Supplementary Materials for
Inhibition of the Prostaglandin Degrading Enzyme 15-PGDH Potentiates Tissue Regeneration

Yongyou Zhang, Amar Desai, Sung Yeun Yang, Ki Beom Bae, Monika I. Antczak, Stephen P. Fink, Shruti Tiwari, Joseph E. Willis, Noelle S. Williams, Dawn M. Dawson, David Wald, Wei-Dong Chen, Zhenghe Wang, Lakshmi Kasturi, Gretchen A. Larusch, Lucy He, Fabio Cominelli, Luca Di Martino, Zora Djuric, Ginger L. Milne, Mark Chance, Juan Sanabria, Chris Dealwis, Debra Mikkola, Jacinth Naidoo, Shuguang Wei, Hsin-Hsiung Tai, Stanton L. Gerson, Joseph M. Ready, Bruce Posner, James K. V. Willson, Sanford D. Markowitz

Correspondence to: SXM10@cwru.edu (SDM); James.Willson@UTSouthwestern.edu (JKVW); SLG5@cwru.edu (SLG); Joseph.Ready@utsouthwestern.edu (JR); Bruce.Posner@UTSouthwestern.edu

Materials and Methods

Statistical comparisons

Graphical depictions of data plot mean values of experimental replicates. Error bars depict standard errors of the mean. Statistical comparisons shown in figures and quoted in text are calculated using two tailed t-tests. In experiments involving multiple group comparisons, overall significance level from ANOVA is also provided. In figures where groups of comparisons are provided, but only a single * value is indicated, the * is labeled as corresponding to the least significant P value that is <0.05.

Mice colonies

Mice studies were conducted in the Case Animal Resource Center with all animal care and procedures performed under a protocol approved by and in accordance with guidelines of Case Western Reserve University's Institutional Animal Care and Use Committee. DSS and partial hepatectomy studies were carried out in male FVB mice (8 to 12 weeks of age) obtained from Jackson laboratories. The animals were housed in standard microisolator cages and maintained on a defined irradiated diet (Prolab Isopro RMH 3000) and autoclaved water. 15-PGDH knock-out mice were maintained on an FVB background as previously described (*39*) and were always compared to control FVB wild-type mice. Identities of 15-PGDH knockout and wild-type mice were confirmed by genotyping as previously described (*22*). Bone marrow and bone marrow transplantation studies involving treatment with SW033291 were done in female C57BL/6J mice obtained from Jackson laboratories at ages of 8-10 weeks.

Assays of tissue prostaglandin levels

Solid tissues were harvested, rinsed in ice-cold PBS containing indomethacin (5.6 μgm/ml), and snap frozen in liquid nitrogen. Marrow was flushed into 1.5ml ice cold PBS with indomethacin; pelleted at 2000 rpm for 5 minutes in an Eppendorf centrifuge; and snap frozen over liquid nitrogen. Frozen samples were pulverized over liquid nitrogen. The powder transferred to an Eppendorf tube with 500 μl of cold PBS with indomethacin, and then homogenized using a tissue homogenizer. The suspension was sonicated in an ice water containing bath sonicator for 5 min using cycles of 20 seconds of sonication with 20 seconds of cooling, followed by centrifugation for 10 minutes at 12,000 rpm. The PGE2 level of the supernate was measured using a PGE2 ELISA Kit (R&D Systems, cat. # KGE004B) and normalized to protein concentration measured by BCA assay (Thermo Scientific, cat. #23225) and expressed as ng PGE2/mg protein. Each sample was assayed in triplicate. PGD2 levels were measured using a Prostaglandin D2 EIA Kit (Cayman, cat. #512031). PGF2a was measured using a PGF2a ELISA Kit (Enzo life Sciences, cat. #ADI-900-069).

SW033291

For biochemical and cell culture studies, SW033291 was dissolved in DMSO and used as a 50 mM stock, with straight DMSO used for control comparisons. For in vivo studies, SW033291 was administered by intraperitoneal injection and was prepared in a vehicle of 10% ethanol, 5% Cremophor EL, 85% D5W at a concentration of 125 μg/200

µl for use in dosing a 25 gm mouse at 5 mg/kg. Corresponding adjustments were made for administering higher doses.

Activity assay of cellular 15-PGDH

15-PGDH enzyme activity was measured in lysates of Vaco-503 cells by following transfer of tritium from a tritiated PGE2 substrate to glutamate by coupling 15-PGDH to glutamate dehydrogenase (*42*). Average values of duplicate determinations were recorded graphically.

Recombinant 15-PGDH protein

The pET-28b vector was modified to express 15-PGDH with addition of a C-terminal 6XHis tag with a TEV cleavage site (sequence: GSKENLYFQGHHHHHH) and three extra amino acids, MAH, at the N-terminus. The protein was expressed in E. coli BL21(DE3)-Rosetta cells and purified using immobilized Ni-affinity chromatography followed by proteolytic cleavage of the 6Xhis tag and size exclusion chromatography. As judged by SDS-PAGE, the sample was >95% pure. Recombinant 15-PGDH protein was frozen at 8 mg/mL protein in a buffer of 50 mM Tris-HCl, pH 7.5, 0.5 mM DTT and 10% glycercol, and stored as aliquots at −80°C until use.

