SUPPLEMENTARY EXPERIMENTAL PROCEDURES

PNAC mouse model

All animal procedures were approved by Institutional Animal Care and Use Committee of the University of Colorado Anschutz Medical Campus and all animals were treated humanely throughout the experimental procedures. As previously described, the PNAC mouse model (7,12) employed C57BL/6 WT adult male mice (8 weeks old, 22–23 g body weight) (Jackson Laboratories, Bar Harbor, ME, USA) that were housed in specific pathogen-free conditions in individual metabolic cages and maintained on a 12 h light/dark cycle. To induce mild intestinal injury and increased permeability, mice were exposed ad libitum to 2.5% DSS (MP Biomedicals, Santa Ana, CA, USA) in the drinking water for 4 days during which time they had access to chow and water ad libitum (referred to as “DSS pretreatment”). After DSS pretreatment, PNAC mice underwent pentobarbital anesthesia during surgical placement of a central venous catheter (CVC) (Silastic tubing, 0.012 inches internal diameter; Dow Corning, IL, USA) into the right jugular vein while the proximal end was tunneled subcutaneously and exited between the shoulder plates. While still under anesthesia, mice were subsequently placed in a rubber mouse harness (Instech Laboratories, Plymouth Meeting, PA, USA) and the CVC was threaded through a swivel apparatus and connected to an infusion pump (Harvard Apparatus, Holliston, MA, USA). Mice were recovered from anesthesia on a heating pad under continuous supervision and allowed to further recovery from surgery for 24 h with intravenous (i.v.)

normal saline (NS) infusion at a rate of 0.23 ml/h and were given ad libitum access to chow and water. After 24 h chow was removed, access to water was continued and mice were continuously infused for 14 days with PN (DSS-PN mice) at a rate of 0.29 ml/h providing a caloric intake of 8.4 kcal/day and 20% soybean lipid emulsion (Intra lipid, Fresenius, Bad Homburg, Germany) at dose of 5 g/kg/day (7,12). Mice were randomly assigned to several groups in addition to the DSS-PN mice. PN mice did not have DSS pretreatment but underwent CVC placement and received PN in the same manner as DSS-PN mice. DSS-chow mice were DSS-pretreated mice that did not have a CVC placed and were given free access to chow and water for 14 days. All PN infused mice had access to water ad libitum but not to chow during the PN infusion period. Chow fed control mice (referred to as Chow mice) had free access to chow and water for a period of 19 days. In some experiments DSS-PN, DSS-chow, and Chow treatments were given to *Il1r*$^{-/-}$mice on the C57BL/6 background (Jackson Laboratories, Bar Harbor, ME, USA). In one group, DSS pretreated mice were administrated PN containing the LRH-1 agonist, 1,2-dilauroyl-sn-glycero-3-phosphocholine (DLPC; Cat No:4378; Tocris, Minneapolis, MN, USA) dissolved in ethanol, at dose of 30 mg/kg/body weight per day (20) during day 4 through 14 of PN infusion. On the day of sacrifice, mice were anesthetized with i.p. pentobarbital and 100–400 µl of blood was collected from the retro-orbital plexus and liver was removed, one piece placed in formalin for 4 h followed by placement in 70% alcohol and another piece of liver was snap frozen in liquid nitrogen, coded, and subsequently stored at −80 °C until analyzed. From all mice

similar lobes of liver were placed in formalin while similar lobes were used for snap freezing to ensure comparability and reproducibility. Coded serum samples were analyzed by the University of Colorado Hospital Clinical Chemistry Laboratory for AST and ALT (12). Total serum bile acids and total serum bilirubin were analyzed in coded specimens using a Total Bile Acid and Bilirubin Detection Kit (Diazyme Laboratories, Poway, CA, USA;(Cat No;DZ042A-KY1) Biovision Inc,Milpitas,CA (Cat No K553)) and according to the manufacturer's instructions (7,12)

Primary mouse hepatocyte isolation and plating

Hepatocytes were isolated from fresh liver tissue as previously described with some modifications (19). Briefly, mouse liver was perfused through a 24G catheter inserted into the portal vein using sequential buffers as follows: 1) EGTA containing EBSS (Cat No: 14155 Gibco, Lafayette, CO, USA), 2) Liver perfusion media for 6 minutes (Cat No: 17701 Gibco), and 3) Liver digest media containing collagenase for 15 minutes (Cat No: 17703 Gibco) at 37^{o}C. Following perfusion, liver tissue was liberated and filtered using 70uM strainer and washed with William E media (Cat No: 32551, Gibco). After centrifugation at 25G for 5 minutes, the hepatocytes were resuspended in Williams E media and plated at a density of $7.5X10^{5}$/cells/well on rat-tail collagenase coated (6 well) plates overnight. The following morning, primary hepatocytes were treated as indicated in the Experimental Procedures section.

LC-MS Quantification of Phytosterols

Cell pellets were extracted in ice-cold methanol containing stable isotope labeled standards (0.65 µM stigmasterol-d5 and 1.3 µM sitosterol-d7; Avanti Polar Lipids, Alabaster, Alabama) at $6x10^6$ cells/mL. Retention time and linearity over six orders of magnitude was pre-determined using both light and deuterated standards in methanol and confirmed with spiked-in standards. Samples were agitated (30 min, 4°C) then centrifuged (18,213 g, 10 min, 4 °C). Protein pellets were discarded, and supernatants were injected (15 µL) into a Thermo Vanquish UHPLC system (Thermo Fisher) coupled to a Thermo Q Exactive mass spectrometer (Bremen, Germany). Metabolites were separated on a Kinetex C18 column (150 x 2.1 mm, 1.7 µm – Phenomenex, Torrance, CA, USA) at 50°C using a twelve minute gradient method at 350 µl/min and mobile phase supplemented with formic acid (A: 1:1 acetonitrile:isopropanol 0.1% formic acid; B: acetonitrile 0.1% formic acid). Solvent gradient: 0-2 min hold at 60% B; 2-8.5 min 60-5% B; 8.5-10.5 min hold at 5% B; 10.5-10.6 min 5-60% B; 10.6-12 min hold at 60% B. The Q Exactive mass spectrometer was operated using an APCI source in positive ion mode, scanning in Full MS mode (1 µscan) from 250 to 500 m/z at 70,000 resolutions, with 10-sheath gas, 20 auxiliary gas. Samples were randomized, technical mixes were generated by pooling aliquots of cell extracts, and were run every 10 analytical runs, to control for technical variability, as judged by coefficients of variation (CV). CV was determined by calculating the ratios of standard deviation divided by mean measurements for compounds of interest across all technical mix runs. Data files were converted to mzXML format then analyzed in Maven (Princeton, NJ, USA) and metabolites were identified and validated as previously described (1-3).

