Supporting Information

Environmental eustress promotes liver regeneration via the sympathetic regulation of type 1 innate lymphoid cells to increase IL-22 in mice

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Supplementary Material and Methods

Behavioral test

To monitor the eustressful response in EE mice, we examined the anxiety levels of mice after a 5-week housing period under EE or SE conditions using the behavioral test elevated plus maze, according to the protocol described previously (ref. 13 in the main text). The behavioral parameters included the time spent in open arms and the percentages of the distances traveled in the open arms during the 5-minute test session. Anxiolytic activity was indicated by the increases in time spent or distances traveled in the open arms.

Quantification of physical activity

The in-cage wheel-running activities of mice were recorded using a video monitoring system whose infrared video cameras were placed on the top of cages. The running wheels were marked with a tape to aid the counting rotations. We reviewed the video recording and counted the total rotations of the running wheels for a period of three consecutive days using the Slow Motion Playback function. Thereafter, the running distance was calculated using the formula: $\pi \times$ diameter of running wheel $\times$ total wheel-running rotations.

Quantitative real-time PCR

The total RNA from regenerating livers were extracted using TRIzol reagent (TaKaRa, Otsu, Japan) and subjected to reverse transcription using the FastKing RT Kit (with gDNase; Tiangen Biotech, Beijing, China). Quantitative real-time PCR analysis of the mRNA expression of interested genes was performed using the StepOne Plus Real-time PCR system (Applied Biosystems, Foster City, CA) with the FastStart Universal SYBR Green Master kit (Roche Diagnostics, Mannheim, Germany). The relative expression levels of the target genes were normalized against that of the reference gene *Gapdh*. The primers used are listed in Table S2.

Enzyme-linked immunosorbent assay

Liver samples, brain samples or serum samples were collected before PHx or 24 h after PHx or CCl_4 administration. Tissue lysates from the liver or brain were prepared using Lysis Buffer (RayBiotech, Norcross, GA), and the total proteins were quantified using the Pierce BCA Protein Assay kit (Thermo Fisher, Waltham, MA). The concentration of interleukin-22 (IL-22) or IL-22 binding protein (IL-22bp, also termed IL-22 receptor α2) was determined using the Mouse IL-22 ELISA Kit (RayBiotech or eBioscience) or Mouse IL-22 receptor, α2 ELISA kit (MyBioSource, San Diego, CA). The concentrations of IL-6, bFGF, GM-CSF, EGF, and BDNF were determined using commercially available ELISA kits from MultiSciences (Hangzhou, China) or Elabscience (Wuhan, China).

Alanine aminotransferase test

To assess liver injury, the serum activity of ALT was measured using an automatic biochemistry analyzer (Hitachi High-Technologies, Tokyo, Japan) and Wako reagents (Wako Pure Chemical Industries, Tokyo, Japan).

Overexpression of BDNF in the brain

We used recombinant adeno-associated virus with PHP.eB capsids (AAV-PHP.eB),

which can effectively cross the blood-brain barrier (1), to deliver a FLAG-tagged murine BDNF gene to the brain. For brain-specific gene expression, the BDNF gene was placed under the control of the neuron specific *SYN1* promoter. The BDNF-expressing AAV vector (AAV-PHP.eB-BDNF) was produced by OBiO Technology Corp., Ltd (Shanghai, China). To deliver the BDNF gene *in vivo*, mice were administered 8×10^{10} viral genomes/mouse of AAV-PHP.eB-BDNF (50 μl total volume) via the lateral tail vein with. Mice administered the same dose of green fluorescent protein (GFP)-expressing virus (AAV-PHP.eB-GFP) were used as controls. Four weeks after viral injection, mice were subjected to PHx to induce liver regeneration.

In vitro functional characterization of the IL-22bp-expressing plasmid

The mammalian expression vector containing a FLAG-tagged murine IL-22bp gene under the control of the CMV promoter was obtained from OBiO Technology Corp., Ltd (Shanghai, China). The IL-22bp-expressing plasmid was functionally characterized *in vitro* in the murine hepatoma cell line Hepa1-6, which was obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and maintained in DMEM supplemented with 10% FBS. Hepa1-6 cells were transfected with the IL-22bp-expressing vectors using Lipofectamine 3000 (Thermo Fisher), according to the manufacturer's instructions. Cells transfected with empty vectors (EV) were used as controls. Forty-eight hours after transfection, the cells were subjected to western blot analysis using an anti-FLAG antibody to verify the expression of exogenous IL-22bp. Meanwhile, the Hepa1-6 cells transfected with IL-22bp-expressing vectors or EV were serum-starved overnight, and then treatment with recombinant murine IL-22 (50 ng/ml, Peprotech, Cranbury, NJ) for 30 min. The cells were finally analyzed for STAT3 phosphorylation via western blotting.

