Title: Optimization of Canalicular ABC Transporter Function in HuH-7 Cells by Modification of Culture Conditions

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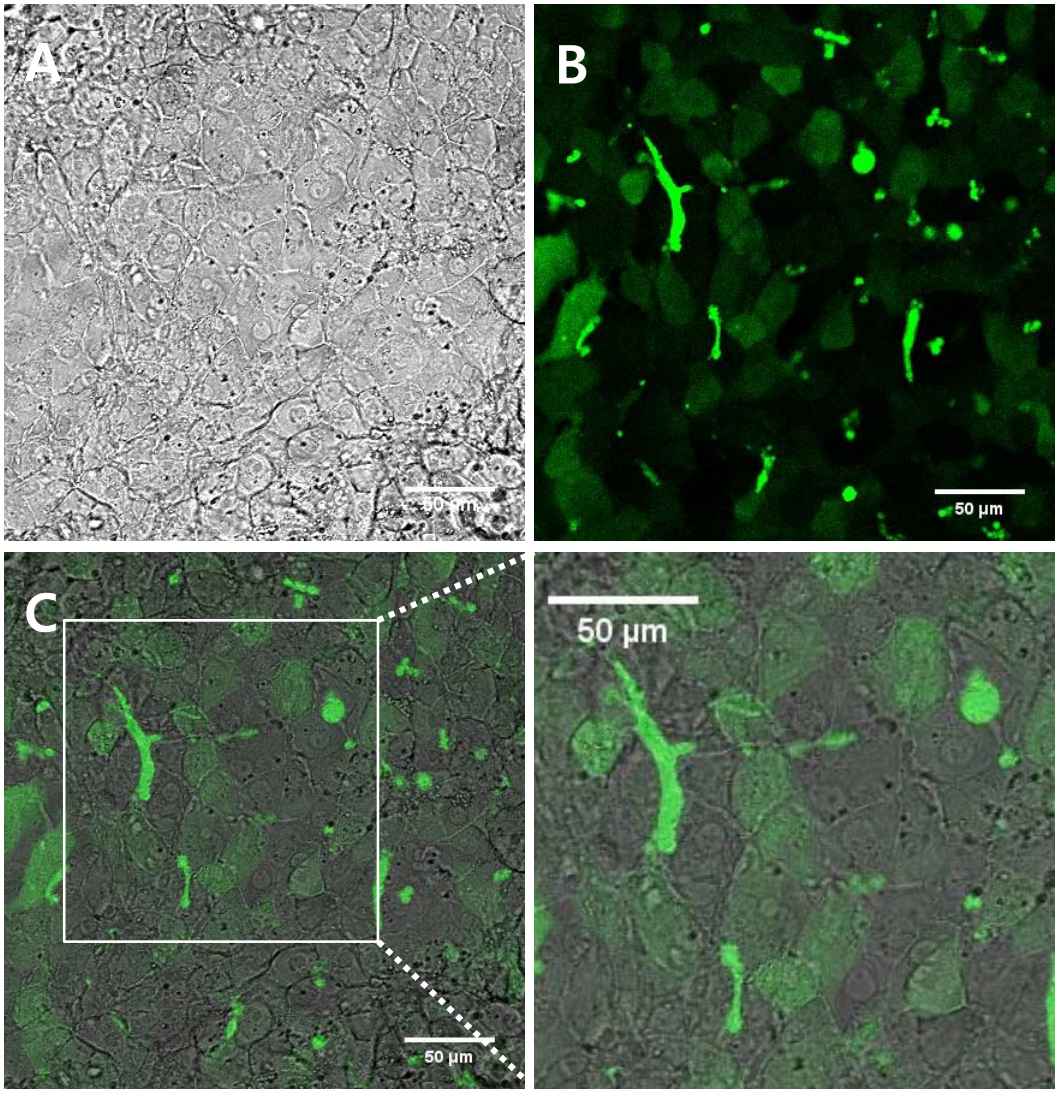
Supplemental figure legends:

Supplemental Fig. 1. Bile canaliculi-like structures of confluent 4-week HuH-7 cultures overlaid with Matrigel extracellular matrix. (A) Differential interference contrast (DIC) image, (B) accumulation of fluorescent canalicular marker, 5(6)-carboxy-2',7'-dichlorofluorescein (CDF, green), and (C) their merged image visualizing cellular localization of CDF accumulation in 4-week HuH-7 cultures with Matrigel overlay (25X magnification). Appearance of bile canaliculi is associated with decreased intracellular CDF accumulation, whereas a few cells that were not surrounded by canaliculi-like structures showed substantial CDF accumulation in their cytoplasmic compartment.