IL-1β and TNF ELISA

The human IL-1β and TNF Legend Max ELISA Kit (Bio Legend, San Diego, CA, USA) were used according to the manufacturer's instructions to detect cell culture media human IL-1β (Cat No: 437007) and TNF (Cat No: 430207) respectively.

Histological analysis

Liver and ileal tissues were removed at sacrifice, formalin-fixed, paraffin-embedded and sectioned at 5 microns. Slides were either stained with Hematoxylin and Eosin or analyzed immunohistochemically using the pan macrophage marker F4/80 (clone BM8; BMA Biomedicals, Switzerland) at a dilution of 1:200. Slides were treated with a Proteinase K (Cat No 25530-049, Invitrogen, Carlsbad, CA) antigen retrieval technique at 20ug/ml in TE $CaCl_2$ buffer, pH8 in a 37°C water bath for 6 minutes. Standard immunohistochemical protocols were used thereafter as we have previously described (12). CD-68 staining was done as previously described (24). Histologic images were captured on an Olympus BX51 microscope equipped with a four- megapixel Macrofire digital camera (Optronics; Goleta, CA) using the Picture Frame Application 2.3 (Optronics). All images were cropped and assembled using Photoshop CS2 (Adobe Systems, Inc.; Mountain View, CA).

Supplementary Table 1. List of TaqMan Probes

Mouse

Gene	Probe Set
Nr5a2	Mm00446088_m1
Nr0b2	Mm00442278_m1
Abcg5	Mm00446241_m1
Abcg8	Mm00445980_m1
Abcb11	Mm00445168_m1
Cyp7a1	Mm00_m1

Human

Gene	Probe Set
NR5A2	Hs00187067_m1
NR0B2	Hs00222677_m1
ABCG5	Hs00223686_m1
ABCG8	Hs00223690_m1
ABCB11	Hs00994811_m1
ABCC2	Hs00960489_m1
IL-1b	Hs01555410_m1
TNF	Hs00174128_m1

Supplementary Table 2. List of Antibodies

Antibody	Catalog No	Dilution	Vendor
LRH-1	sc-393369	1:1000	Santa Cruz Biotechnology
SHP	sc-271470	1:500	Santa Cruz Biotechnology
FXR	252165	1:1000	Abbiotec
Total NFκB	8242s	1:1000	Cell Signaling
Phosphor-NFκB	3033S	1:1000	Cell Signaling
Cyp7a1	Ab65596	1:1000	Abcam
ABCG8	NBP1-71706	1:1000	Novus Biologicals
GRB2	3972	1:1000	Cell Signaling
Anti-Mouse- IgG-HRP	HAF007	1:5000	R&D systems
Anti-Rabbit -IgG-HRP	3972	1:5000	Santa Cruz Biotechnology

Supplementary Table 3. List of Primers

Name of Promoter	Primers	Reference
Nr5a2	Epitech ChIP qPCR assays for GPM1042424(+)08A	QIAGEN
Abcg5/8	F:GCTCCAGGGCAGCTTTAACT; R:GCCTTTCTCCCAGCATTCCT	Reference 17

SUPPLEMENTARY FIGURE LEGENDS

Supplemental Figure S1: Expression of *IL-1B* and *TNF* in THP-1 cells and *ABCC2* in HepG2 cells

Co-culture of human monocyte/macrophage THP-1 and HepG2 cells as in Figure 1. mRNA expression of (A) *IL-1B* and (B) *TNF* in THP-1 cells, and (C) *ABCC2/*MRP2 in HepG2 cells*.* Statistical analysis was performed, and adjusted P values obtained using one-way ANOVA with Tukey's correction for multiple comparisons. *p < 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001. (D & E) Cytokines secreted by THP-1 cells from co-culture experiments with HepG2 cells incubated with and without LPS for 4h and with or without stig+sito. IL-1B and TNF were analyzed by ELISA from supernatant in the upper and bottom wells. Statistical analysis was performed, and adjusted P values obtained using one-way ANOVA with Tukey's correction for multiple comparisons.(F) Chromatin immunoprecipitation (ChIP) assay for LRH-1 binding to the promoter of *Cyp7a* in liver homogenate from Chow, DSS-Chow, PN and DSS-PN mice. Data presented as fold change over IgG. Statistical analysis was performed, and adjusted P values obtained using (A, D) one-way ANOVA with Tukey's correction for multiple comparisons. **p< 0.01.(G) Gene expression analysis of *NR5A2/*LRH-1*, ABCG8, and ABCG5* in liver tissue from 6 children with PN-associated cholestasis (PNAC) vs. 5 control children (Table 2). mRNA expression was determined after normalization to *HPRT1* as an endogenous control gene and expressed relative to

results obtained from Controls.(H) Representative image of CD68 immunostaining (20X) in Control children and PNAC children.

Supplemental Figure S2: Effect of siRNA knockdown of *NFκB1* and *NR5A2* on gene expression in HepG2 cells.