Hydrodynamic tail vein injection

The IL-22bp-expressing plasmid DNA was prepared using the EndoFree Plasmid Maxi

Kit (Qiagen, Hilden, Germany). Hydrodynamic tail vein injection was performed according to the protocol described previously (2). Plasmid DNA suspended in 1.8 ml sterile phosphate buffered saline was injected for 5–8 s into the tail vein of SE or EE mice (10 μg per mice) 1-2 days before PHx. An equal amount of empty vector was used as a control. Hepatic overexpression of IL-22bp after hydrodynamic injection was confirmed using ELISA on the day 1 of PHx.

Western blot

The total proteins from liver tissues or Hepa1-6 cells were extracted using RIPA buffer. After quantification using the BCA Protein Assay kit (Thermo Scientific), equal amounts of proteins were subjected to western blot analyses as described previously (3). The blots for the detected proteins were semi-quantified using ImageJ software, and quantitative results were presented as the relative expression levels of target proteins normalized to the pan-protein levels. Information regarding the primary antibodies is provided in Table S1.

Flow cytometry

The liver harvested from SE or EE mice was mechanically dissociated and passed through a 70 μm cell strainer. The liver single-cell suspensions were obtained using a Percoll (Sigma-Aldrich, St Louis, MO) density centrifugation, as described previously (4). For flow cytometric analysis, cells were incubated with anti-CD16/CD32 antibodies to block nonspecific and Fc-mediated binding, and then stained with anti-CD3ε, anti-NK1.1, anti-CD49b, and anti-CD49a antibodies. For intracellular IL-22 staining, the cells were fixed, permeabilized (using the Cyto-Fast Fix/Perm buffer set from Biolegend), and stained with anti-IL-22 antibody after surface staining. Viability Dye eFluor 450 (eBioscience) was used to exclude dead cells. All data were acquired on a FACSCelesta flow cytometer (BD Bioscience, San Jose, CA) and analyzed using FCS Express 4.0 software (De Novo Software, Glendale, CA). The gating strategy for flow cytometric analysis is shown in Fig. S10. Information

regarding the antibodies used is provided in Table S1.

Catecholamine staining

The sucrose–phosphate–glyoxylic acid (SPG) histofluorescence method was performed as described previously (5) to visualize catecholamines in the liver sections from 6-OHDA-treated mice or control mice. SPG fluorescence was observed using an Axio Vert.A1 fluorescence microscope (Carl Zeiss, Jena, Germany).

Noradrenaline measurement

The Noradrenaline High Sensitive ELISA kits (Eagle Biosciences, Amherst, NH) was used to measure noradrenaline in liver tissue lysates according to the manufacturer's protocol. The fresh frozen liver tissues were homogenized using 20 mM Tris·Cl buffer containing 1 mM EDTA for noradrenaline measurement.

Reference

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4. **Wang J**, **Sun R**, Wei H, Dong Z, Gao B, Tian Z. Poly I:C prevents T cell-mediated hepatitis via an NK-dependent mechanism. *J Hepatol*. 2006;44(3):446-454.
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Supplementary Tables

Table S1. Primary antibodies used in this study.

Antigen	Source	Identifier	Application
Ki67	Cell Signaling	Clone: D3B5	Immunohistochemistry
PCNA	Proteintech	#10205-2-AP	Immunohistochemistry
phospho-STAT3	Signalway Antibody	#11045	Western blot
phospho-STAT3	Cell Signaling	Clone: D3A7	Western blot
STAT3	Proteintech	#60199-1-Ig	Western blot
CD3ε	eBioscience	Clone: 145-2C11	Flow cytometry
NK1.1	eBioscience	Clone: PK136	Flow cytometry
CD49b	Biolegend	Clone: DX5	Flow cytometry
CD49a	Biolegend	Clone: HMα1	Flow cytometry
IL-22	Biolegend	Clone: Poly5164	Flow cytometry
CD16/CD32	eBioscience	Clone: 93	Flow cytometry