Activity assays of recombinant 15-PGDH protein

For initial characterization of inhibition of 15-PGDH enzyme activity by SW033291, reactions were assembled with experiment specific concentrations of 15-PGDH enzyme, and experiment specific concentrations of SW033291, plus 150 µM NAD(+) and 25 µM PGE2 in reaction buffer (50 mM Tris-HCl, pH7.5, 0.01% Tween 20). The reaction mix was incubated for 15 min at 25 ºC in an Envision Reader (PerkinElmer). Enzyme activity was determined by following generation of NADH as assayed by recording fluorescence at Ex/Em=340 nM/485 nM every 30s for 3 minutes, commencing immediately after addition of PGE2 (*43*). IC_{50} values were calculated with GraphPad Prism 5 software (http://www.graphpad.com/scientific-software/prism/) using the sigmoidal dose-response function and plotted against SW033291 concentration. The linear increase in IC_{50} value with increasing enzyme concentration indicated a tight-binding inhibition with the dependence on 15-PGDH:SW033291 stoichiometry rather than absolute SW033291 concentration.

For analysis of initial reaction rates, kinetic reactions containing 10 nM 15-PGDH enzyme, 150 µM NAD(+), 25 µM PGE2, and varying concentrations of SW033291 were assembled in a total volume of 200 µL in reaction buffer (50 mM Tris-HCl pH7.5, 0.01% Tween 20). The generation of 15-keto-PGE2 was calculated by following the change of NADH fluorescence (Ex/Em=340 nM/485 nM) every 15s for 195s (*43*) and was plotted versus reaction time. Successive reactions contained SW033291 concentrations of 0, 0.2 nM, 0.25 nM, 0.4 nM, 0.5 nM, 0.8 nM, 1 nM, 1.6 nM, 2 nM, 3.25 nM, 5 nM, 7.5 nM, 10 nM, 15 nM, 20 nM.

To derive the Ki^{App}, relative initial reaction velocities were plotted against SW033291 concentration and fitted to the Morrison equation for a tight-binding inhibitor (*23*) using GraphPad Prism 5 software. This analysis gave an active enzyme concentration value [E]T of 8.52 nM, indicating 85.2% activity in the enzyme preparation, and Ki^{App}=0.10 nM.

Determining effect of PGE2 concentration on SW033291 IC_{50}

Assays of 15-PGDH enzyme activity were done at 5 nM 15-PGDH, 150 µM NAD(+), 50 mM Tris-HCl, pH7.5, 0.01% Tween 20, and PGE2 concentrations of 5 µM, 10 µM, 20 µM, 40 µM. Activity was determined as the rate of NADH generation as determined by fluorescence (Ex/Em=340 nM/485 nM) measured every 30s for 15 mins. The IC_{50} values were calculated with GraphPad Prism 5 software (http://www.graphpad.com/scientific-software/prism/) as described above.

15-PGDH thermal denaturation

Thermal denaturation of 15-PGDH was monitored by differential scanning fluorimetry using SYPRO orange dye (*43*). Briefly, the protein was diluted to a final assay concentration of 10 µM in 100 mM Tris buffer pH 8.0, containing 0.01% Tween-20 and 1:1000 SYPRO orange dye (Invitrogen). The final assay volume was 20 µL, with or without 100 µM of NADH. SW033291, in assay buffer plus 0.4% (v/v) DMSO, was added to 20 µM final concentration. Heat denaturation curves were recorded using a real-time PCR instrument (CFX-96, Bio-Rad) applying a temperature gradient of 2 C/min. Analysis of the data was performed using default Bio-Rad CFX Manager V3.1 software. Melting temperatures of 15-PGDH were determined by the inflection points of the plots of –d(RFU)/dT.

Thermal denaturation of HSD17B10 and BDH2

Specificity of interaction of SW033291 with 15-PGDH was assessed by testing SW033291 effect on melting temperature of HSD17B10 and BDH2, two short chain dehydrogenases that are both closely structurally related to 15-PGDH. Recombinant HSD17B10 and BDH2 were purchased from Syd Labs, Boston, MA.

Assay of SW033291 affect on PGE2 level

The A549 cell line was maintained in F12K medium supplemented with 10% fetal calf serum (FBS) and 50 µg/mL gentamicin in a humidified atmosphere containing 5% CO2 at 37° C. Cells were plated in duplicate in a 24-well plate (1 mL per well) at $1X10^5$ cells per well and grown for 24 h before stimulation with IL-1β (1 ng/mL) overnight (16 h) to induce COX2 expression and PGE2 production. Cells were then treated for an additional 8 hours with fresh medium containing the indicated concentration of SW033291. Medium was then collected and the level of PGE2 analyzed using a PGE2 enzyme immunoassay Kit (R&D System). Data were collected from four independent experiments. Results were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests. Parallel determinations of cell viability were performed using the CellTiter-Glo® assay (Promega).