(A). Immunoblotting analysis demonstrating that siRNA knockdown of *NFκB1* increases LRH-1 expression in HepG2 cells. (B). Quantification of the integrated density values (IDV) of LRH-1 immunoblot in A following normalization to *GRB2* and expressed relative to untreated control. (C and D). siRNA knockdown of *NR5A2*/LRH-1 in HepG2 cells reduced (C) *NR5A2* mRNA and (D) LRH-1 protein by immunoblotting. (E, F, G). *ABCG5, ABCG8 and NR0B2*/SHP mRNA were similarly reduced by *NR5A2* siRNA in HepG2 cells. Gene expression was normalized to *HPRT1*. Statistical analysis was performed by one-way ANOVA with Tukey's correction for multiple comparisons. **p< 0.01, ***p< 0.001, ****p< 0.0001.

Supplemental Figure S3: Effect of LRH-1 inhibitor on FXR target genes in cultured hepatocytes

Cultured cells were incubated with LRH-1 inverse agonist ML179 for 4 hrs followed by +/- addition GW4064 and +/- stig+sito overnight and mRNA expression was then analyzed. (A) *NR0B2*/SHP mRNA in HepG2 cells. $^{\#}$p<0.0001 vs all other groups. (B) *ABCC2*/MRP2 in HepG2 cells. $^{\#}$p<0.0001 vs all other groups. (C) *ABCG8* in HepG2 cells. $^{\#}$p<0.0001 vs all groups. (D) *Abcb11*/BSEP in in primary

mouse hepatocytes (Mouse 1^{o}). (E) *Nr0b2*/SHP in primary mouse hepatocytes. $^{\#}$p<0.01 vs all other groups. (F,G) *Abcg5* and *Cyp7a1* in primary mouse hepatocytes. (H) Immunoblot of *Cyp7a1* in primary mouse hepatocytes.. For all above experiments, statistical analysis was performed by one-way ANOVA with Tukey's correction for multiple comparisons but in Fig G (by Student's unpaired *t*-test).. *p<0.05, **p<0.01, ***p<0.001, ****p< 0.0001.(I) Gene expression analysis of *Fgf15, Abcg5* and *Abcg8 from* terminal ileum from Chow, DSS-Chow, PN, DSS-PN and DSS-PN/DLPC treated mice. mRNA expression was normalized to *Hprt1* as an endogenous control gene and expressed relative to results obtained from untreated Chow controls.

(J) Liver sections from Chow, DSS-PN, and DSS-PN/DLPC treated-mice were stained with hematoxylin and eosin or immune-stained for macrophages with F4/80, Representative images are shown of at least 3 mice per treatment (CV, central vein, PT, portal triad).

Supplemental Figure S4: IL-1β-induced reduction of *ABCG5/8* mRNA expression enhances the inhibitory effect of phytosterols on FXR target gene expression

HepG2 cells (A) and primary mouse hepatocytes (Mouse 1^{o}) (B) were incubated with IL-1β for 4 hrs followed by +/- GW4064 and +/- stig+sito overnight, cells were harvested and mRNA was analyzed. (A) *ABCG5* in HepG2 cells. $^{\#}$p<0.05 vs all

other groups except GW4064 + IL-1β. (B) *Abcg5* in primary mouse hepatocytes. #p<0.0001 vs all other groups. (C) *NR0B2*/SHP in HepG2 cells. #p<0.05 vs all other groups. *p< 0.05. For all above experiments, statistical analysis was performed by one-way ANOVA with Tukey's correction for multiple comparisons.

Supplemental Figure S5: **The effect of LXR inhibition on phytosterol mediated suppression of *NR0B2*/SHP in HepG2 cells**