Table S2. Primers for the quantitative real-time PCR

Target gene	Primers sequence (5’ - 3’)
Gapdh	Forward: AGGTCGGTGTGAACGGATTTG
	Reverse: TGTAGACCATGTAGTTGAGGTCA
Ccnb1	Forward: AAGGTGCCTGTGTGTGAACC
	Reverse: GTCAGCCCCATCATCTGCG
Foxm1	Forward: CTGATTCTCAAAAGACGGAGGC
	Reverse: TTGATAATCTTGATTCCGGCTGG
Birc5（Survivin）	Forward: GAGGCTGGCTTCATCCACTG
	Reverse: CTTTTTGCTTGTTGTTGGTCTCC

Table S3. Cytokine array analysis of liver tissues from EE and SE mice

Cytokine	Fold change (EE/SE)	Regulation	*P* value
4-1BB	0.96889	Down	0.8123
6Ckine	0.01124	Down	0.0464*
ACE	0.99251	Down	0.9283
Activin A	0.13920	Down	0.3295
ADAMTS1	0.01339	Down	0.0595
Adiponectin	0.93297	Down	0.5180
ALK-1	0.74363	Down	0.1750
ANG-3	2.15325	Up	0.0308*
ANGPTL3	2.71178	UP	0.0632
AR	0.85895	Down	0.6531
Artemin	0.00121	Down	0.0002*
Axl	6.65170	Up	0.5239
B7-1	0.17539	Down	0.4369
BAFF R	1.64475	Up	0.0482*
bFGF	1.93284	Up	0.0036*
BLC	1.32778	Up	0.0222*
BTC	1.09097	Up	0.2801
C5a	0.93954	Down	0.6078
CCL28	0.01974	Down	0.0921
CCL6	1.49967	Up	0.0590
CD27	1.32244	Up	0.2638
CD27L	1.33298	Up	0.4450
CD30	0.91342	Down	0.4499
CD30L	1.17601	Up	0.0744
CD36	0.04860	Down	0.3704
CD40	0.79083	Down	0.5794
CD40L	0.92673	Down	0.7238
CD48	1.01206	Up	0.8943
CD6	1.19160	UP	0.5513
Chemerin	1.28529	Up	0.4038
Chordin	0.83701	Down	0.5137
Clusterin	1.25601	Up	0.0439*
CRP	0.97835	Down	0.8909
CT-1	0.95867	Down	0.7661
CTLA4	0.72712	Down	0.5629
CXCL16	1.12925	Up	0.3419
Cystatin C	1.05260	Up	0.4064
DAN	1.04954	Up	0.8880
Decorin	1.37133	Up	0.2199
Dkk-1	0.60787	Down	0.1085
DLL4	1.27934	Up	0.2348

Table S3. Cytokine array analysis of liver tissues from EE and SE mice (continued)

Cytokine	Fold change (EE/SE)	Regulation	*P* value
Dtk	1.54027	Up	0.0884
E-Cadherin	0.98181	Down	0.7971
EDAR	0.92344	Down	0.7046
EGF	1.50620	Up	0.0498*
Endocan	1.42028	Up	0.0739
Endoglin	1.17038	Up	0.1293
Eotaxin	1.36617	Up	0.9169
Eotaxin-2	0.17495	Down	0.4974
Epigen	0.71929	Down	0.3334
E-selectin	6.75160	Up	0.4628
Fas	1.16205	Up	0.8360
Fas L	0.08037	Down	0.3760
Fcg RIIB	1.13842	Up	0.1229
Fetuin A	1.11082	Up	0.6434
Flt-3L	0.62700	Down	0.1561
Fractalkine	1.36749	Up	0.6200
Galectin-1	0.95342	Down	0.5566
Galectin-3	1.05626	Up	0.5123
Galectin-7	0.90815	Down	0.7824
Gas 1	0.60846	Down	0.1600
Gas 6	1.04140	Up	0.7547
GITR	0.66319	Down	0.0101*
GITR L	1.23079	Up	0.3573
GM-CSF	1.28711	Up	0.0218*
gp130	0.74648	Down	0.5334
Granzyme B	5.06667	Up	0.4943
Gremlin	0.02557	Down	0.0876
H60	0.83549	Down	0.4088
HAI-1	0.86707	Down	0.3238
HGF	1.03209	Up	0.8306
HGF R	1.08109	Up	0.2862
ICAM-1	1.31915	Up	0.1707
Ifna11	1.22729	Up	0.2072
IFNg	1.36023	Up	0.0273*
IFNg R1	1.15496	Up	0.4206
IGF-1	0.92495	Down	0.7195
IGFBP-2	0.95767	Down	0.5963
IGFBP-3	1.15321	Up	0.0604
IGFBP-5	1.08807	Up	0.5294
IGFBP-6	1.07352	Up	0.5632
IL-1 R4 (Il1RL1)	1.32157	Up	0.0318*