Bone marrow colony forming assays

To assay for colony forming capacity, whole bone marrow was isolated from 8-12 week old mice. In studies of 15-PGDH knockout and control mice, twenty thousand bone marrow cells from each mouse were directly plated in duplicate 3 cm^2 plates coated with complete methylcellulose media containing IL3, IL6, SCF, Epo (MethoCult® , Stem Cell Technologies M3434). In studies from SW033291-treated mice (10 mg/kg injected

twice daily IP for 5 doses), twenty thousand bone marrow cells from each treated and control mouse were harvested at 6 hours after the last SW033291 or vehicle injection and then plated in duplicate 3 cm^2 plates. After 14 days, colonies were counted, scored, and subtyped, in blinded fashion, by specially trained personal from the Case Comprehensive Cancer Center Hematopoietic Biorepository and Cellular Therapy Core Facility. Bone marrow cellularity was determined at the time of harvest from a 1:100 dilution in PBS by counts performed using a hemocytometer under a light microscope. Using marrow cellularity values, CFU counts were normalized to per femur values. CFU counts were tabulated graphically with error bars corresponding to standard error of the means and different treatments were compared using 2-tailed t-tests.

Ex Vivo treatment of murine bone marrow with SW033291

Whole bone marrow was isolated from 8-10 week old female littermate FVB mice that were either 15-PGDH wild-type or knockout, and incubated with either 0.5 μM SW033291 or vehicle-control for 2 hours on ice. For assay of colony forming activity twenty thousand cells were plated in 3 cm^2 plates coated with complete methylcellulose media containing IL3, IL6, SCF, Epo (Stem Cell Technologies M3434) and scored after 14 days. Marrow from 3 mice were individually treated, and then plated in duplicate into a total of 6 separate wells. CFU counts were tabulated graphically with error bars corresponding to standard error of the means and different treatments were compared using 2-tailed t-tests.

Hematopoietic analysis of SW033291-treated mice

8-10 week old female C57BL/6J mice were injected IP with either vehicle or SW033219 (10 mg/kg) twice daily for 5 doses. Peripheral eye blood was taken from mice 6 hours after the last treatment and blood counts were recorded using the Hemavet 950fs (Drew Scientific). Blood counts were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests. In addition, mice were sacrificed and marrow flushed 6 hours following the final treatment for SKL and SLAM analysis as described below.

Hematopoietic analysis of 15-PGDH knockout mice:

Female 15-PGDH knockout mice on an FVB background and control female FVB mice, 8-10 weeks of age, were characterized for peripheral blood counts as described above and for bone marrow stem cells as described below.

Bone marrow stem cell analyses

Bone marrow cellularity was determined via 1:100 dilution in PBS and counting performed using hemocytometer under light microscope. To identify cell populations, total marrow was incubated with lineage markers (CD3, CD4, B220, Ter119, CD11b) and the percentage of Sca1+/c-Kit+ cells in lineage negative cells was determined using flow cytometry on a BD LSRII cytometer (BD Biosciences). The SLAM cell population was characterized by further sub-gating the SKL population and measuring the frequency of CD48-/CD150+ cells within SKL. Data was analyzed on Flowjo Version 8.8 software (Treestar). Cellularity values were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

Bone marrow gene expression

Mice were treated with 5 IP doses administered twice daily of either: vehicle, SW033291 (10 mg/kg), SW033291 (10 mg/kg) + EP1 antagonist SC-51322 (10 μg/mouse) (Cayman), SW033291 (10 mg/kg) + EP2 Antagonist PF04418948 (10 μg/mouse) (Cayman), SW033291 (10 mg/kg) + EP3 antagonist L-798,106 (10 μg/mouse) (Santa Cruz), or SW033291 (10 mg/kg) + EP4 Antagonist L-161982 (10 μg/mouse) (Cayman). Bone marrow was harvested at 2 hours after the last injection. Whole marrow was incubated with antibodies to isolate the SKL population (FITC-sca1; APC-ckit; lineage markers PE- CD3,B220,CD11b,Ter119,Cd4- ; BD Bioscience) or the $CD45^-$ population (PE-CD45- ; BD Bioscience) on ice for 20 minutes. Populations were sorted using the BD FACSAria cell sorter and RNA obtained from the isolated populations. Gene expression in different experiments was measured using Applied Biosystems TaqMan® primers and probe sets to mouse CXCR4 (Mm01996749_s1), CXCL12 (Mm00445553_m1), SCF (Mm00442972_m1), JAG1 (Mm00496902_m1), Survivin (MM00599749_m1), and endogenous controls GAPDH(Mm03302249_g1).

Cyclic AMP analyses in bone marrow:

CD45- cells were isolated from bone marrow from mice treated with 5 doses given IP twice daily of SW033291 (10 mg/kg) or vehicle control, with marrow harvested at 3 hours after the last injection. CD45- cells were prepared by depletion using CD45 MicroBeads (Miltenyi Biotec, Cat # 130-052-301) and cyclic AMP levels measured cAMP Parameter Assay Kit (R&D System, Cat# KGE002B) and normalized to total protein concentration.

