Supporting Material:

Material and Methods:

Flow Cytometric Analysis of Hepatic Leukocyte Populations Expressing TSLPR. The surface expression of TSLPR and IL7Rα on NKT cells, $CD4^+$ T cells, $CD8^+$ T cells, and eosinophils were determined by flow cytometry. Hepatic leukocytes from naïve and vehicle and halothane treated mice were isolated and stained using established procedures (*1*). All staining specified below was performed in 5% fetal bovine serum (FBS) in sterile PBS at 4°C in the dark. Hepatic leukocytes were stained with violet LIVE/DEAD® Fixable Aqua Dead Cell stain kit (Invitrogen, Carlsbad, CA) for 30 minutes then blocked with 0.5 µg of anti-CD16/CD32 (2.4G2, BD Pharmingen, San Diego, CA) for 15 minutes. They were then stained for 45 minutes with combinations of 0.5 µg each of APC-Cy7-conjugated anti-CD11b (M1/70, BD Pharmingen), Alexa 488-conjugated anti-CD11c (N418, eBioscience, San Diego, CA), eFluor 450-conjugated anti-Gr-1 (RB6-8C5, eBioscience), PE-conjugated anti-sialic acid-binding immunoglobulin-like lectin-F (Siglec-F) (E50-2440, BD Pharmingen), PE-Cy5-conjugated anti-CD3 (145-2C11, eBioscience), Pacific Blue-conjugated anti-CD4 (RM4-5, BD Pharmingen), PerCP-Cy5.5-conjugated anti-CD8 (53-6.7, eBioscience), PE-labeled PBS-57-loaded mouse CD1d tetramer (α-GalCer Tet) (The NIH Tetramer Facility at Emory University, Atlanta, GA), carboxyfluorescein-conjugated anti-TSLPR (R&D Systems, Minneapolis, MN) and APC-conjugated anti-CD127 (IL-7Rα) (A7R34, eBioscience) or corresponding isotype controls. Cells were then fixed with BD Cytofix™ solution (BD Biosciences). Live cell events (2×10^5 per

liver) were measured on a LSRII flow cytometer (BD Biosciences) and data was analyzed with FCS Express 3 (De Novo Software, Los Angeles, CA). Briefly, hepatic leukocytes from individual mice were stained with combinations of α-GalCer Tet, CD3, CD4, CD8, CD11c, CD11b, Gr-1, and Siglec-F antibodies. Cells gated as $CD3^+$ α-GalCer Tet^+, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$ and $CD11c^-$ $CD11b^+$ $Gr\text{-}1^{low}$ $Siglec\text{-}F^{high}$ were determined to be NKT cells, $CD4^+$ T cells, $CD8^+$ T cells, and eosinophils, respectively. The surface expression of TSLPR and IL-7Rα was determined on each of the above population of cells and plotted as histogram plots depicting staining with anti-TSLPR or isotype control (polyclonal goat IgG) or IL-7R and Rat IgG2a isotype control. The mean fluorescence intensity was obtained from the histograms and plotted to assess changes in TSLPR and IL-7Rα expression in individual cell types. Following surface staining to distinguish NKT, $CD4^+$ T cells, $CD8^+$ T cells and eosinophils in some samples, intracellular staining was performed to assess TSLP levels using Foxp3 Staining Buffer set (eBioscience) according to the manufacturer's instructions. Briefly, cells were fixed and permeabilized and then stained in permeabilization buffer with either anti-TSLP or isotype control (polyclonal goat IgG) (R&D Systems) for 45 mins. Samples were washed twice in permeabilization buffer and stained for 45 mins with APC-conjugated donkey anti-goat IgG (R&D Systems). Samples were washed and analyzed as described previously. No TSLP intracellular staining was detected in any of the cell types examined.