Table S3. Cytokine array analysis of liver tissues from EE and SE mice (continued)

Cytokine	Fold change (EE/SE)	Regulation	*P* value
IL-10	1.20323	Up	0.0787
IL-12p40	1.18387	Up	0.0487*
IL-12p70	1.13723	Up	0.3341
IL-13	0.78401	Down	0.6475
IL-15	1.02684	Up	0.9635
IL-17	1.17968	Up	0.2155
IL-17B	8.77459	Up	0.3421
IL-17B R	1.02198	Up	0.9181
IL-17E	0.98904	Down	0.9481
IL-17F	0.84511	Down	0.5623
IL-1a	2.72588	Up	0.0074*
IL-1b	1.26582	Up	0.0626
IL-1ra	1.29498	Up	0.2313
IL-2	1.15396	Up	0.1907
IL-2 Ra	0.92512	Down	0.7333
IL-20	1.06626	Up	0.5928
IL-21	0.98457	Down	0.9765
IL-22	1.23996	Up	0.0462*
IL-23	1.14122	Up	0.3813
IL-28	1.10570	Up	0.3588
IL-3	1.18494	Up	0.0250*
IL-3 Rb	0.92957	Down	0.5244
IL-33	0.93230	Down	0.4939
IL-4	1.16728	Up	0.2120
IL-5	1.19717	Up	0.1652
IL-6	1.19177	Up	0.0129*
IL-7	0.96089	Down	0.8690
IL-7 Ra	1.24966	Up	0.0428*
IL-9	1.09850	Up	0.3661
I-TAC	0.82360	Down	0.5476
JAM-A	0.95562	Down	0.5042
KC	3.76619	Up	0.0339*
Kremen-1	1.27274	Up	0.4432
Leptin R	0.77272	Down	0.2512
Lipocalin-2	0.96980	Down	0.5540
LIX	0.96847	Down	0.8531
LOX-1	1.35755	Up	0.9173
L-Selectin	1.03492	Up	0.7764
Lungkine	1.26644	Up	0.2230
Lymphotactin	0.95497	Down	0.6798
MadCAM-1	0.82159	Down	0.2076

Table S3. Cytokine array analysis of liver tissues from EE and SE mice (continued)

Cytokine	Fold change (EE/SE)	Regulation	*P* value
Marapsin	1.28080	Up	0.5247
MBL-2	0.96681	Down	0.6210
MCP-1	1.08945	Up	0.3305
MCP-5	1.12775	Up	0.5112
MCSF	1.08393	Up	0.6416
MDC	1.02605	Up	0.9135
Meteorin	1.34538	Up	0.3764
MFG-E8	1.04799	Up	0.8137
MIG	0.10003	Down	0.2830
MIP-1g	1.09763	Up	0.3585
MIP-2	1.25752	Up	0.1604
MIP-3a	0.02551	Down	0.0850
MIP-3b	1.19110	Up	0.7579
MMP-10	1.99711	Up	0.2981
MMP-2	0.01280	Down	0.1198
MMP-3	13.28823	Up	0.3127
Neprilysin	0.92330	Down	0.5067
Nope	0.87344	Down	0.4833
NOV	1.17544	Up	0.5477
OPG	0.92664	Down	0.6006
OPN	1.21047	Up	0.1495
Osteoactivin	1.04622	Up	0.7515
OX40 Ligand	0.92973	Down	0.8452
P-Cadherin	1.07442	Up	0.7266
PDGF-AA	0.41445	Down	0.4339
Pentraxin 3	1.00550	Up	0.9715
Periostin	1.70092	Up	0.1501
Persephin	2.32468	Up	0.7972
PF4	2.63142	Up	0.1055
PlGF-2	1.02475	Up	0.8680
Progranulin	1.08023	Up	0.6760
Prolactin	0.74384	Down	0.0648
Pro-MMP-9	1.31393	Up	0.3455
Prostasin	0.79718	Down	0.5517
P-selectin	1.05172	Up	0.3814
RAGE	0.89616	Down	0.3898
RANTES	1.30576	Up	0.3627
Renin 1	0.77837	Down	0.1968
Resistin	1.09482	Up	0.3217
SCF	1.83030	Up	0.2563
SDF-1a	1.13007	Up	0.4678

