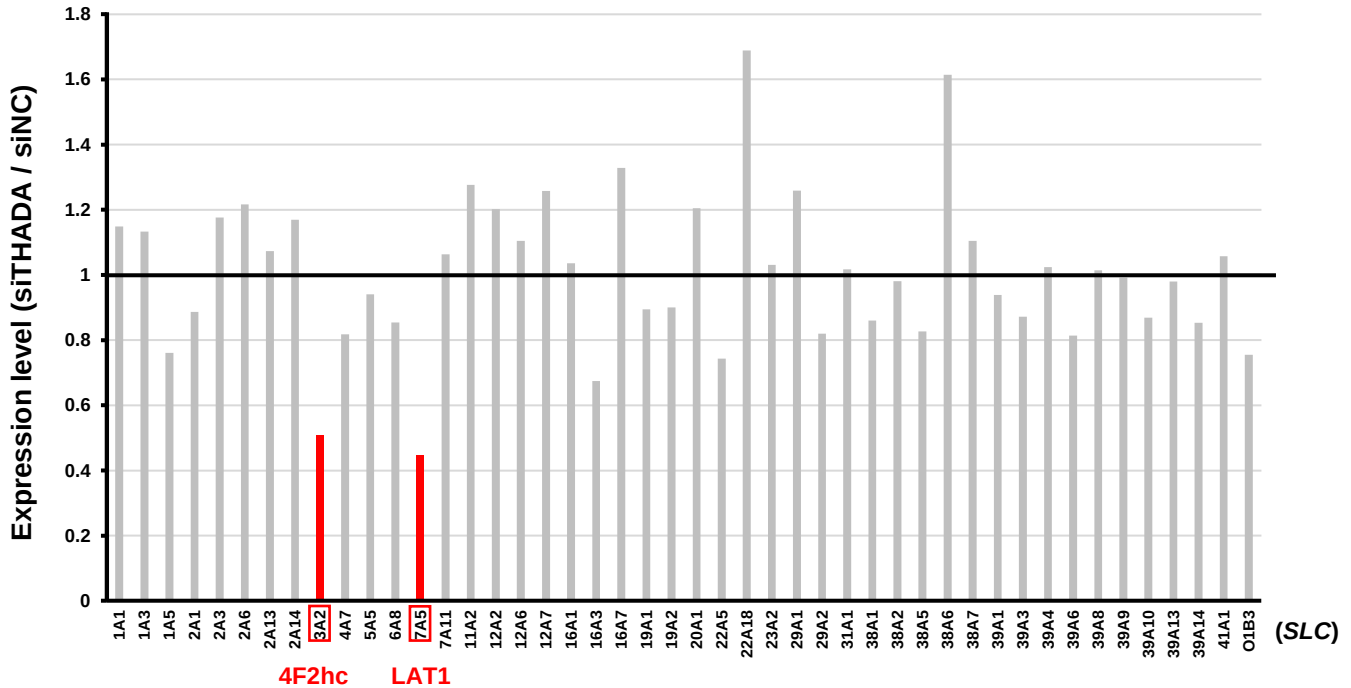


Fig. S2 Change in expression of SLC transporters involved in nutrient uptake by THADA-knockdown. KB cells were treated with THADA siRNA (siTHADA) or negative control siRNA (siNC) for 72 h, and then total RNA samples were prepared. Microarray gene expression analysis was performed using these samples. In the graph, relative expression levels of the SLC transporters (siTHADA/siNC) were shown.

