Supplementary information

Induction of blood-circulating bile acids supports recovery from myelosuppressive chemotherapy

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SUPPLEMENTARY METHOD

Measurement of bile acid using mass spectrometry

For measuring specific BA contained in the samples, 20 mg of adult mouse liver tissue or 100 μl of blood plasma was weighted and 0.1 ml of deuterated internal standards (d_4-CA, d_4-GCA, d_4-TCA) were spiked to each sample. Liver tissues were homogenized in 4 ml of cold 50 % ethanol at 10,000 rpm for 5 minutes on ice. The suspended solutions were sonicated for 5 minutes and then heated at 100 ºC for 30 min. The treated solution was diluted with 12 ml of water and briefly purified in the InertSep C18 cartridge (G&L Science). BA fraction eluted with 90 % ethanol was dried up and dissolved in 1ml of 10 % acetonitrile (ACN).

Mass spectrometry analysis was carried out using TSQ Quantum Discovery Max mass spectrometer equipped with an ESI probe and Surveyor HPLC system (Thermo Fisher Scientific). A chromatographic separation column, InertSustain C18 column (150 mm x 2.1 mm I.D., 3 μm particle size; G&L Science) were employed at 35 ºC. After injection of the sample solution, compounds were separated using an ACN-aqueous ammonium acetate gradient. The mass spectrometry conditions, spray voltage, sheath gas pressure, collision energy etc. were set according to the previous reports.[40,41]

Antibodies

The cells were stained with the following antibodies conjugated to FITC, PE, PE-Cy5, PE-Cy7, APC, APC-eFluor780 or Brilliant Violet: -CD150 (TC15-12F12.2, BioLegend), -CD48 (HM48-1, BioLegend), -C-kit (2B8, eBiosciences), -Sca-1 (D7, BioLegend), -CD3 (145-2C11, BioLegend), -Gr-1 (RB6-8C5, BioLegend), -CD11b (M1/70, BioLegend), -B220 (RA3-6B2,

BioLegend) and Ter119 (TER119, BioLegend). Anti-CD3, -B220, -CD11b, -Gr-I and Ter119 antibodies were used as lineage antibody mix.

qRT-PCR primers

Following primers obtained from Applied Biosystems were used: *Cyp7a1* (Mm00484150_m1), *Cyp8b1* (Mm00501637_s1), *Cyp27a1* (Mm00470430_m1), *Cyp1a1* (Mm00487218_m1), *Cyp3a11* (Mm00731567_m1), *Tbxas1* (Cyp5a1, Mm00495553_m1), *Nr1h4* (FXR, Mm00436425_m1), *Baat* (Mm00476075_m1), *Slc10a1* (NTCP, Mm00441421_m1), *Slco1a1* (Mm01267415_m1), *Slco1b2* (Mm00451510_m1), *Slco1a4* (Mm01267407_m1), *Slco4a1* (Mm00455754_m1), *Abcg2* (Mm00496364_m1), *Abcc3* (Mm00551550_m1), *Abcc4* (Mm01226381_m1), *Abcc5* (Mm01343626_m1), *Actb* (Mm026119680_g1). All ΔCt values were normalized using *Actb* (ΔΔCt).