Murine Hepatocyte Isolation and Cell Culture:

Female Balb/cJ mice aged 7-10 weeks (19-23g) were euthanized and liver was exposed following dissection. The liver was perfused using a 20-gauge cannula through the inferior vena cava and out of the hepatic portal vein with oxygenated HBSS buffer (pH 7.4) containing 0.6 mM EGTA and 25 mM $NaHCO_3$ to remove the blood. The perfusate was replaced with William's Medium E containing collagenase IV (>125 U/mg, Sigma) at 0.1 mg/ml dilution for 3-5 min at which point the livers appeared to be significantly digested. The livers were then excised and placed in William's Medium E containing 50% fetal calf serum (FCS) (Invitrogen) and hepatocytes were liberated from the tissue by gentle raking and agitation. Cells were passed through a 100 μm cell strainer and washed by centrifugation twice for 5 min at 60g at 4°C with Complete William's Medium E (William's Medium E with Glutamax supplemented with 10% bovine insulin solution (0.3 U/ml, Sigma), 10% FCS, 100 U/ml penicillin, and 100 ug/ml streptomycin). After washing, cells were resuspended in Complete William's Medium E and viability of the cells was determined by Trypan blue exclusion. Primary murine hepatocytes were plated at 100,000 cells/cm2 in 12-well cell culture plates that were pre-coated with rat tail Collagen I (Invitrogen) and cultured in Complete William's Medium E overnight. Media was changed 24 hours post seeding and every other day thereafter. Hepatocytes in culture became 100% confluent by 24 hours post seeding. Primary mouse hepatocytes were treated with recombinant mouse IL-1β (1 ng/ml, R&D Systems), IL-4 (10 ng/ml), or tumor necrosis factor-α (TNF-α) (10 ng/ml, R&D Systems) or with

combinations of these cytokines for 24 hours in Complete William's Medium E supplemented with 1% BSA Cohn Fraction V instead of 10% fetal calf serum.

Cell Culture Conditions of Mouse Hepa 1-6 cells. Hepa 1-6 cells (CRL-2026, ATCC, Manassas, VA) were cultured in DMEM, High Glucose media (Life Technologies, Carlsbad, CA) supplemented with 10% FBS (Life Technologies) and 1% antibiotics/antimycotics (Life Technologies). Cells were plated at 100,000 cells/cm^2 into 12-well plates (Corning Costar, Tewksbury, MA) and cultured for 3 days. On day 4, cells were treated with recombinant mouse cytokines for stimulation studies. Cultured Hepa 1-6 cells were treated with recombinant mouse IL-1β (1 ng/ml, R&D Systems), IL-4 (10 ng/ml), or tumor necrosis factor-α (TNF-α) (10 ng/ml, R&D Systems) or with combinations of these cytokines dissolved in DMEM, High Glucose media supplemented with 1% BSA Cohn Fraction V.

Cell Culture Conditions of Human Primary Hepatocytes. Plateable cryopreserved human hepatocytes from three different donors (donors: HH1020, HH1026, HH1031; In Vitro ADMET Laboratories, Columbia, MD) were used in this study. The hepatocytes had > 90% viability upon thawing, and were observed to form >95% confluent monolayer cultures throughout the study. Studies were performed in 24-well collagen-coated plates (In Vitro ADMET Laboratories). Briefly, human hepatocytes were thawed and plated at approximately 500,000 cells per well in 0.5 ml of Hepatocyte Plating Medium (APSciences, Columbia,

MD). Cells were cultured in an incubator maintained at 37°C with a humidified atmosphere of 95% air and 5% carbon dioxide. Approximately 4 hours after plating, the plating medium was replaced with Hepatocyte Induction Medium (APSciences) containing 0.25 mg/ml Matrigel. The next day, medium was changed with dexamethasone-free Hepatocyte Induction Medium. On the third day, cells were ready for treatment with recombinant cytokines. Hepatocytes were cultured in the presence or absence of recombinant human IL-1β (1 ng/ml, R&D Systems), recombinant human TNF-α (10 ng/ml, R&D Systems), and recombinant human IL-4 (10 ng/ml, R&D Systems) individually or in combination in modified Hepatocyte Induction Medium containing 1% BSA for 24 hours. Triplicate samples were used for each condition.