Figure S3

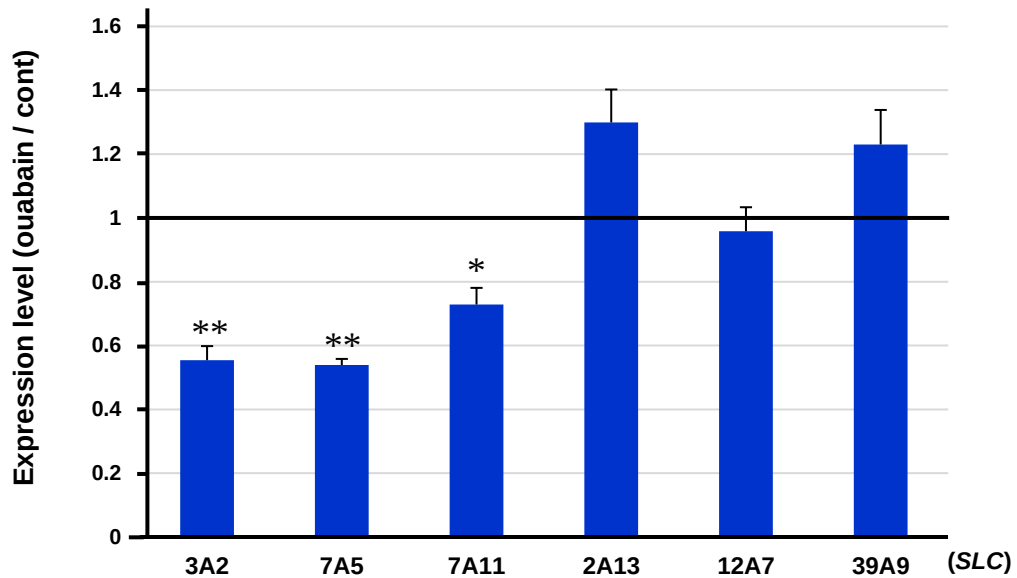


Fig. S3 Change in expression of SLC transporters (picked up from Fig. S2) in the ouabain-treated KB cells. KB cells were treated with or without (control) ouabain (100 nM) for 48 h, and then total RNA samples were prepared. Real-time PCR was performed using these samples. Expression levels of SLC transporters were normalized by corresponding GAPDH expression, and the quantitative data were shown. $n = 3-4$. * $P < 0.05$ and ** $P < 0.01$ versus control cells.

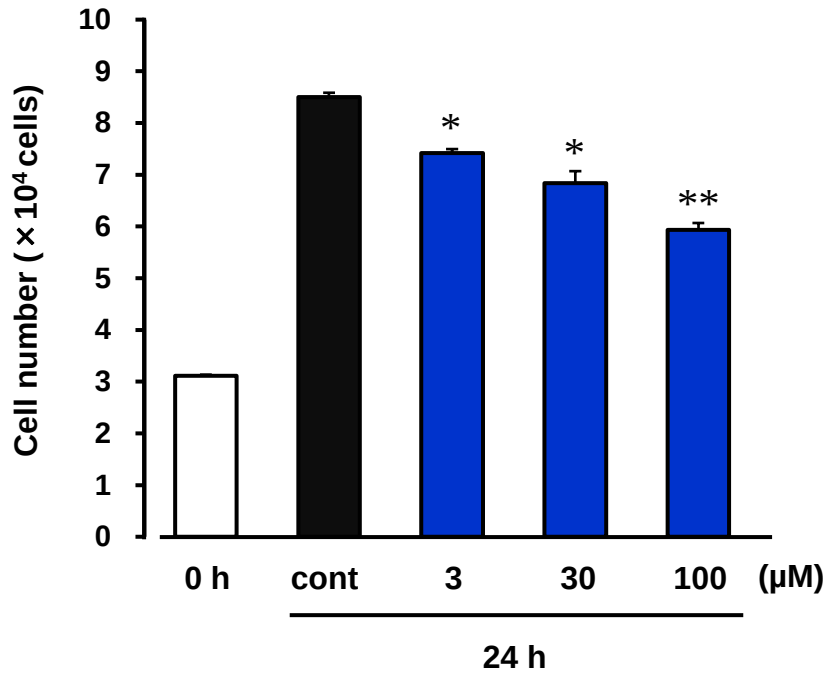


Fig. S4 Inhibition of cancer cell proliferation by JPH203, a LAT1 inhibitor. KB cells (3×10^4 cells) were treated with 3-100 μ M JPH203 for 24 h. As a control, the cells were cultured without JPH203 (cont). The cell number was counted just after (0 h) and 24 h after the treatment. $n = 3$. * $P < 0.05$ and ** $P < 0.01$ versus control (cont).