Supplemental Fig. 2. Effect of dexamethasone (DEX) on the expression of BSEP in standard HuH-7 cultures. (A) Confluent HuH-7 cells were cultured without Matrigel for 4 weeks and exposed to indicated concentrations of DEX for the last 3 weeks. BSEP expression in membrane extracts of HuH-7 cells (15 μg protein) was analyzed by Western blotting. Na^+/K^+ ATPase was used as a loading control. (B) Corresponding densitometric analysis of BSEP expression. Each value represents the mean ± SD ($n = 3$, each). $^{***}p < 0.001$ significantly different from the cultures without DEX (one-way ANOVA with Tukey's test).

Supplemental Fig. 3. Effect of Matrigel lots and phenol red on TCA canalicular excretion in 4-week HuH-7 cultures supplemented with 1 μM dexamethasone (DEX). Confluent HuH-7 cells were cultured for 4 weeks, supplemented with 1 μM of DEX for the last 3 weeks, and overlaid with two different lots of Matrigel (Matrigel™ 354234; Lot1:5008302, Lot2:8274014) on day 22. For without phenol red condition, cells were grown with media with phenol red for the first 3 weeks, and then switched to phenol red-free media for the last 1 week. Cells were pre-incubated for 10 min in standard or Ca^{2+}-free HBSS and then incubated with 2 μM [^{3}H]-TCA (200 nCi/ml) in standard HBSS for 10 min. Results represent mean ± SD of triplicate determinations. Biliary excretion index (BEI, %) was calculated as described in the Materials and Methods.



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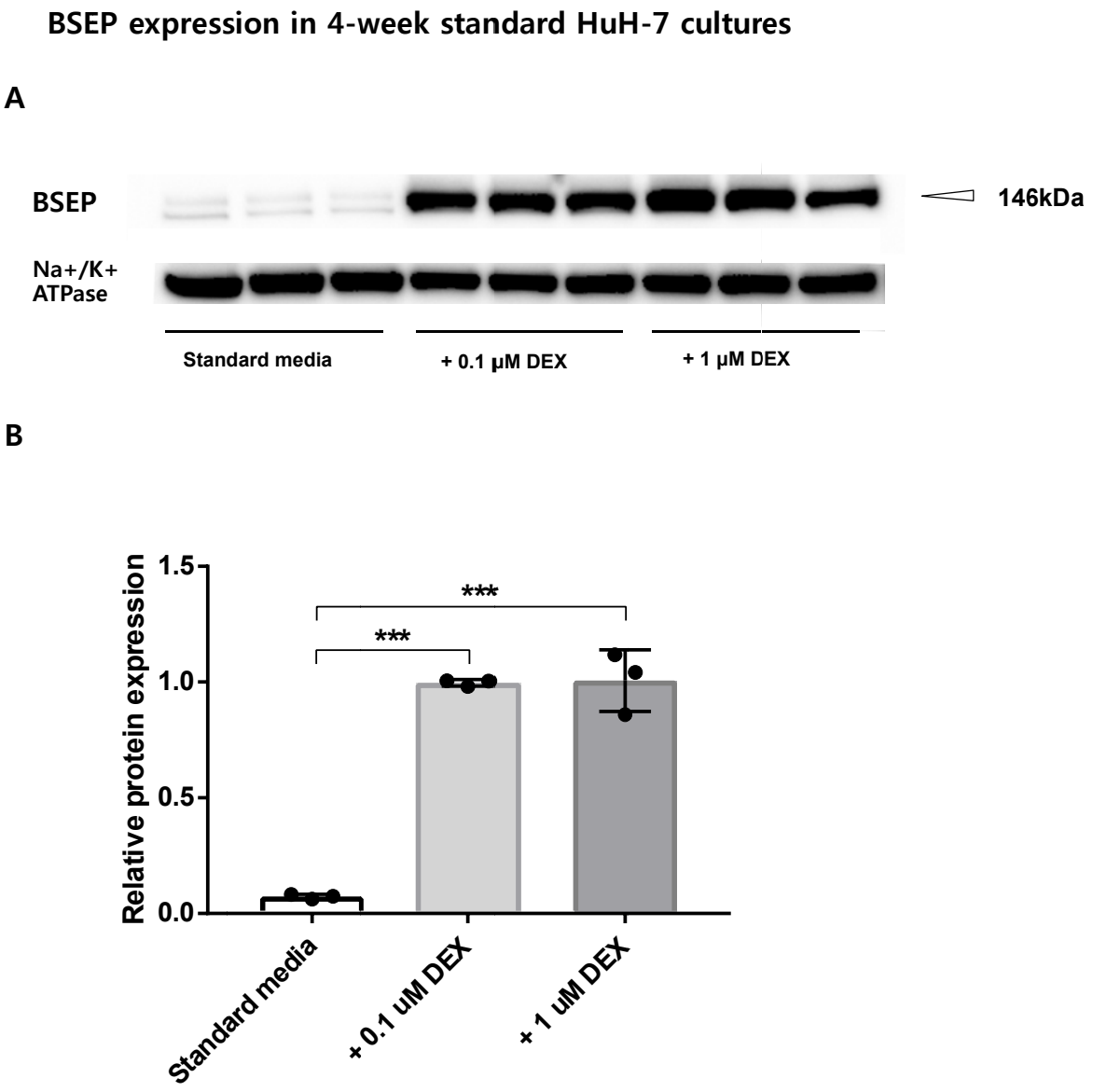