References:

1. W. R. Proctor *et al.,* Eosinophils mediate the pathogenesis of halothane-induced liver injury in mice. *Hepatology* **57**, 2026 (May, 2013).

Figure Legends:

Figure S1: Expression of TSLPR on the surface of hepatic leukocytes isolated from naïve WT mice. (A) Total liver, hepatocytes, and hepatic leukocytes were isolated from naïve WT Balb/cJ mice (age 7-10 weeks). Expression of mRNA was determined for TSLP, TSLPR, and IL7Ra mRNA in total liver homogenates (Liver), isolated hepatocytes (Hep), and hepatic leukocytes (Leuk). Data reported as means ± SEM of 5-9 per group. **P*<0.05 compared with liver homogenates and $^{\#}P$<0.05 compared with isolated hepatocytes, ND: not detected. (B-C) Hepatic leukocytes from individual naïve female WT Balb/cJ mice

or mice treated with vehicle or halothane were stained with combinations of α-GalCer Tet, CD3, CD4, CD8, CD11c, CD11b, Gr-1, and Siglec-F antibodies. (B) Representative density dot plots for NKT-cells ($CD3^+$ α-GalCer Tet^+), $CD4^+$ T cells ($CD3^+$ $CD4^+$), $CD8^+$ T cells ($CD3^+$ $CD8^+$) and eosinophils ($CD11c^-$ $CD11b^+$ $Gr\text{-}1^{low}$ $Siglec\text{-}F^{high}$) from individual mice. (C) Representative histogram plots depicting staining with anti-TSLPR (black fill) or isotype control (polyclonal anti-goat IgG) (grey fill) on hepatic NKT cells, $CD4^+$ T cells, $CD8^+$ T cells, and eosinophils. (D) Comparison of mean fluorescence intensity of hepatic NKT cells, $CD4^+$ T cells, $CD8^+$ T cells, and eosinophils stained for TSLPR or IL-7Rα 24 hours after treatment with vehicle or halothane and assessed by flow cytometry. Data are reported as mean fluorescence intensity ± SEM (N=5 per group). $*P<0.05$ compared with vehicle treated control mice.

Discussion:

TSLPR is expressed on a wide array of leukocytes and while at the timepoint we examined we detected no treatment related changes in TSLPR protein expression, altered expression on cell types not assessed here may partially explain the differences we observed between mRNA levels (Figure 1C) and protein expression (Figure S1D) after halothane treatment. Further study is required to identify which cell types are responsive to TSLP after halothane treatment.

Figure S2. The effects of IL-1β, TNF-α, and IL-4 treatment on plated human hepatocytes from individual donors. The expression of mRNA (A) of TSLP,

CCL11, and CCL26 and protein levels in the cell culture supernatant (B) from cultured hepatocytes from individual donors (HH1020, HH1026, and HH1031) treated for 24 hours individually or in combination with recombinant human IL-1β (1 ng/ml), TNF-α (10 ng/ml), and IL-4 (10 ng/ml). All data reported as means ± SEM with an N=3 wells from hepatocytes isolated from three individual donors. *P<0.05 vs. same IL-4 treatment in the absence of IL-1β and TNF-α, $^{\#}P$<0.05 vs. same IL-1β and TNF-α treatment in the absence of IL-4. $^{\dagger}P$<0.05 vs. the two bracketed treatment groups.

