Supplemental data to:

Establishment and characterization of a novel Caco-2 subclone with a similar low expression level of human carboxylesterase 1 to human small intestine

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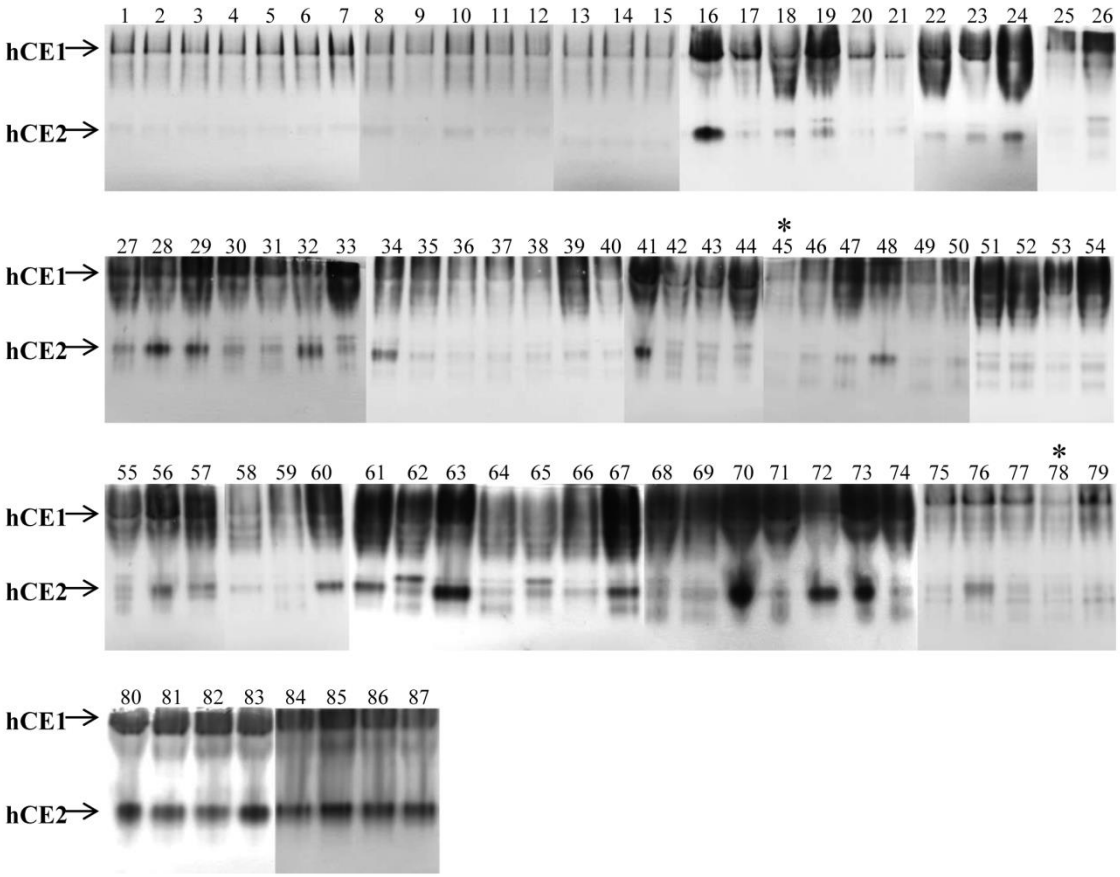
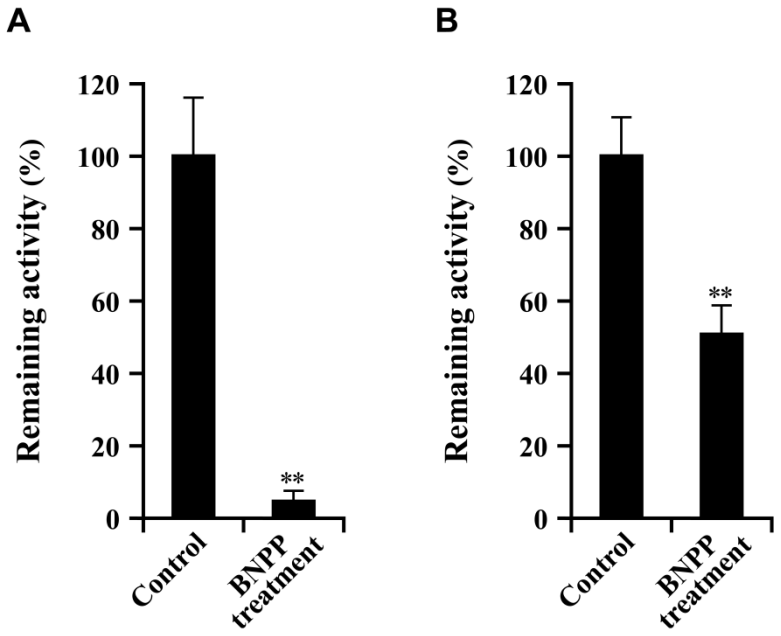