Corticosterone measurement and treatment of mice

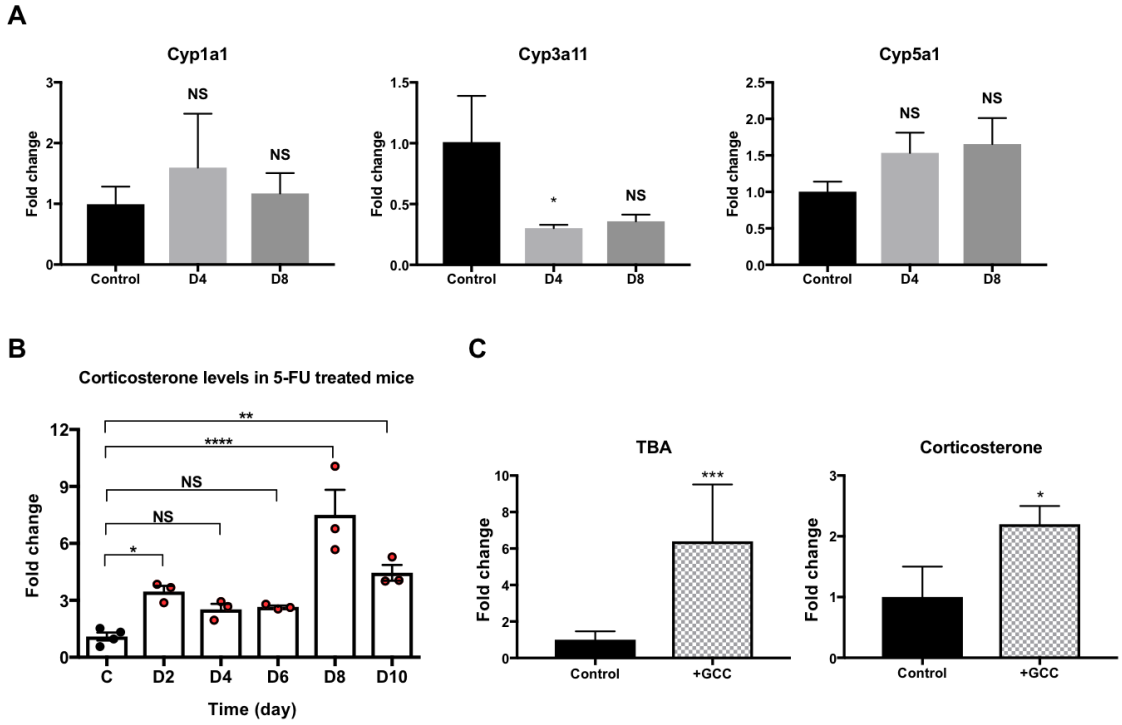
Blood plasma from mice was collected and levels of corticosterone were measured using an ELISA kit (Abcam). Mice were injected with a total dose of 200 μg corticosterone (Sigma) i.p. and analyzed 24 hr later for TBA levels.

SUPPLEMENTARY REFERENCES

40. Muto A, Takei H, Unno A, et al. Detection of Δ4-3-oxo-steroid 5β-reductase deficiency by LC-ESI-MS/MS measurement of urinary bile acids. *J. Chromatogr. B. Analyt. Technol. Biomed. Life. Sci.* 2012;900:24-31.

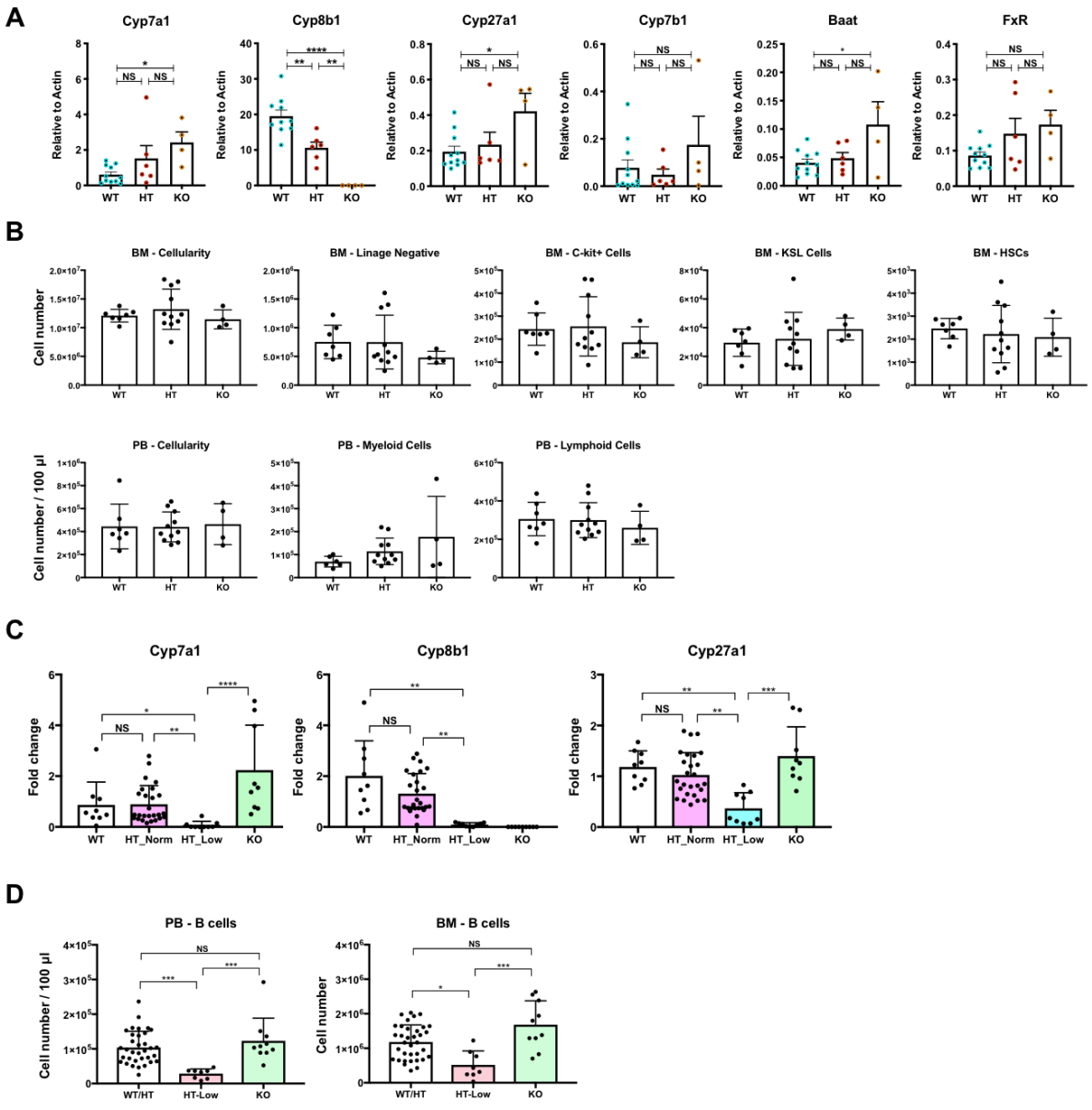
41. Naritaka N, Suzuki M, Sato H, et al. Profile of bile acids in fetal gallbladder and meconium using liquid chromatography-tandem mass spectrometry. *Clin. Chim. Acta.* 2015;446:76-81.