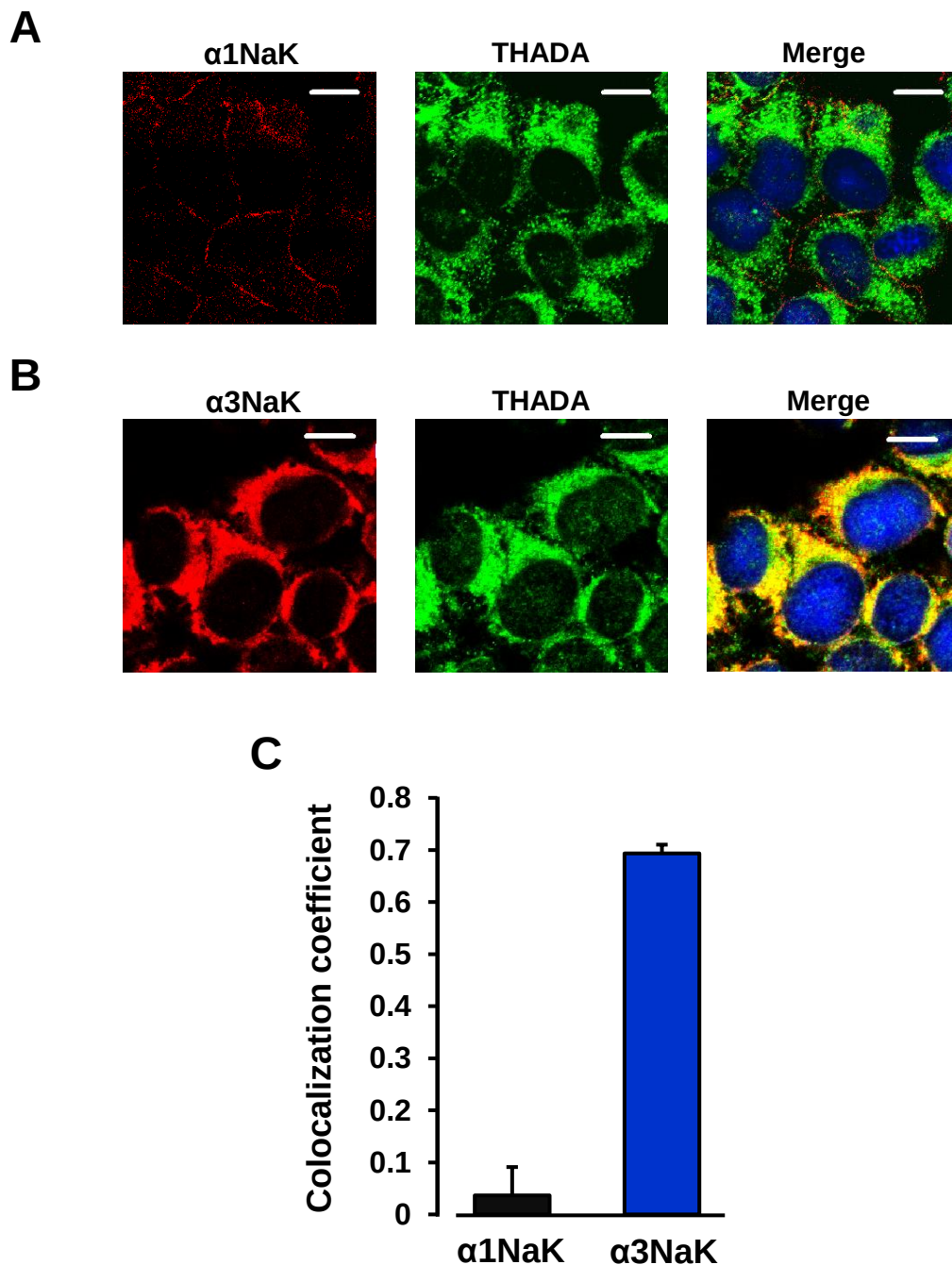


Fig. S5 Localization of Na⁺,K⁺-ATPases and THADA in KB cells. A Immunocytochemistry using anti-Na⁺,K⁺-ATPase $\alpha 1$ -isoform ($\alpha 1\text{NaK}$) (red) and anti-THADA (green) antibodies in the cells. DNA in the nucleus was visualized with DAPI (blue). Scale bars, 10 μm .

B Immunocytochemistry using anti-Na⁺,K⁺-ATPase $\alpha 3$ -isoform ($\alpha 3\text{NaK}$) (red) and anti-THADA (green) antibodies in the cells. DNA in the nucleus was visualized with DAPI (blue). Scale bars, 10 μm .

C Colocalization coefficient of THADA with $\alpha 1\text{NaK}$ or $\alpha 3\text{NaK}$ was calculated with Pearson correlation coefficient analysis. n = 11-13.