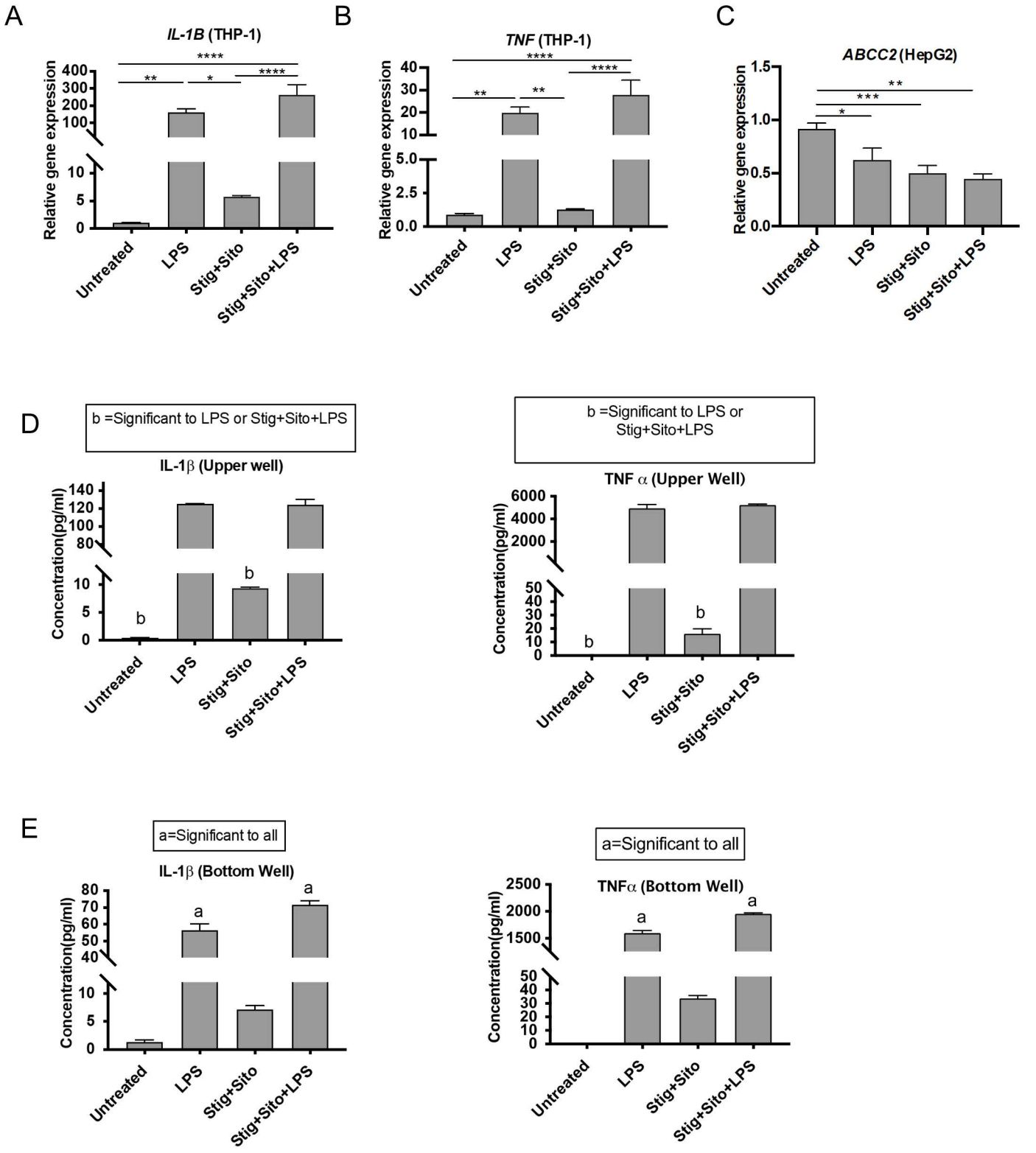
HepG2 cells and primary mouse hepatocytes were incubated in the presence or absence of the LXR antagonist GSK2033 for 4 hrs followed by addition of +/- GW4064 or +/- stig or +/- sito or +/- stig+sito overnight, cells were harvested, and mRNA analyzed. (A) *ABCG5*. #p<0.01 vs all other groups except GSK2033. (B) *NR0B2*/SHP. #p<0.001 vs all other groups. (C) *NR0B2*, effect of stig in presence of GW4064. #p<0.01 vs all other groups. (D) *NR0B2*, effect of stig in absence of GW4064. #p<0.05 vs all other groups (E) *NR0B2*, effect of sito in presence of GW4064. #p<0.01 vs all other groups. (F) *NR0B2*, effect of sito in absence of GW4064. (G) *Abcg5* (H) *Nr0b2* #p<0.05 vs all other groups. For all above experiments, statistical analysis was performed by one-way ANOVA with Tukey's correction for multiple comparisons. *p<0.05, **p<0.01, ***p<0.001. (I) ChIP assay demonstrating FXR binding to the promoter of *NR0B2*/SHP in cell homogenate from HepG2 cells incubated with GW4064 or GW4064+sito overnight. Data presented as fold change over IgG. Statistical analysis was performed by one-way ANOVA with Tukey's correction for multiple comparisons. **p< 0.01.

SUPPLEMENTARY FILE REFERENCES

1. Flynn AR, Mays SG, Ortlund EA, Jui NT. Development of Hybrid Phospholipid Mimics as Effective Agonists for Liver Receptor Homologue-1. ACS Med Chem Lett 2018;9:1051-1056.

2 Mays SG, Stec J, Liu X, D'Agostino EH, Whitby RJ, Ortlund EA. Enantiomer-specific activities of an LRH-1 and SF-1 dual agonist. Sci Rep 2020;10:22279.

Supplementary Figure 1



Supplementary Figure 1

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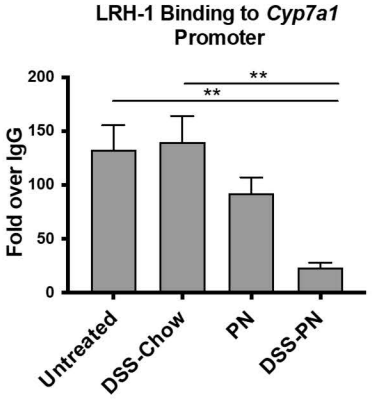

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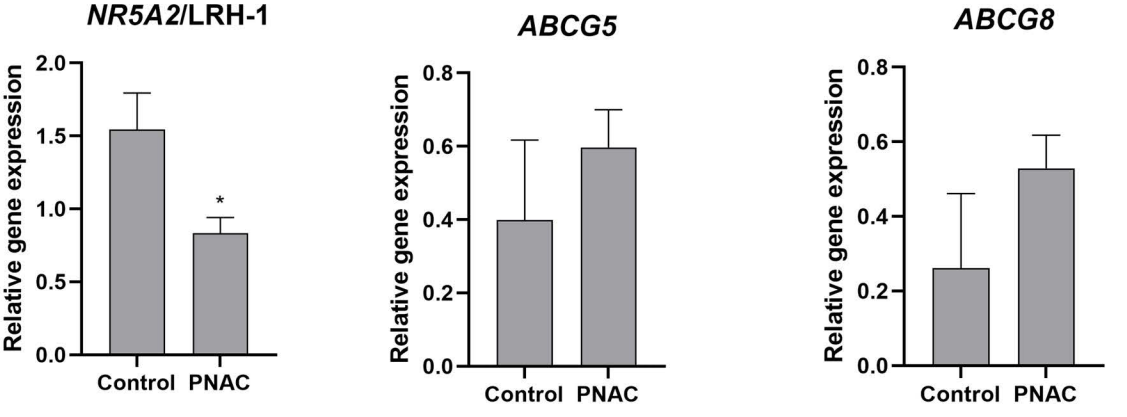