Figure S1

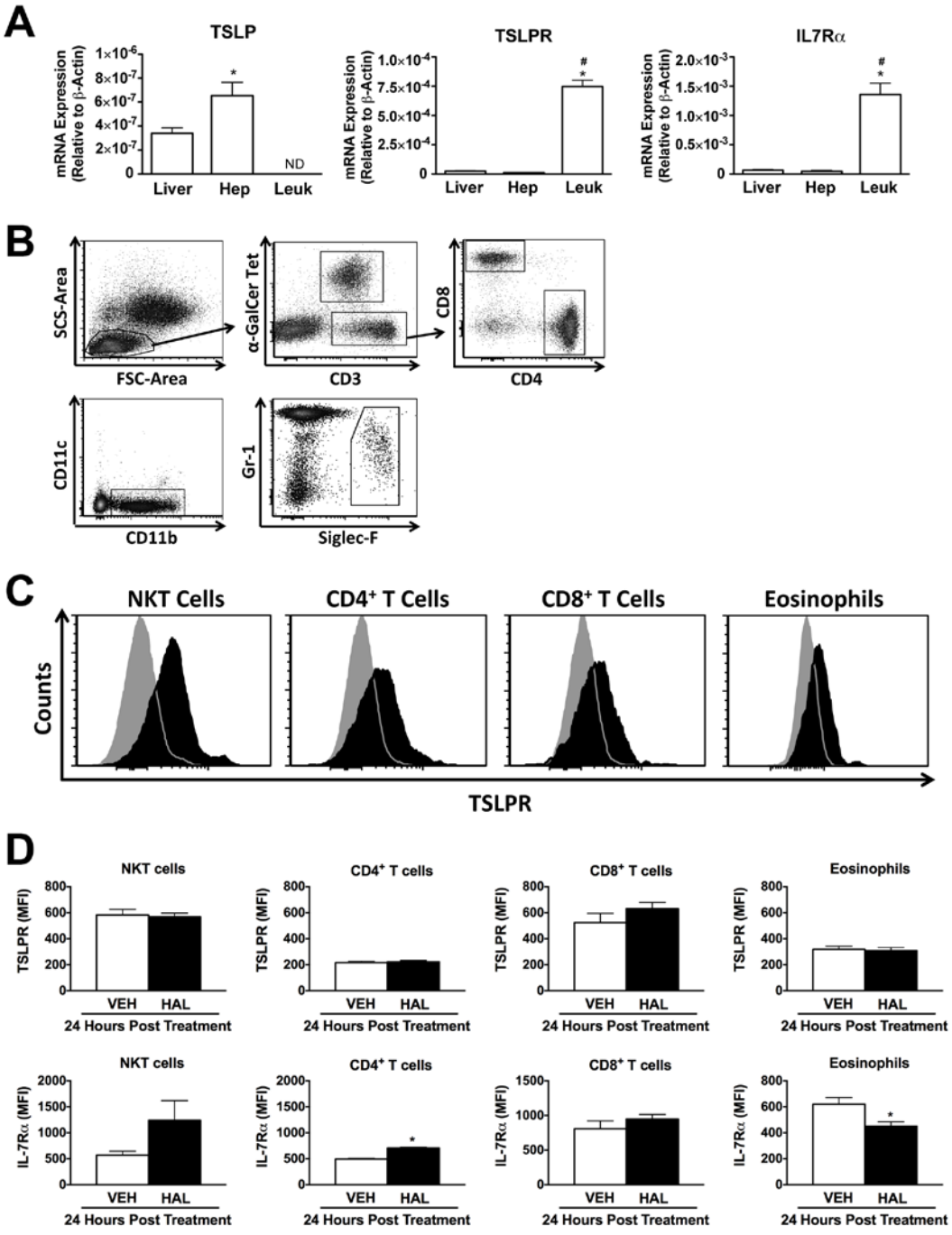
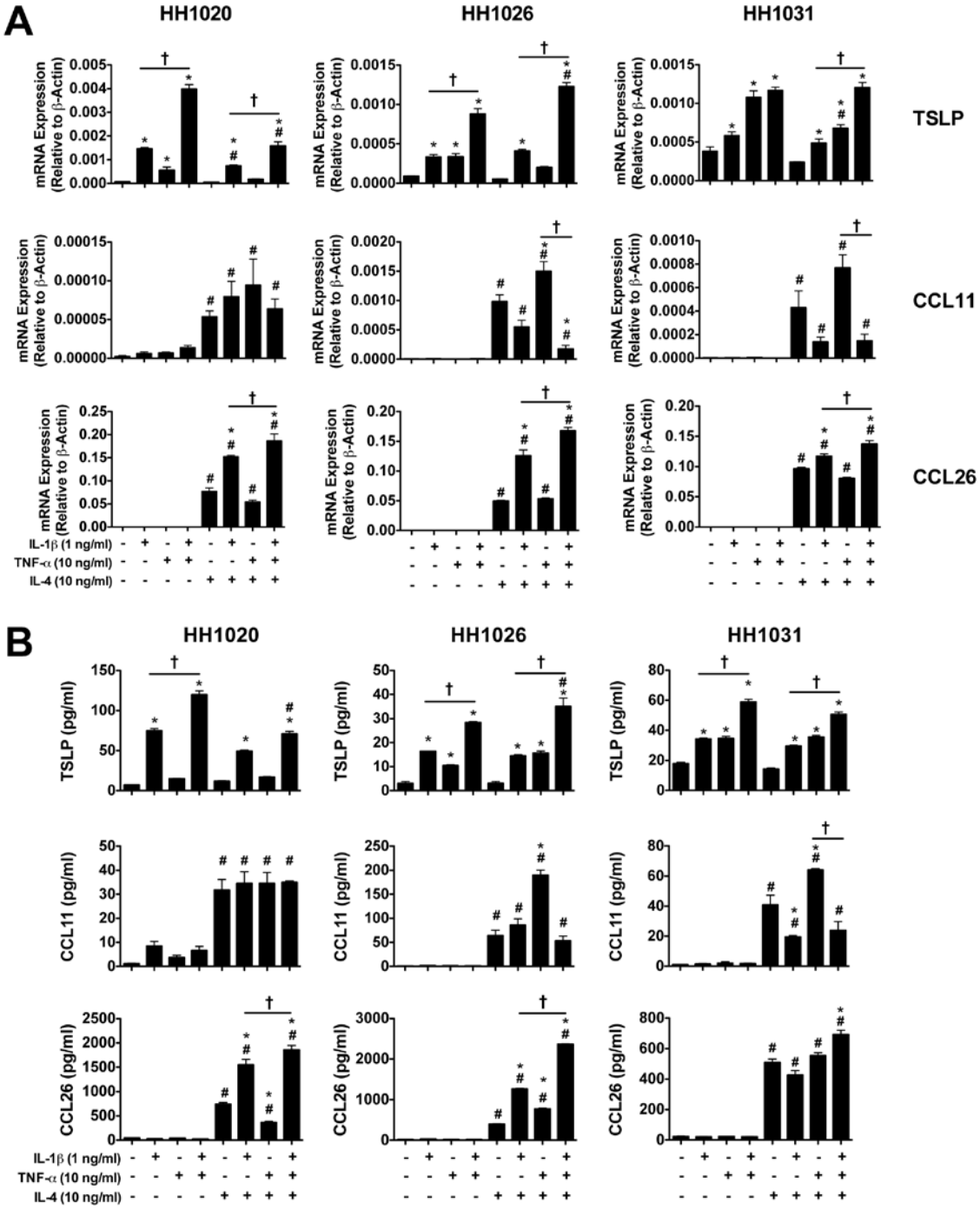

A
TSLP
TSLPR
IL7Rα
mRNA Expression (Relative to β-Actin)
Liver
Hep
Leuk
ND
B
SCS-Area
FSC-Area
α-GalCer Tet
CD3
CD8
CD4
CD11c
CD11b
Gr-1
Siglec-F
C
NKT Cells
CD4+ T Cells
CD8+ T Cells
Eosinophils
Counts
TSLPR
D
TSLPR (MFI)
IL-7Rα (MFI)
VEH
HAL
24 Hours Post Treatment

Figure S2


A
HH1020
HH1026
HH1031
mRNA Expression (Relative to β-Actin)
TSLP
CCL11
CCL26
IL-1β (1 ng/ml)
TNF-α (10 ng/ml)
IL-4 (10 ng/ml)
B
HH1020
HH1026
HH1031
TSLP (pg/ml)
CCL11 (pg/ml)
CCL26 (pg/ml)
IL-1β (1 ng/ml)
TNF-α (10 ng/ml)
IL-4 (10 ng/ml)