Bone marrow homing assays

Whole bone marrow from 8 week old female C57BL/6J mice was labeled with 5 μM CellTrace CFSE (Life Technologies) and transplanted into lethally irradiated recipient mice (of same age, gender, and strain). Mice were irradiated with 11Gy total body irradiation 12 hours prior to transplant. Recipient mice were treated with either vehicle, 10 mg/kg SW033291, or a combination of Indomethacin (5 mg/kg) + SW033291, Plerixafor (10 mg/kg)(Sigma) + SW033291, EP2 Antagonist PF04418948 (10 μg/mouse) (Cayman) + SW033291, or EP4 Antagonist L-161982 (10 μg/mouse) (Cayman) + SW033291 for three doses. The three treatment doses were administered immediately following 11Gy IR, immediately following transplant, and 8 hours post transplant. After 16 hours whole marrow was flushed from recipient mice and the total percentage of CFSE positive bone marrow was analyzed on a BD LSRII flow cytometer.

Murine bone marrow transplantation

For survival analysis, 8-10 week old female C57BL/6J recipient mice were lethally irradiated at 11 Gy and transplanted with 200,000 whole bone marrow cells from 8-10 week old female C57BL/6J donor mice. Following transplant the recipient mice received twice daily IP injections with either vehicle or 5 mg/kg SW033291. Animal survival was monitored and recorded daily, and displayed graphically. Significance of differences between survival curves was determined using a two tailed Log-rank (Mantel-Cox) test. To follow recovery of blood counts, 8 week old mice were lethally irradiated at 11 Gy

and transplanted with 500,000 whole bone marrow cells. Mice received twice daily IP injections with either vehicle or 5 mg/kg SW033291. Animals were sacrificed at days 5, 8, 12, and 18 and blood counts, bone marrow cellularity, and SKL percentage was measured at each time-point using the Hemavet 850fs. Blood counts were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

Long term survival following bone marrow transplantation

0.5 million whole bone marrow cells from 8 week old wild-type mice were transplanted into recipient mice lethally irradiated with 11Gy total body irradiation 12 hours prior to transplant. Recipient mice were treated with either vehicle (N=10) or 5 mg/kg SW033291 (twice daily IP) (N=10) for 21 days. Animal survival was recorded in recipient mice at seven months post transplant.

Serial Transplantation

1 million whole bone marrow cells from 8 week old wild-type mice were transplanted into recipient mice lethally irradiated with 11Gy total body irradiation 12 hours prior to transplant. Recipient mice were treated with either vehicle or 5 mg/kg SW033291 (twice daily IP) for 21 days. 8 weeks post-transplant recipient mice were sacrificed, marrow harvested, and 1 million whole marrow cells were transplanted into second cohort of lethally irradiated recipient mice. This process was serially repeated to generate 3 successive generations of mice descended from the initial transplant recipients. Animal survival was recorded at each round of transplant.

DSS colitis studies

Groups of male FVB mice 8-12 weeks of age were administered 7 days of drinking water containing 2% dextran sodium sulfate, MW 36-50kDa (Cat. #160110, MP Biochemical), formulated in autoclaved distilled water, and then switched to regular drinking water for 7 to 14 days. Mice were followed for daily body weights, recorded as a percentage of day 1 body weight. Results were tabulated graphically with error bars corresponding to standard error of the means. Different treatment arms were compared across the time course of study using 2-tailed paired t-tests.

Mouse colonoscopy

Colonoscopy was done under isofluorane anesthesia. The colonoscopy system consisted of a high resolution video-endoscopic system (Olympus VISERA, OTV-S7pro) and a miniature endoscope (1.9 mm in diameter) that was originally designed for human pediatric urethroscopy. Video-endoscopic procedures were monitored and recorded. Narrow band imaging was used when required to increase contrast and to confirm identity of small ulcerations. Mice followed by colonoscopy underwent serial examinations on study days 8, 11 and 15. Mice used for colonoscopy studies were maintained as a separate cohort and analyzed only in comparison to other mice in the colonoscopy group. Ulcer counts were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

Analysis of colon ulcer lengths

On study day 15, mice colons were flushed, opened, cut into longitudinal thirds from distal, middle, and proximal colon, laid flat for 30 minutes on a Whatman paper soaked with neutral buffered formalin, and then layered one level above the next in alternating layers of tissue and agar, with the mucosa of one tissue level facing the serosa of the next tissue layer, to create a "*petit four*" arrangement. Blocks are solidified by 30 minutes of refrigeration, and then fixed overnight at 4 degrees C in neutral buffered formalin. Petit four blocks were then paraffin embedded, and 5 μM sections cut such that slides contained vertical sections through the colonic mucosa from each tissue layer in the block, thus capturing the entire length of the colon from cecum to anus. Slides representing random longitudinal sections through the colon were then stained with hematoxylin and eosin and examined under the microscope to identify mucosal ulceration (characterized by complete loss of colonic epithelium and colonic crypts with erosion and purulent inflammatory exudate, see Fig. 5B). Ulcer measurements were obtained from photomicrographs taken using the Nikon E 800 microscope in one to three sections (of each colon segment - proximal, middle and distal) and lengths calculated using the MetaMorph (Molecular Devices) image analysis program free form line tool with pixel number calibrated for each magnification. Ulcer measurements were summed and expressed as a percent of total colon length. The colonic lengths were obtained from an 8 x11 print of the high resolution flatbed scan image of a single hematoxylin & eosin stained 5 micron section. Each slide section contained a split mirror image of the colon *petit four* block composed roughly of equally divided -proximal, middle and distal colon (6 segments total). Measurements were performed on the print using an engineering ruler calibrated to 0.5 mm. The final measurements were averaged over the two slide sections and a reduction factor performed for the printed image. Lengths of average ulcerated mucosa were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed T-tests.