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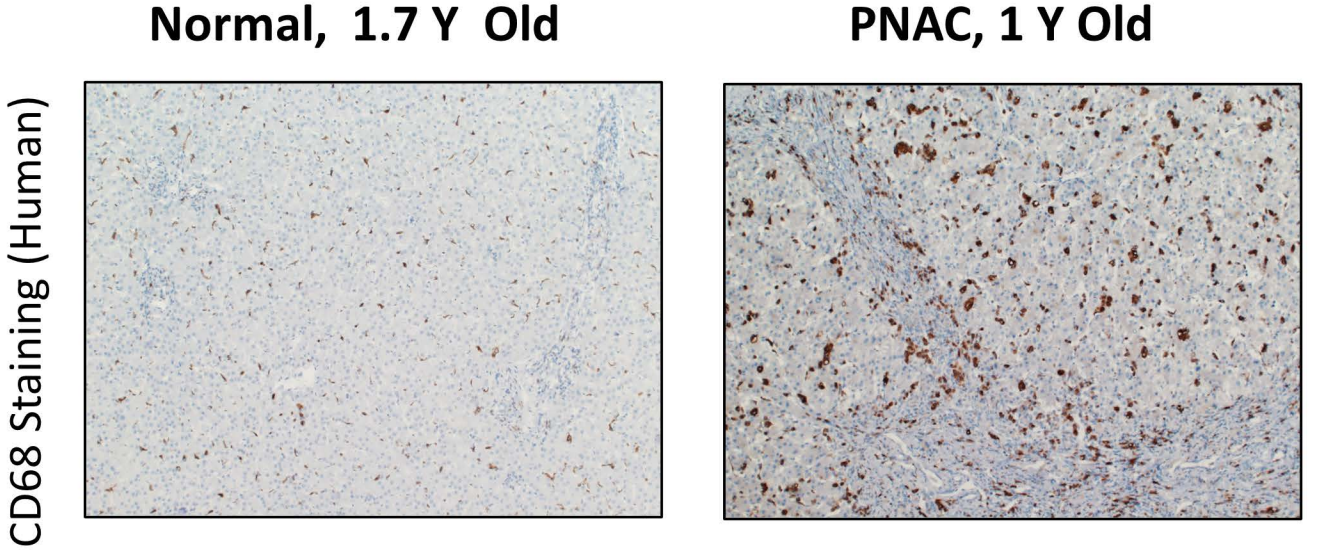

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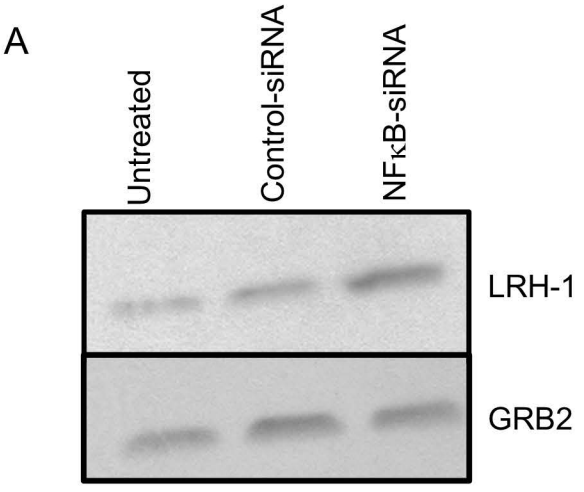