SUPPLEMENTARY FIGURES



Supplementary Figure S1. Increased glucocorticoid levels are connected to higher circulating BA levels.

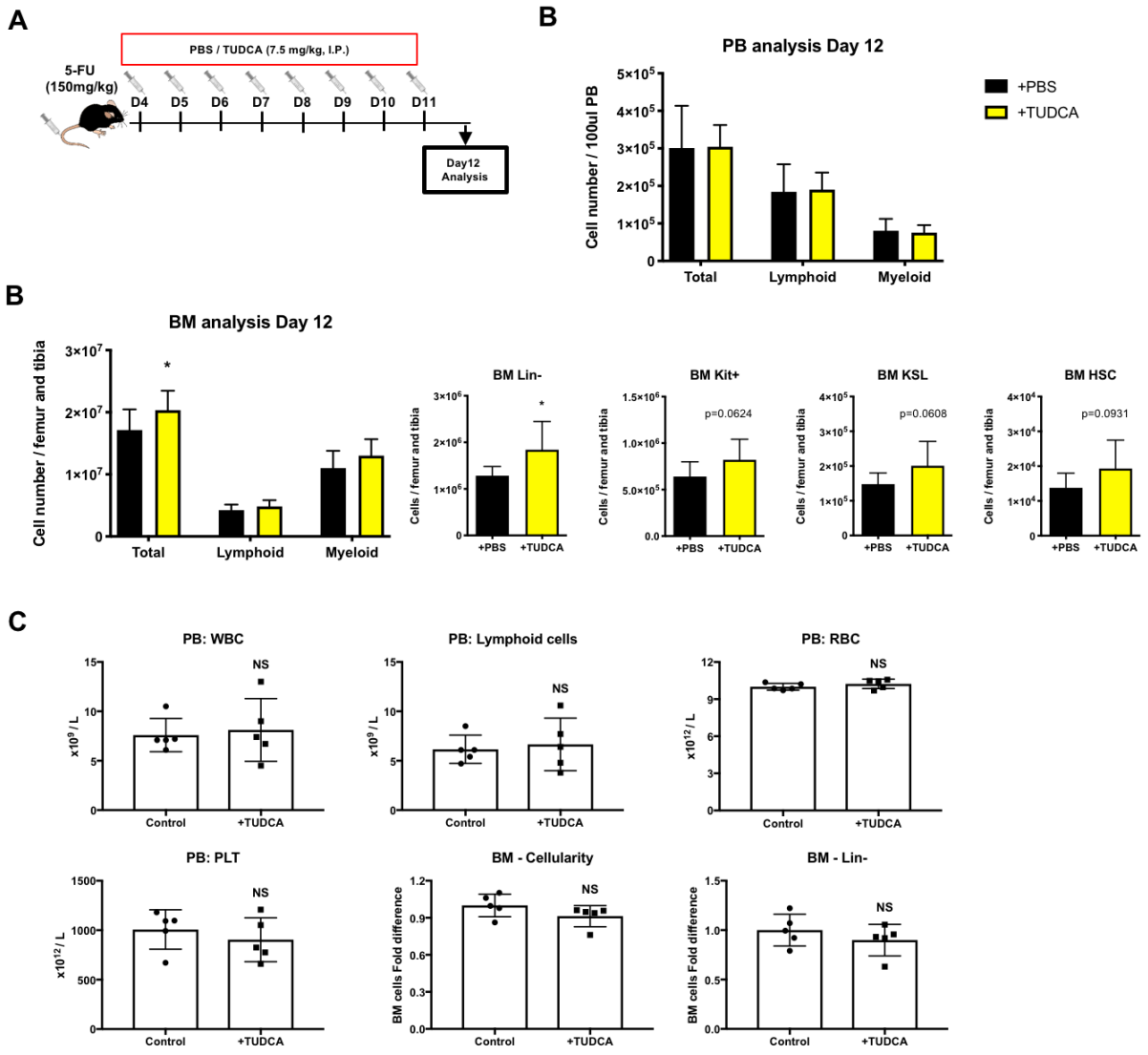
(**A**) qRT-PCR analysis for expression levels of non-BA related CYP enzymes. Liver samples of untreated mice (Control), day 4 after 5-FU treatment (D4) and day 6 after 5-FU treatment (D6) are shown. (**B**) Corticosterone levels in mouse plasma during 5-FU treatment. (**C**) Injection of steady state mice with PBS (control) or Corticosterone (+GCC). Levels of TBA (left) and total Glucocorticoid (right) in plasma of the injected mice are shown. Results represent means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, NS; not significant.