Murine Endoscopic Index of Colitis Severity (MEICS) Score

The MEICS score, assessed during colonoscopy, grades colitis severity on a scale of 0-15 and is derived by combining 5 component scores that were each graded on a 0-3 scale: mucosal thickening, change in mucosal vascular pattern, fibrin exudate, mucosal granularity, and stool consistency (*31*). MEICS scores were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

Cryptitis score assessment

Each of the distal, middle and proximal colon segments from DSS treated mice were divided into 4 equal sections (12 sections total). For each section, a cryptitis severity grade was assigned using the grading system of Cooper and colleagues (*32*) as: 0 (no damage), 1 (basal 1/3 damaged), 2 (basal 2/3 damaged), 3 (crypt lost with intact epithelium), 4 (crypt lost and surface epithelium lost). For each section the percent of area affected by cryptitis was further scored (area score) as 0 (none), 1 (1-25% involvement), 2 (26-50% involvement), 3 (51-75% involvement), 4 (75-100% involvement). A cryptitis section score was obtained by multiplication of the (severity grade)*(area score) yielding a section score of 0-16. For each of the distal, middle, and

proximal colon segments, the average cryptitis score was calculated by averaging the cryptitis scores for all of the sections across that segment. A whole colon cryptitis score was determined for each mouse by summing the average cryptitis scores from the distal, middle and proximal colons to yield final cryptitis score ranging from 0-48. In practice DSS largely spares the proximal colon, yielding an effective experimental range of 0-32. Mean cryptitis scores were determined on study day 8 for cohorts of 15-PGDH wild-type mice treated with SW033291 at 5 mg/kg twice daily IP, 10mg/kg twice daily IP, or vehicle control twice daily IP, and for 15-PGDH knock out mice. Cryptitis scores were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed T-tests.

BrdU labeling of colon crypts

Mice were injected IP with BrdU 100 mg/kg (Sigma #B5002-1g) 3 hours prior to sacrifice. Colons were excised, flushed, opened and formalin fixed and paraffin embedded in "petit four" blocks as described above. Slides that captured longitudinal sections through the colonic mucosa from the entire length of the colon, from cecum to anus were cut, deparaffinized, and rehydrated through graded water: ethanol mixtures. Antigen retrieval was done in 0.01M sodium citrate buffer pH 6.0 at 95^0C for 20 minutes. Nuclei with BrdU incorporation were visualized by staining with rat-anti-BrdU primary antibody (Abcam Ab6326) and with Alexa594 conjugated goat anti rat secondary antibody. Colonic crypts were visualized by staining for E-Cadherin using FITC-conjugated mouse anti-E-Cadherin antibody (BD Biosciences #BD612131). Slides were counterstained with DAPI. Distal, middle, and proximal colon segments were separately examined. For each segment, BrdU positive cells were counted across a minimum of 50 crypts distributed across 5 random 20X microscopic fields, to determine an average number of BrdU positive cells per crypt per segment. For each colon, an overall colon average of BrdU positive cells per crypt was calculated as the average value of the proximal, middle, and distal colon determinations. Overall colon BrdU labeling counts were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

Disease Activity Index (DAI)

A modified DAI, based on that of Cooper and colleagues (*32*), scored diarrhea and rectal bleeding on a scale of 0-6, and was calculated as the sum of two indexes that on a scale of 0-3 respectively score diarrhea, and bleeding. DAI scores were tabulated graphically with error bars corresponding to standard error of the means. Different treatment arms were compared across the time course of study using 2-tailed paired t-tests.

DSS induction of colonic cytokines

The DSS-induced colitis procedure was the same as described above with mice treated with an initial 7 days of DSS containing drinking water followed by regular drinking water for 7 days. On day 15, mouse colons were collected, flushed with chilled 1xPBS, and opened length-wise. After removal of the rectum, a 0.5 cm section of the distal colon was collected and snap frozen in liquid nitrogen for storage until RNA processing. RNA was isolated from frozen colon samples using the RNAqueous Total