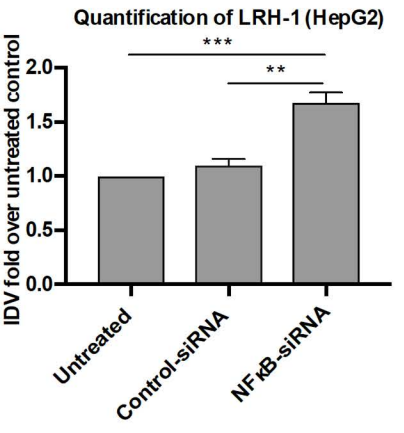

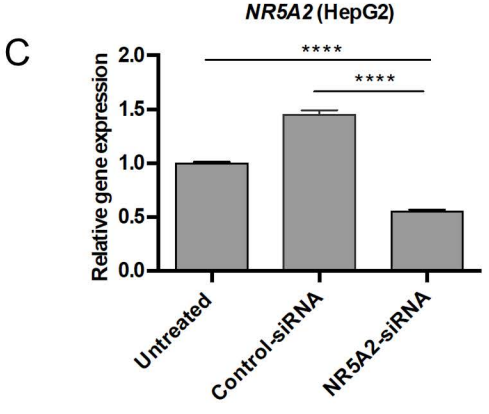

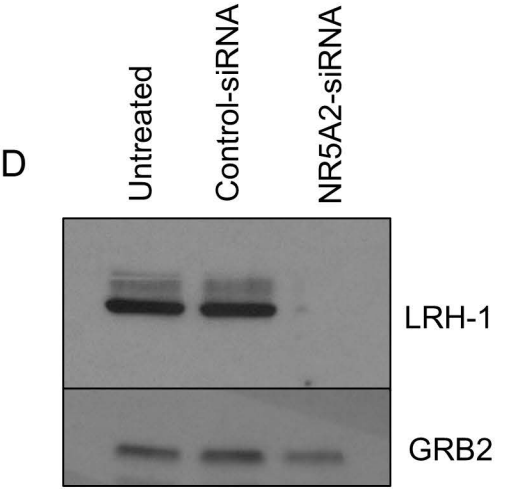

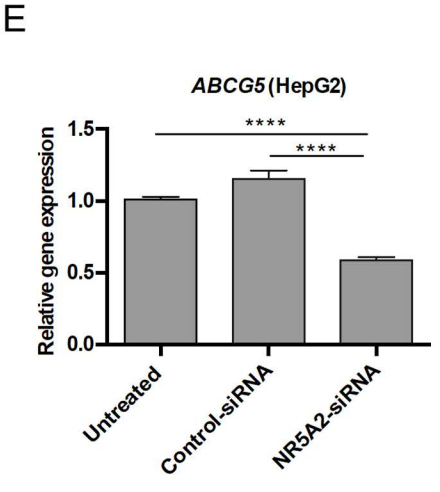

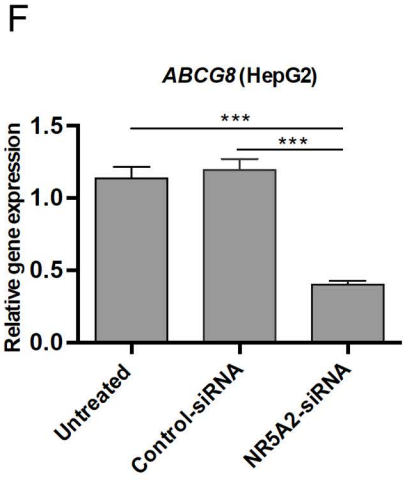

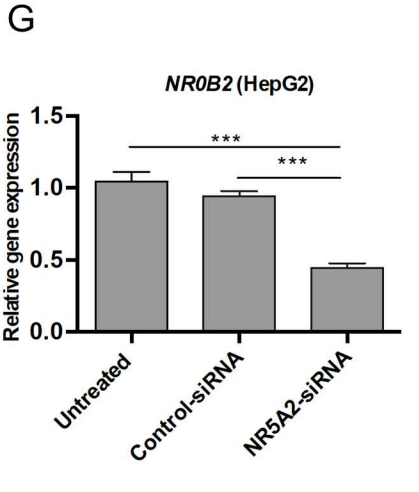

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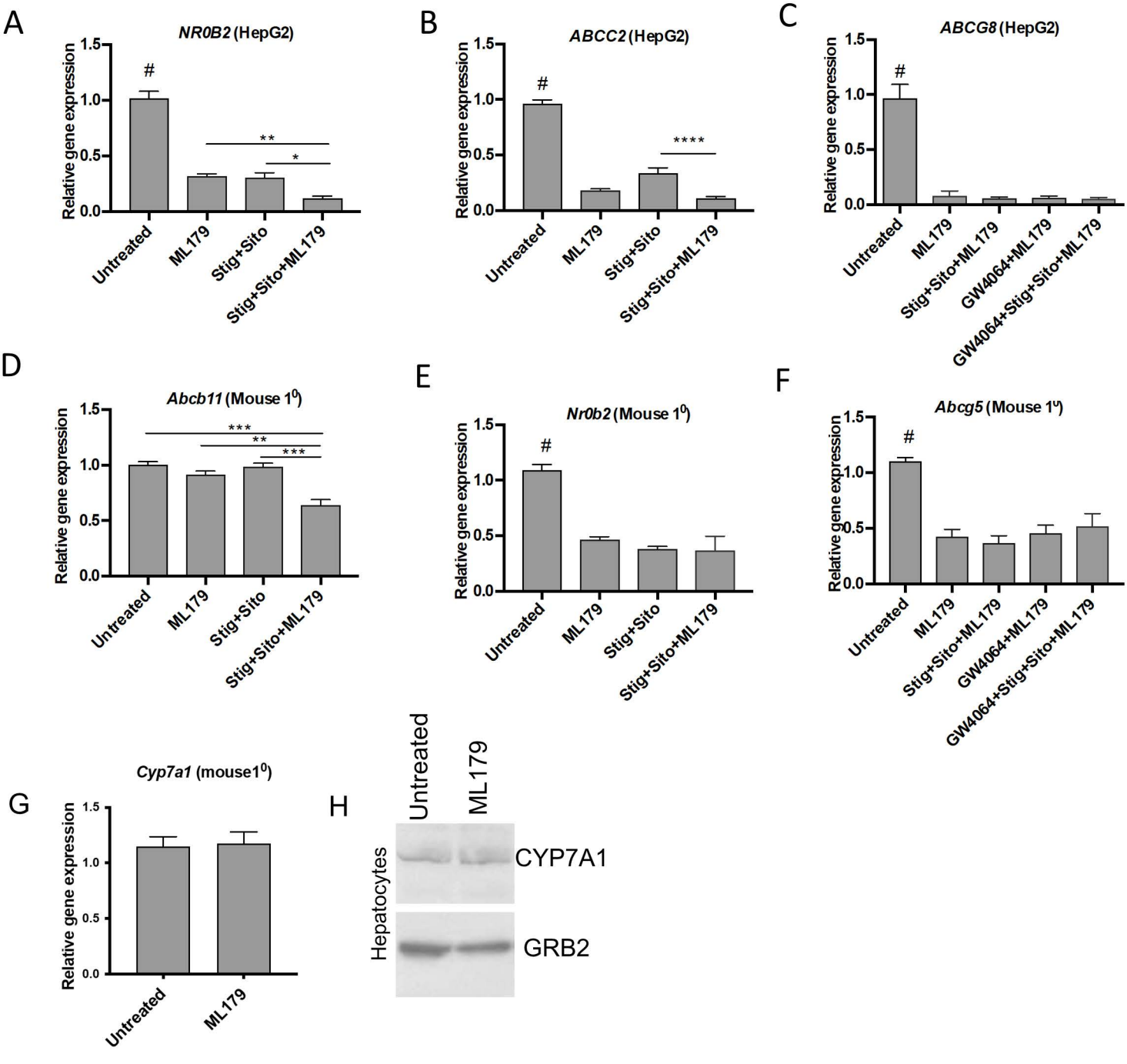