Supplementary Figure S2. Analysis of *Cyp8b1* KO mice under steady state conditions and stress.

(**A**) Expression of key BA synthetic enzymes in the liver of *Cyp8b1* WT, HT and KO mice under steady state conditions. (**B**) BM and PB cellularity of WT, HT and KO mice under steady state conditions. (**C**) Expression of key BA synthetic enzymes in the liver after 5-FU treatment and categorization of HT animals into HT-Norm and HT-Low subgroups based on their enzyme

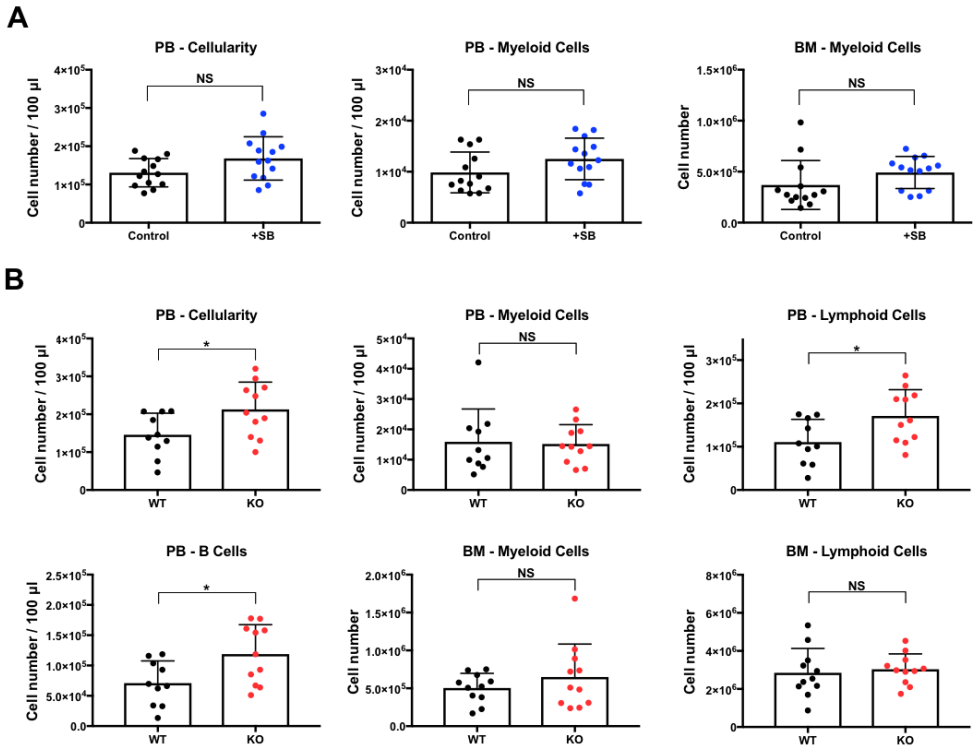
expression. (**D**) The number of B-cells in PB and BM in *Cyp8b1* KO mice on day 8 after 5-FU treatment. Results represent means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, NS; not significant.