RNA Isolation kit (Life Technologies) with the following modifications to the protocol. A volume of 350 µl of lysis buffer was added to the frozen sample, pulverized using a tissue homogenizer, and further sheered by passing through a 22 gauge needle 10 times followed by 10 times through a 26 gauge needle. RNA was eluted in two successive 40 µl and 20 µl volumes of elution buffer and then pooled for a total of 60 µl collected. RNA concentrations were determined using a Qubit 2.0 Fluorometer using the Qubit RNA Assay Kit (Life Technologies). All RT-qPCR assays were performed following the MIQE guidelines. cDNA was synthesized using AMV RT (Roche) following the manufactures recommended protocol. Real-time PCR measurement was performed in a 20 µl reaction mix containing 1 µl of cDNA template and a 1:20 dilution of primer/probe set in 1X Supermix (Bio-Rad) and was run on a CFX96 optical module (Bio-Rad). Thermal cycling conditions were 95ºC for 3 min, followed by 50 cycles of 95ºC for 15 sec and 60ºC for 1 min. Mouse hydrolysis probe/primer sets for all cytokines assayed were obtained from Life Technologies and were as follows, Mm00443258_m1 (Tnf, NM_013693), Mm01168134_m1 (Ifng, NM_008337), Mm00434228_m1 (Il1b, NM_008361), Mm00434256_m1 (Il2, NM_008366), Mm00441242_m1 (Ccl2, NM_011333), and Mm00443111_m1 (Ccl4, NM_013652). Hprt (NM_013556), Mrpl19 (NM_026490), and Ubc (NM_019639) were used as the reference gene set for normalization and were amplified using the hydrolysis probe/primer kits Mm00446968_m1, Mm00452754_m1, and Mm01201237_m1, respectively, from Life Technologies and followed the same reaction conditions above for amplification. The geometric mean (GEO3) of the Cq values for HPRT, UBC, and MRPL19 was used for normalization. The level of Gene Of Interest (GOI) expression was determined as the ratio of GOI:GEO3 = $2\exp-(Cq_{GOI} - Cq_{GEO3})$. For each reverse transcription reaction, Cq values were determined as the average values obtained from three independent real-time PCR reactions. RNA that had not undergone the reverse transcriptase step, as well as a water sample that was carried through the reverse transcriptase step, were used as negative controls. Both controls were negative for all assays performed.

Construction of chimeric mice

Whole bone marrow was harvested from 8 week old 15-PGDH wild-type FVB and 15-PGDH knockout FVB Mice. 1 million total cells from each group was transplanted into lethally irradiated 15-PGDH wild-type FVB mice. At 8 weeks post transplantation the resulting chimeric mice were placed on DSS treatment.

Partial hepatectomy

10-12 week old male FVB mice, or 10-12 week old male 15-PGDH knockout mice on an FVB background, were placed under isoflurane anesthesia and underwent a two-thirds partial hepatectomy through resection of the median and left lateral hepatic lobes as described by Mitchell and Willenbring (*35*). Mice that were treated with SW033291 received injections in a vehicle of 10% ethanol, 5% Cremaphor EL, 85% D5W, or with vehicle control. The SW033291 injections were commenced at the time of surgery and continued twice daily throughout the study. Following sacrifice, livers were removed, and weights determined for whole mouse and for isolated livers. Liver weights and ratios of liver weight to body weight were tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

BrdU labeling of regenerating liver

Mice that had undergone partial hepatectomy were injected with BrdU at 50 mg/kg IP 2 hours prior to sacrifice. Tissues were formalin fixed and paraffin embedded. Antigen retrieval on deparaffinized 5 μm sections was performed at 98^0C in Antigen Unmasking Solution (Vector Laboratories, Cat # H-3300). Endogenous peroxidases were inactivated using BioCare Peroxidazed I (Cat #PX968M). Endogenous immunoglobulin was blocked using BioCare Rodent Block M (Cat # RBM961). BrdU incorporation was detected using Rat-Anti-BrdU monoclonal antibody (Abcam, Cat# Ab6326) with detection using BioCare reagents RTP629L, RTH630L, BDB2004L, followed by counterstaining with hematoxylin. BrdU labeling counts were determined at each data point by counting 10 random high powered (40X) fields per mouse, with from 6 to 10 mice assessed for each data point. Counts of BrdU positive cells were normalized to an average count of 87.5 hepatocytes per high powered field to derive the percent of BrdU positive cells. BrdU labeling was tabulated graphically with error bars corresponding to standard error of the means and compared using 2-tailed t-tests.

Cyclic AMP analyses in regenerating liver

Cyclic AMP levels were measured in liver lysates using cAMP Parameter Assay Kit (R&D System, Cat# KGE002B) and normalized to total protein concentration.

High throughput screen for 15-PGDH interacting compounds

A high throughput screen to identify 15PGH interacting compounds was performed at the University of Texas High Throughput Screening Core, as described in steps 1-8 below.

1. 15-PGDH reporter assay: A targeted knock-in vector (*44*) was constructed to replace exon 7 of the HPGD gene, that encodes for 15-PGDH, with HPGD exon-7 fused in frame to a Renilla luciferase coding cassette, such that the HPGD locus would now encode for a 15-PGDH-luciferase fusion protein. The vector was packaged into adeno-associated virus and transducted into Vaco-9m (V9m) cells to generate V9m-SC3, a clonal derivative that showed low background and good inducibility of the reporter construct. Additional reporter lines were constructed using the same knock-in targeting vector recombined into colon cancer cell lines LS174T and Vaco-503 (V503).

2. Cell culture: V9m-SC3 cells were grown in minimum essential medium (MEM – Invitrogen, Cat# 10370-021) supplemented with 8% FBS (Hyclone, Cat# SH30071-03), 2 mM L-glutamine (Invitrogen, Cat# 25030-081), and 50 μg/ml gentamicin (Invitrogen, Cat# 15750-078). V9M-TK-Renilla cells were cultured in the same medium as above plus 5 μg blasticidin S (Invitrogen, Cat# R210-01). V503 cells were cultured in MEM supplemented with 2 % FBS, 2 mM L-glutamine, 2 μg/ml transferrin (Invitrogen, Cat# 13008-016), 0.86 ng/ml selenium (Sigma, Cat# S-5261), 10 μg/ml insulin (Sigma, Cat# I-0516), 1 μg/ml hydrocortisone (Sigma, Cat# H-0396), and 50 μg/ml gentamicin. LS174T-SC1 cells were cultured in MEM – Eagle (ATCC, Cat# 30-2003) supplemented with 10% FBS, 2 mM L-glutamine, and 50 μg/ml gentamicin.

