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**A novel role for OATP2A1/*SLCO2A1* in a murine model of colon cancer**

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**Supplementary Figures**

1 **Supplementary Figure S1**

2

3 **EP4 expression in polyps in the small intestine**

4 **Method**

5 Tissue samples were excised, and then fixed with 4 % paraformaldehyde. Briefly, for light-microscopic  
6 analysis, paraffin-embedded sections were incubated with rabbit anti-mouse EP4 IgG (1:10 dilution,  
7 overnight at 4°C, Cayman Chemical, Ann Arbor, MI), and followed by horseradish peroxidase-conjugated  
8 secondary antibodies (1: 100-200 dilution). DAB stain was developed with 3,3'-diaminobenzidine (Nacalai  
9 Tesque, Kyoto, Japan). The sections were observed with a light microscopy (Nikon E200, Tokyo, Japan)

10

11 **Result**

12 EP4 expression was  
13 determined by means  
14 immunohistochemistry in  
15 polyps of the small  
16 intestines from *Slco2a1*<sup>+/+</sup>  
17 and *Slco2a1*<sup>-/-</sup>/*Apc*<sup>Δ716/+</sup>.

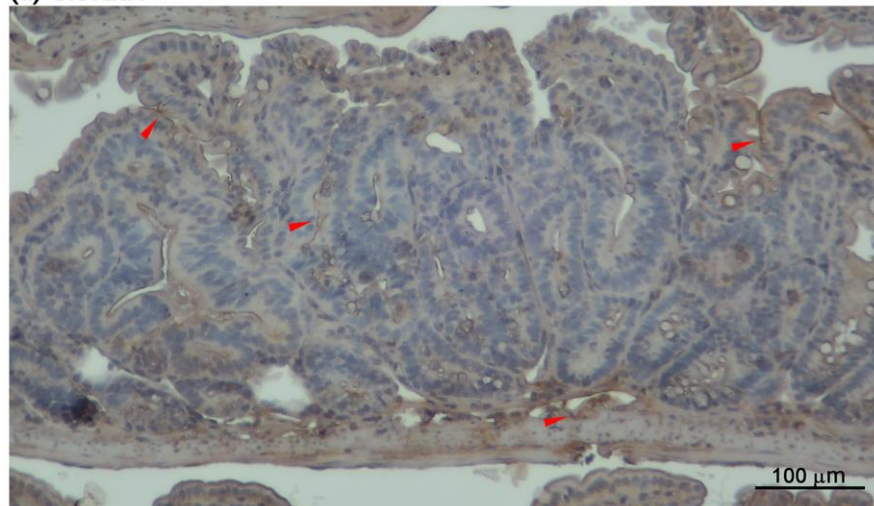
18 The picture shown as  
19 Supplementary Figure S1  
20 represents the at least  
21 three individual animals  
22 in each group. DAB stain  
23 was clearly developed in  
24 the epithelial cells and  
25 some stromal cell, and  
26 blood vessels. Apparently  
27 significant changes were  
28 unlikely observed  
29 between *Slco2a1*<sup>+/+</sup> and  
30 *Slco2a1*<sup>-/-</sup>/*Apc*<sup>Δ716/+</sup> mice.

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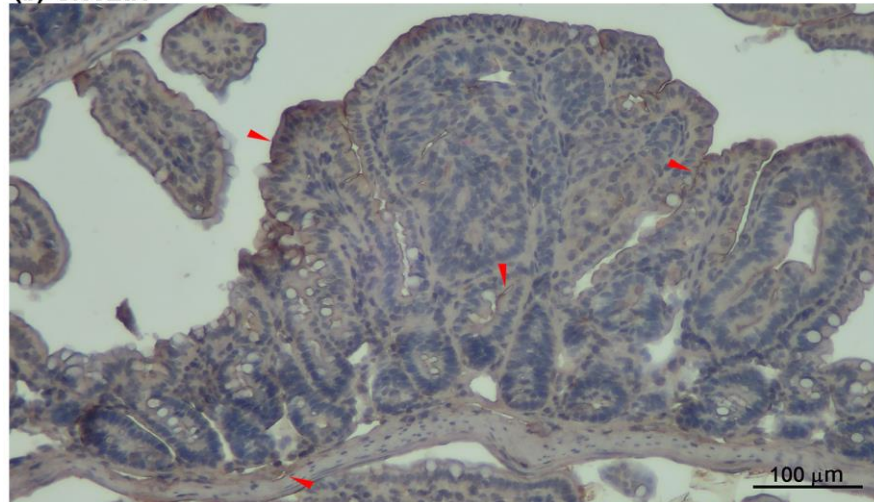
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**Supplementary Figure S1**

(a) *Slco2a1*<sup>+/+</sup>



(b) *Slco2a1*<sup>-/-</sup>



1 **Supplementary Figure S2**

2

3 **Analysis of *SLCO2A1* expression in human colon cancer patients**

4 To assess any association of *SLCO2A1* expression with colorectal cancer survival, the Oncomine gene  
5 expression database was interrogated for mRNA levels of *SLCO2A1* related to colon cancer patient  
6 outcome using datasets from published studies (1). The one year survival data from the Cancer Gene Atlas  
7 (TCGA) was analysed by an unpaired t-test using Welch's correction. The survival data was analysed by  
8 the Mantel-Cox log-rank test.