Table S3. Cytokine array analysis of liver tissues from EE and SE mice (continued)

Cytokine	Fold change (EE/SE)	Regulation	*P* value
sFRP-3	1.20121	Up	0.6755
Shh-N	1.50441	Up	0.0070*
SLAM	8.86063	Up	0.3426
TACI	1.04979	Up	0.8806
TCA-3	1.05568	Up	0.6253
TCK-1	1.21980	Up	0.1463
TECK	1.74358	Up	0.4274
Testican 3	1.26356	Up	0.5016
TGFb1	1.15037	Up	0.7079
TIM-1	1.16637	Up	0.2559
TNF RI	0.82061	Down	0.4493
TNF RII	1.14149	Up	0.3146
TNFa	0.84558	Down	0.0463*
TPO	0.99968	Down	0.9989
TRAIL	1.04088	Up	0.8872
TRANCE	0.87980	Down	0.7420
TREM-1	0.84063	Down	0.2591
TremL1	1.49059	Up	0.5100
TROY	0.85517	Down	0.0291*
Tryptase ε	12.65857	Up	0.3010
TSLP	1.05757	Up	0.7206
TWEAK	0.73783	Down	0.4400
TWEAK R	0.98010	Down	0.9279
VCAM-1	0.93038	Down	0.2237
VEGF	1.06241	Up	0.7388
VEGF R1	0.74655	Down	0.1213
VEGF R3	1.00531	Up	0.9833
VEGF-B	1.04096	Up	0.9099
VEGF-D	1.43488	Up	0.2256

A total of 193 cytokines were detectable by cytokine microarray and presented in the table.

*$P < 0.05$ was considered statistically significant.

Supplementary Figure Legends

Figure S1. Post-PHx body weight of EE and SE mice (related to Figure 1).

Mice were housed under the EE or SE condition for 5 weeks and then subjected PHx to induce liver regeneration. The body weights of EE or SE mice (n = 11 for SE_{12h} and SE_{144h}, n = 12 for the other groups) were measured before or after PHx. All data are presented as mean ± SEM. *, $P < 0.05$.

Figure S2. Voluntary wheel running does not promote liver regeneration after PHx.

Mice were housed with or without running wheels for 5 weeks and then subjected to PHx to induce liver regeneration. Liver weight to body weight ratios (A) and the percentages of Ki67-positive hepatocytes (B-C) at 48 h after PHx were compared between the voluntary wheel-running group and control group (n = 8 per group). Representative Ki67-stained liver sections are shown in B. All data are presented as mean ± SEM.

Figure S3. Brain overexpression of BDNF mimics the pro-regeneration effect of EE.

(A) Mice were housed under EE or SE for four weeks and their brain BDNF levels were then determined using ELISA (n = 8 per group). (B-E) Mice were intravenously administered AAV-PHP.eB-BDNF or AAV-PHP.eB-GFP through the tail vein. (B) Brain and liver levels of BDNF were determined using ELISA (n = 4 per group). Of note, the injection of AAV-PHP.eB-BDNF resulted in a significant increase in brain

BDNF expression, but did not change the levels in the liver. (C-E) Four weeks after injection, mice were subjected to PHx. Liver weight to body weight ratios (C) and the percentages of Ki67-positive hepatocytes (D-E) at 48 h after PHx are compared between the groups (n = 6 per group). Representative Ki67 stained liver sections are shown in D. All data are presented as mean ± SEM. *, $P < 0.05$.

Figure S4. ELISA results for the liver regeneration-related cytokines in EE mice or *Tbx21*-deficient mice (related to Figure 2).

(A) Levels of IL-22, IL-6, bFGF, GM-CSF, and EGF in the regenerating liver (24 h post PHx) of EE or SE mice were determined using ELISA (n = 6 per group). The results are expressed as the relative cytokine levels normalized against that of the SE group. (B) ELISA results for the IL-6 level in the regenerating livers (24 h post PHx) of *Tbx21*-deficient mice or wild-type mice (n = 6 per group). All data are presented as mean ± SEM. **, $P < 0.01$; *, $P < 0.05$.