3. UT Southwestern Chemical Library: The UT Southwestern chemical library (~230,000 small molecules) is comprised of small organic molecules purchased from the following vendors: ChemBidge Corporation (75,000), Chemical Diversity Labs (100,000), ComGenex (22,000), TimTek (1200), Prestwick (1100), and NIH (clinical collection, 450) as well as those submitted for screening by UTSW chemistry labs (2,500). The compounds in the library satisfy a relaxed set of Lipinsky's rules, with 99% having a molecular weight less than 550 (average 250-300).

4. HTS assay: V9M-SC3 cells from 60-90 % confluent 15-cm dishes were detached with Accutase (Innovative Cell Technologies, Cat# AT-104) and resuspended in 5 ml of medium. Cells were then vigorously mixed with pipette up and down 10-15 times to minimize clumping. After counting with hemocytometer, cells were plated at 8,400 cells/well in 384-well plate (Corning Cat#3570) in a volume of 60 µl using a Multidrop (MTS Lab Systems). Following a 48-hour incubation period, library compounds (columns 3 to 22), DMSO (columns 2 and 23), and Lapatinib (column 1) were added using a Biomek FX liquid handling system. Final concentration were 0.5% (DMSO), 2.5 µM UTSW library compounds and 0.5 µM (Lapatinib). Column 24 was left untreated. After 24-hour incubation in the presence of library compounds, the medium was removed and cells lysed in 10 µl of lysis buffer (150 mM HEPES, 10% glycerol, 1 mg/ml gelatin, 0.25% Triton X-100, 0.05% Antifoam 204) for 30 minutes. To detect Renilla luciferase activity, coelenterazine substrate (12.5 µM) was added in 50 µl of assay buffer (0.5 M NaCl, 0.1 M KH2PO4, 1 mM Na2EDTA, and 25 mM Thiourea) using a liquid dispenser (BioTek) and luminescence was read immediately in an Envision (Perkin Elmer) reader. The UTSW library was screened in singleton (1 compound per well, 1 replicate) and the results analyzed as described below. Assay quality was monitored during the primary screen (*45*). The average Z' value was 0.7. Assay plates with unacceptable plate statistics (Z' < 0.45) and systematic errors (e.g. plate effects, addition errors, etc.) were repeated.

After hit selection and cherry-picking, confirmation studies were carried out using the primary assay, a counter screen assay, and secondary assays (as described below) were performed as per the methods above, except that each compound was tested at two concentrations (2.5 and 7.5 µM) with triplicate measurements at each dose. A counter screen was conducted using V9M-TK-Renilla cell line in which a TK promoter has been cloned upstream of Renilla luciferase. Secondary reporter assays were conducted with two added colon cancer reporter cell lines constructed in V503 and LS174T-SC1 background. All cell lines were plated at 9,000 cells per well.

5. Viability counter assay: On day 1, V9m-SC3 cells from one 15-cm dish were detached with Accutase (10-15 min), counted with hemocytometer and plated in 384-well plates at 4,000 cells per well in 60 µl of medium. On day 2, 0.3 µl of cherry picked compounds (columns 3-22), DMSO (columns 2 and 23), and Brefeldin A (column 1) as positive control were added with a Biomek FX liquid handler. Final concentrations were 2.5 µM (cherry picked compounds), 0.5 %, (DMSO), and 400 µg/ml (Brefeldin A). Column 24 was left untreated. On day 6, plates were assayed for cell viability by adding 10 µl of the Cell Titer Glo reagent (Promega, Cat# G7573) into the medium. Prior to measuring luminescence with an EnVision plate reader, plates were incubated at room temperature and shaken for 4 minutes.

6. Analysis of high throughput screening data: All data was analyzed using the Genedata Screener® software (version 10.1, GeneData, Inc. Basel, Switzerland). For analysis of the data from the primary screen of the UTSW chemical library, experimental results obtained from EnVision multi-label plate reader were processed and quality-controlled using the Assay Analyzer module of the Genedata Screener® Suite. For each plate, the raw data values for all wells were normalized using equation 1 which assumes hits are infrequent, structurally unrelated, and randomly distributed on individual library plates:

$$(1)\quad Normalized\ Values = \frac{Raw Values - Median\ of\ Test\ Population}{Median\ of\ Test\ Population} * 100$$

The Test Population consists of the UTSW library located in columns 3 to 22 of the 384-well assay plate. This assumption was applied to all the library plates except for the natural product collection plates which were normalized to the neutral control (DMSO) instead of the test population. Lapatinib, a positive control, was included on every assay plate and DMSO was used as a neutral control (columns 2 and 23). Normalized well values were then corrected, where a correction factor each well was calculated using a proprietary pattern detection algorithm in the Assay Analyzer software (See GeneData user documentation and (*46*)). Z-scores were calculated from the corrected normalized activity for each compound (*46*).