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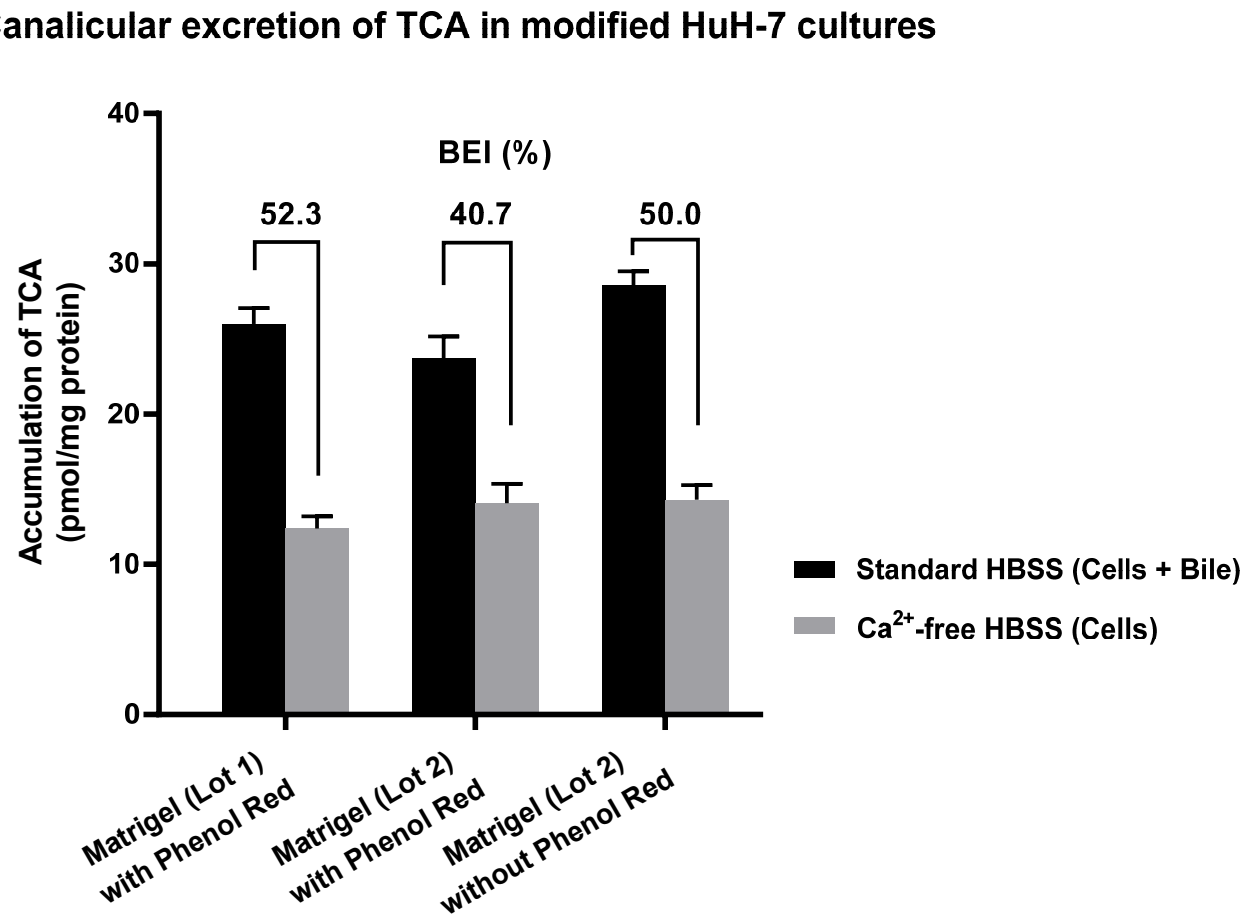

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