Figure S5. *In vitro* functional characterization of the IL-22bp-expressing plasmid (related to Figure 2).

Hepa1-6 cells were transfected with the IL-22bp plasmid or empty vector (EV). The phosphorylation of STAT3 in the IL-22bp-transfected or EV-transfected Hepa1-6 cells treated with or without IL-22 was analyzed by western blotting. Similar results were observed in two independent experiments.

Figure S6. Influence of EE on the NKT, T, cNK, and ILC1 population in hepatic lymphocytes after PHx (related to Figure 4).

Liver mononuclear cells were obtained from EE or SE mice at the indicated time points after PHx and subjected to flow cytometric analysis. Proportions of $NK1.1^+$ $CD3^+$ NKT cells (A), $NK1.1^-$ $CD3^+$ T cells (B), $NK1.1^+$ $CD3^-$ $CD49b^+$ cNK cells, and $NK1.1^+$ $CD3^-$ $CD49a^+$ ILC1s (C) in hepatic lymphocytes were analyzed by flow cytometry (n = 5 for SE_{48h} and EE_{24h}, n = 6 for the other groups). All data are presented as mean ± SEM. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Figure S7. Hepatic overexpression of IL-22bp does not influence the EE-induced changes in ILC1s and cNK cells in the regenerating liver (related to Figure 5).

EE or SE mice were hydrodynamically injected with the IL-22bp-expressing vector or empty vector (EV) via the tail vein, and then subjected to PHx. (A-B) Flow cytometric analyses of the proportion of $NK1.1^+$ $CD3^-$ cells in the hepatic lymphocytes of EE or SE mice at 24 h after PHx (n = 6 per group). Representative flow cytometry plots are shown in A. (C-D) Flow cytometric analyses of the proportion of $CD49b^+$ or $CD49a^+$ cells in hepatic $NK1.1^+$ $CD3^-$ cells of EE or SE mice (n = 6 per group). Representative flow cytometry plots are shown in C. All data are presented as mean ± SEM. **, $P < 0.01$; *, $P < 0.05$.

Figure S8. Determination of the effectiveness of 6-OHDA-indued chemical sympathectomy (related to Figure 6).

(A) Catecholamine staining of the liver sections of 6-OHDA-treated or vehicle-treated EE or SE mice before PHx. Arrows, the periportal catecholaminergic nerve fibers. Similar results were observed in at least three independent experiments. Original magnification: ×200. (B) The noradrenaline level in the regenerating livers (24 h post PHx) of 6-OHDA-treated or vehicle-treated EE or SE mice was determined using ELISA (n =6 per group). All data are presented as mean ± SEM. ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$.

Figure S9. EE fails to increase the proportion of ILC1s in regenerating livers after propranolol treatment (related to Figure 7).

Propranolol-treated EE or SE mice were subjected to PHx. (A) Forty-eight hours after PHx, the proportion of $NK1.1^+$ $CD3^-$ cells in liver lymphocytes and the proportion of $CD49b^+$ or $CD49a^+$ cells in liver $NK1.1^+$ $CD3^-$ cells were analyzed by flow cytometry. Representative flow cytometry plots are shown. (B) Comparison of the proportion of $NK1.1^+$ $CD3^-$ $CD49b^+$ cells or $NK1.1^+$ $CD3^-$ $CD49a^+$ cells in liver lymphocytes between propranolol-treated EE and SE mice (n = 6 per group). All data are presented as mean ± SEM.

Figure S10. Gating strategies for flow cytometric analysis used to define hepatic ILC1s and cNK cells.

Forward scatter area (FSC-A) versus side scatter area (SSC-A) was used to select lymphocytes followed by FSC-A versus forward scatter-height (FSC-H) to select

singlets. The singlets gate was analyzed to determine their uptake of the Viability Dye stain. Live lymphocytes were examined to determine the expression of CD3 and NK1.1. The $CD3^{-}$ $NK1.1^{+}$ cells were selected for further analysis of the CD49a and CD49b expression. The $NK1.1^{+}$ $CD3^{-}$ $CD49a^{+}$ $CD49b^{-}$ cells were defined as ILC1s and $NK1.1^{+}$ $CD3^{-}$ $CD49a^{-}$ $CD49b^{+}$ cells were defined as cNK cells.