9

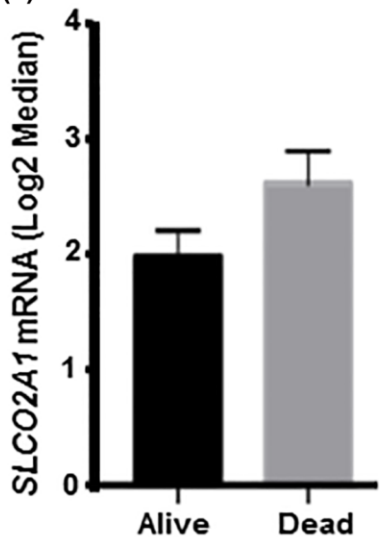
10 **Result**

11 Using publically available Oncomine database (1), we compared patients with colon adenocarcinoma  
12 where tumour *SLCO2A1* mRNA expression and survival data were available (**Supplementary Fig. S2**).  
13 Only 25 cases with one year survival data (**Supplementary Fig. S2a**) and 20 cases with overall survival  
14 data (**Supplementary Fig. S2b**) were found that also had tumour *SLCO2A1* mRNA expression data.  
15 Although not reaching statistical significance, an association was noted between higher expression of  
16 *SLCO2A1* mRNA in tumour tissue and shortened survival ( $p = 0.14$ , **Supplementary Fig. S2b**). These  
17 observations led us to test the hypothesis that *SLCO2A1* expression impacts colon cancer disease  
18 progression in a mouse colon cancer model.

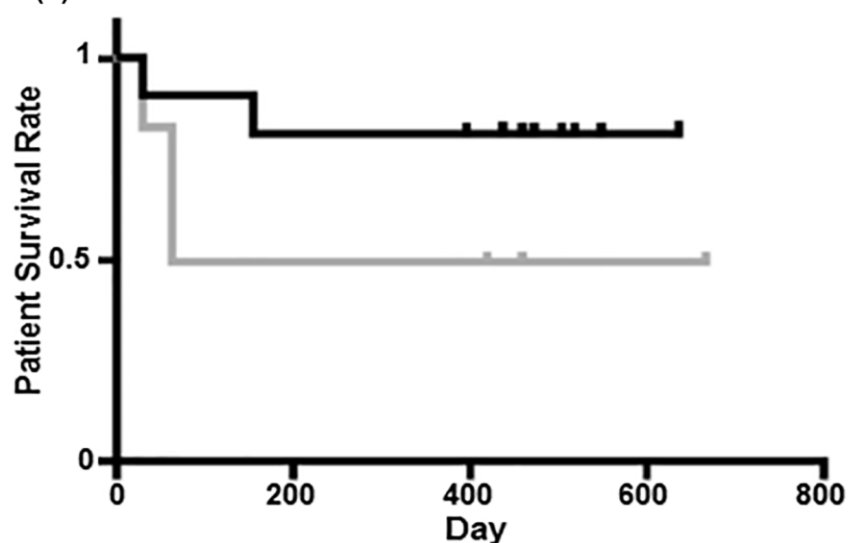
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**Supplementary Figure S2**

(a) *SLCO2A1* mRNA



(b) Patient Survival



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1 **Legend for Supplementary Figure S2**

2 ***SLCO2A1* expression affects colon adenocarcinoma patients**

3 (a) *SLCO2A1* mRNA expression in colon adenocarcinoma patients who survived (black, n = 17) and died  
4 (grey, n = 8) one year according to the Cancer Genome Atlas (p = 0.1). Data was obtained from Oncomine  
5 data base (1). (b) Survival curves of colon adenocarcinoma patients with higher (grey, n = 9) and lower  
6 (solid, n = 11) *SLCO2A1* mRNA expression than median value (p = 0.14).

7

8 **Reference**

- 9 1. Rhodes DR, Kalyana-Sundaram S, Mahavisno V, Varambally R, Yu J, Briggs BB, et al. Oncomine 3.0:  
10 genes, pathways, and networks in a collection of 18,000 cancer gene expression profiles. *Neoplasia*  
11 **2007**;9:166-180.