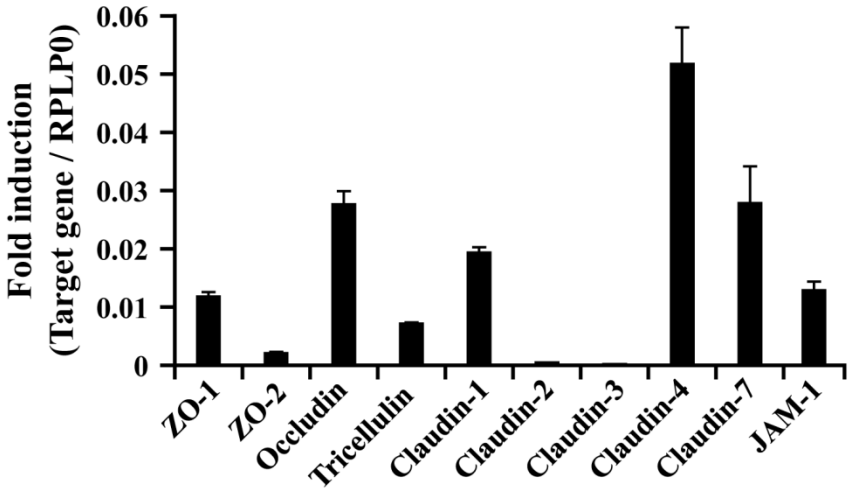
Supplemental Figure 1. Expression of hCE1 and hCE2 in Caco-2 subclones

Esterase activity staining after native PAGE using α-naphthylacetate. Homogenates of the subclones (#1–#15, 10 μg; #16–#87, 50 μg) were loaded onto each lane. Arrows indicate bands corresponding to each CES isozyme. * denotes the selected subclones.



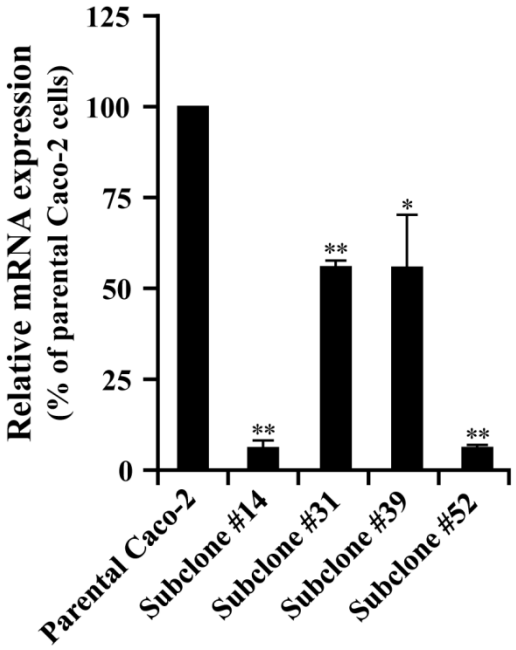
Supplemental Figure 2. Hydrolysis of oseltamivir (A) and PNPA (B) in S9 fractions of Caco-2 cells treated with BNPP

S9 fractions were prepared from Caco-2 cell monolayers treated with HBSS (control) or 200 μM BNPP. S9 fractions were diluted with 50 mM HEPES buffer (pH 7.4) at 100 μg/mL. Substrates (concentration of oseltamivir and PNPA: 500 μM) were incubated with the diluted S9 fractions. Each column represents the mean ± S.D. (n = 3). "**" indicates $p<0.01$ in comparison with control.



Supplemental Figure 3. Expression profile of tight junction components in parental Caco-2 cells

The mRNA expression levels of tight junction components were determined by real-time quantitative PCR and normalized to the expression level of RPLP0 mRNA. Values are means ± S.D. (n = 3).



Supplemental Figure 4. Relative expression levels of ZO-1 in Caco-2 subclones

The mRNA expression levels in subclone #14, #31, #39 and #52, which showed slower growth than parental Caco-2 cells, were determined by real-time quantitative PCR. The fold induction of mRNA level of ZO-1 normalized to the RPLP0 mRNA level in parental Caco-2 cells (control) is set as 100%, and that in each subclone is shown relative to control. Values are means ± S.D. (n = 3). ** indicates $p<0.01$; * indicates $p<0.05$, both in comparison with parental Caco-2 cells.

Supplemental Table 1. Effect of 5-azacytidine and sodium butyrate on the mRNA expression of human CES isozymes in subclone #78

CES isozymes	5-Azacytidine		Sodium butyrate	
	Control (DMSO 0.1%)	5 μM	Control (H_2O 1%)	5 mM
	(% of average expression level of control)			
CES1A1	100 ± 16.9	93.9 ± 33.2	100 ± 36.7	94.0 ± 22.9
CES2A1	100 ± 21.1	83.3 ± 30.5	100 ± 16.2	86.6 ± 22.0

The mRNA expression levels were determined by real-time quantitative PCR and normalized to the expression level of β-actin mRNA. The average relative mRNA expression level in the control was set as 100%. Values represent the mean ± S.D. (n = 3). There were no significant differences in the expression of either CES isozyme between controls and cells treated with gene inducers.