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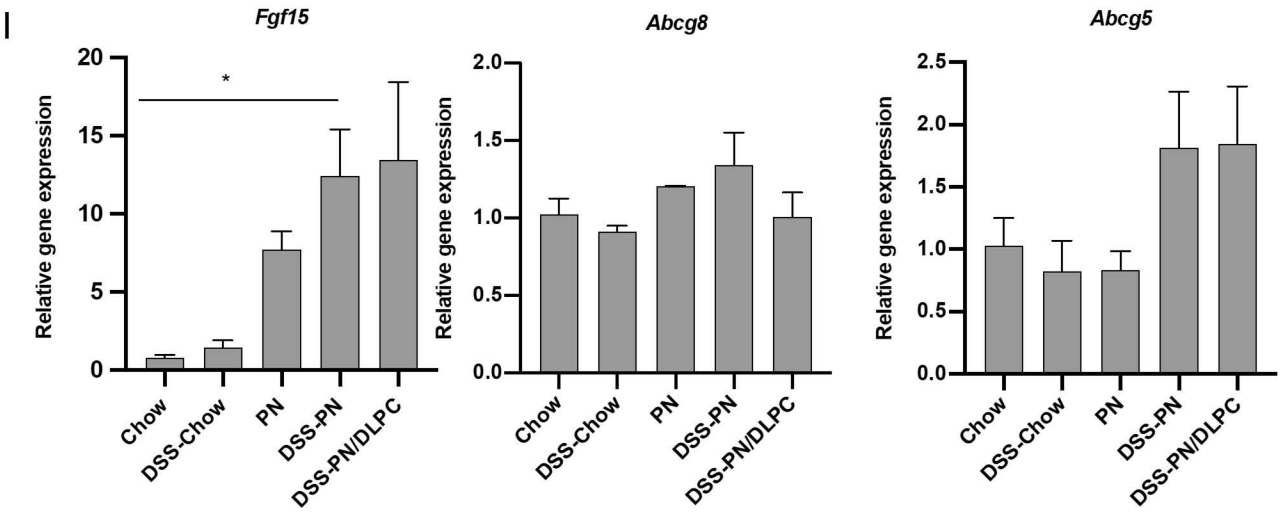

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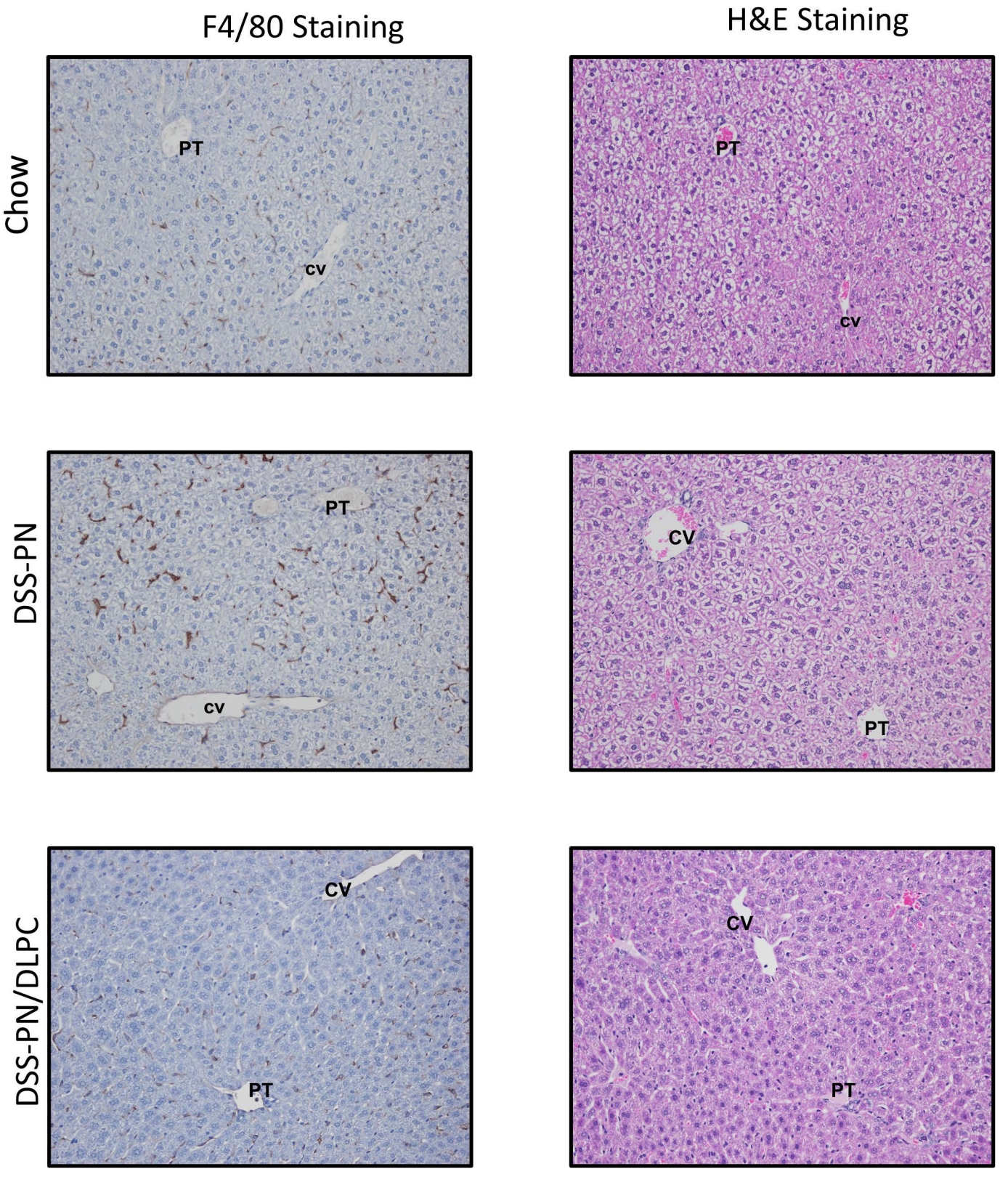