Supplementary Figure S3. Effect of TUDCA injection on long-term recovery and untreated mice.

(**A**) Schematic of the experimental setup of TUDCA supplementation in 5-FU treated mice. (**B**) Cellularity in PB, BM of the TUDCA treated mice on day 12. The number of PB cells, BM cells, Lineage- cells, and HSCs ($CD150^{+}CD48^{-}$KSL cells) in femur and tibia are shown. n=10-11. (**C**) Daily TUDCA injection was performed to untreated mice for 4 days, and PB and BM were analyzed. While blood cell count (WBC), lymphoid cell count, red blood cell cound (RBC), platelet count (PLT) in PB, as well as BM cellularity (Cellularity) and the number of lineage

negative BM cells (Lineage Negative) are shown. Results represent means ± SEM. N=5. NS; not significant.



Supplementary Figure S4. Analysis of Salubrinal treated mice and ddit3 KO mice recovering from 5-FU.

(**A**) Analysis of PB and BM of mice injected with Salubrinal on day 8 after 5-FU treatment. PB cellularity (left), PB myeloid cellularity (middle) and BM myeloid cellularity (right) are shown. (**B**) Analysis of *Ddit3* KO mice on day 8 after 5-FU treatment. PB cellularity (upper left), PB myeloid cellularity (upper middle), PB lymphoid cellularity (upper right), PB B cell cellularity (lower left), BM myeloid cellularity (lower middle) and BM lymphoid cellularity (lower right) are shown. Results represent means ± SEM. *$P < 0.05$, NS; not significant.

Patients	Age	Gender	Diagnosis	Risk/Stage
1	1y4m	F	BCP-ALL	SR
2	3y0m	M	BCP-ALL	SR
3	2y9m	M	BCP-ALL	SR
4	9y4m	F	BCP-ALL	IR
5	4y8m	F	BCP-ALL	SR
6	4y10m	F	BCP-ALL	HR
7	13y0m	F	BCP-ALL	IR
8	4y9m	M	BCP-ALL	SR
9	4y4m	M	BCP-ALL	HR
10	7y2m	F	BCP-ALL	Relapse (BM)
11	4y3m	F	BCP-ALL	Relapse (CNS)
12*	10y1m	F	BCP-ALL	SR
13*	3y8m	M	BCP-ALL	SR
14	13y11m	M	T-ALL	
15	8y2m	M	T-ALL	
16	12y4m	M	Burkitt Lymphoma	B-NHL Stage4
17	8y10m	M	Mature B ALL	B-NHL Stage4
18	15y2m	M	Mature B ALL	B-NHL Stage4
19	1y5m	F	AML	IR
20	12y2m	M	APL	
21	11m	F	ML-DS	
22	10m	M	ML-DS	
23	10y0m	M	Brain tumor (Germinoma)	
24	11m	M	Neuroblastoma	Stage2
25	6m	M	Neuroblastoma	Stage4S
26*	8m	M	Neuroblastoma	Stage4S
27	1y8m	M	Neuroblastoma	Stage4
28*	3y2m	F	Neuroblastoma	Stage4

Supplementary Table S1. List of patients analyzed in the study.

Age, gender, diagnosis, risk/stage of patient are listed. BCP-ALL: B-precursor Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, APL: Acute Promyelocytic Leukemia, ML-DS: Myeloid Leukemia associated with Down Syndrome, NHL: Non Hodgkin Lymphoma, SR: Standard Risk, IR: Intermediate Risk, HR: High Risk. *Subjected to mass spectrometry analysis for BA component.