Supplementary Figure 4

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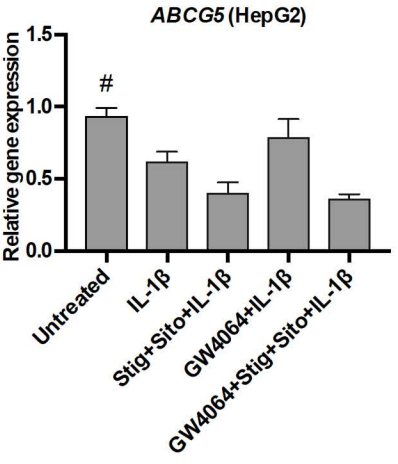

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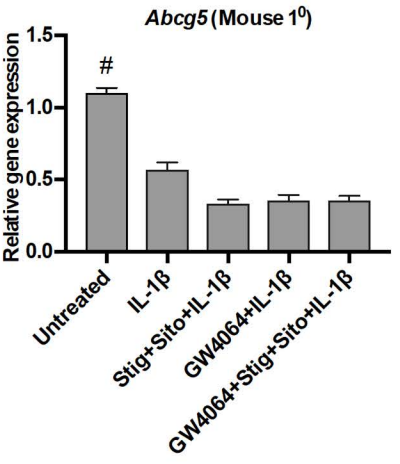

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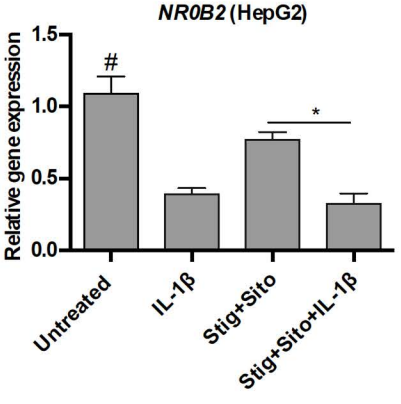

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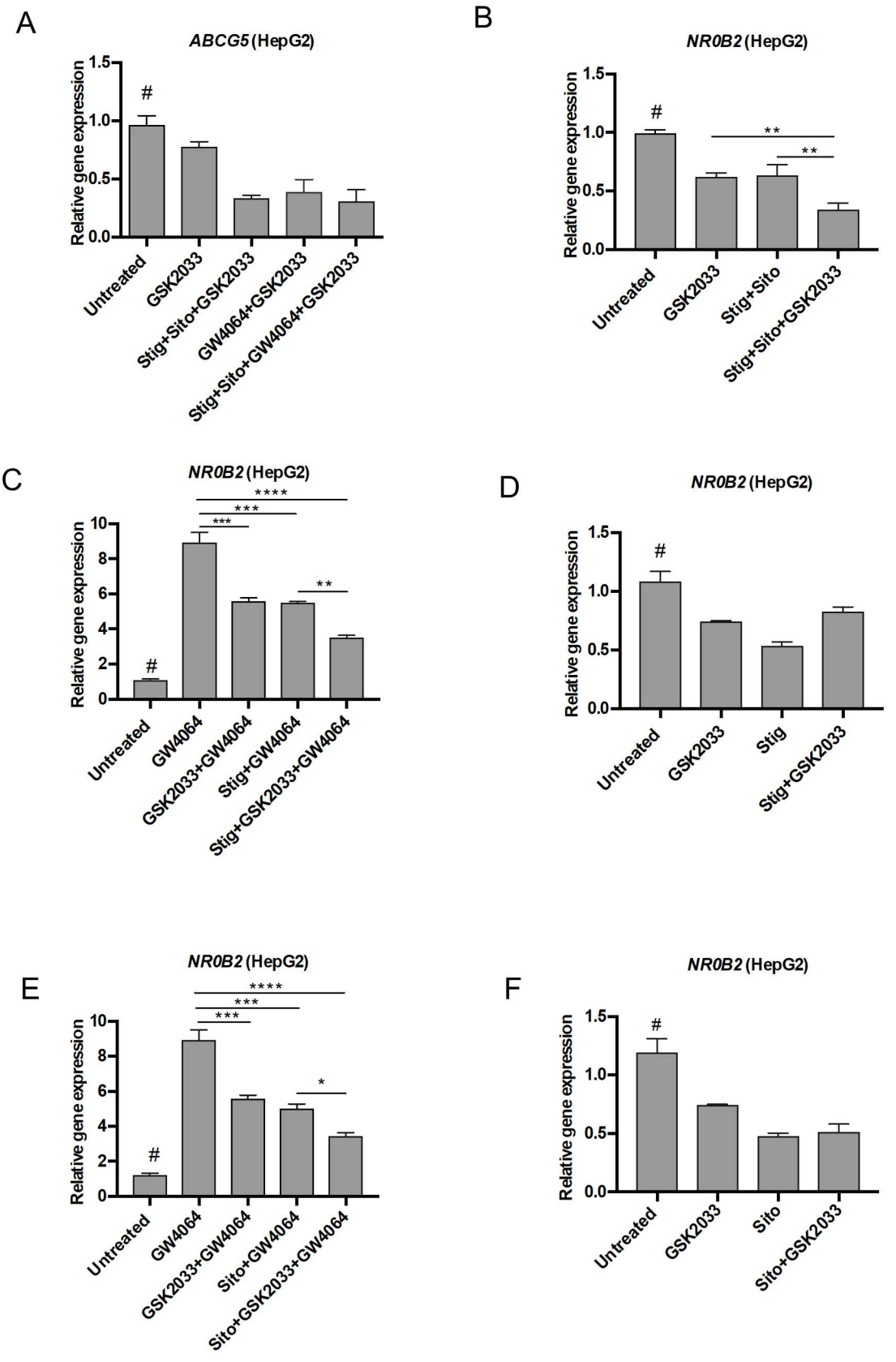

Supplementary Figure 5