After cherry picking primary hits, the selected compounds were assayed in triplicate using the primary reporter assay, a viability assay with the V9M cell line, a counter screen for non-specific reporter activity carried out with the V9M-TK-Renilla cell line, and secondary reporter assays for the V503 and LS174T cell lines. The data for each assay well was normalized to the neutral controls. The normalized activity values (replicates) for each compound in each assay were then condensed to a single value (condensed activity) using the "Robust Condensing" method in Genedata Screener®. The condensed activity is the most representative single value of the triplicates. In general, the triplicates were pre-condensed into a pair of values as follows:

$$(2)\ Values\ (X,Y) = (Median\ of\ Triplicates\ m) \pm Dispersion$$

where: $Dispersion = Median(|X_1 - m|, |X_2 - m|, |X_3 - m|)$

The less X and Y differ ($|X - Y|$), the better the data quality. For data points where $|X - Y| \leq 30\%$, the median of X and Y was used as the condensed activity, which is also the median of the triplicate measurements. Otherwise, a condensing function Max(X,Y) was used to estimate the condensed activity. A robust Z-Score was then calculated for each compound using equation 3:

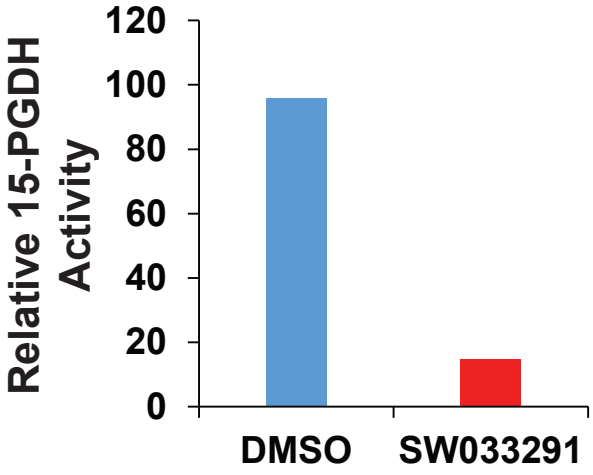
$$(3)\ \text{Robust Z Score} = \frac{Condensed\ Activity - Median\ of\ Neutral\ Controls}{Robust\ Stdev\ of\ Neutral\ Controls}$$

7. Selection and progression of hits from HTS: Primary hits (2,787) were selected using a cutoff for the Z-score of > 3. These hits were segregated into structurally related

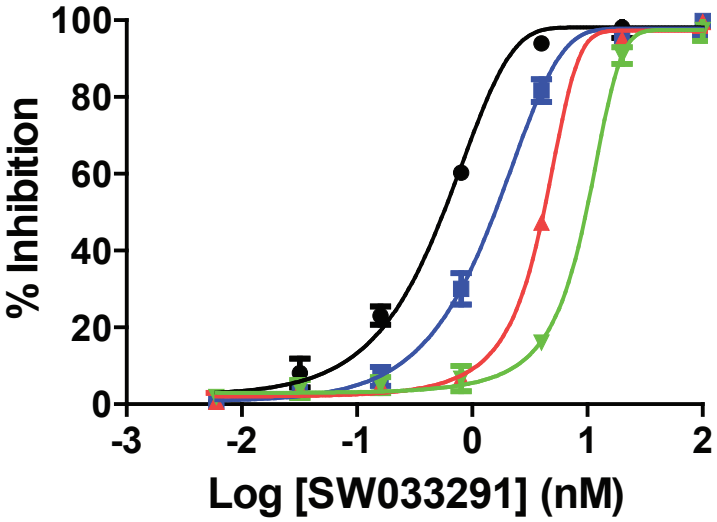
groups (series) using the clustering algorithms available in Biovia's Pipeline Pilot analysis suite. A weighted subsetting approach was used to select 1,280 compounds for cherry-picking: 1) for clusters with more than ten members, the three most active compounds were selected; 2) for clusters with less than 10 members, the most active compound in each cluster was selected; and 3) from the remaining primary hits not selected in 1) and 2), the 183 most active compounds were selected. The cherry-picked compounds were arrayed into four 384-well plates and subsequently tested in several assays designed to select compounds with the following activity profile: at least two-fold activity in three colon cancer cell lines, less than 1.25-fold activity with respect to the DMSO control in the TK-Renilla counter screen, and less than 25% reduction in ATP levels (compared to the DMSO control) in the assay for viability (Cell-Titer GloTM, Promega, Inc.). Twenty-eight (28) compounds fit this profile and were reviewed for chemical attractiveness (lead-like properties) and synthetic tractability. From these compounds, three chemical series were selected for re-synthesis and hit expansion.

8. Reconfirmation of SW033291: SW033291 was re-synthesized and tested for activity in interacting with 15-PGDH as reflected by inducing luciferase activity in the V9m-SC3 reporter cell line. SW033291 increased 15-PGDH luciferase reporter activity 2.4-fold with an EC50 of <20 nM. Functional properties of SW033291 in altering 15-PGDH enzyme activity were then assayed in cell based assays (Fig. S1A) and in incubations in vitro with recombinant 15-PGDH protein (Fig. S1, B and C).

S1A



S1B



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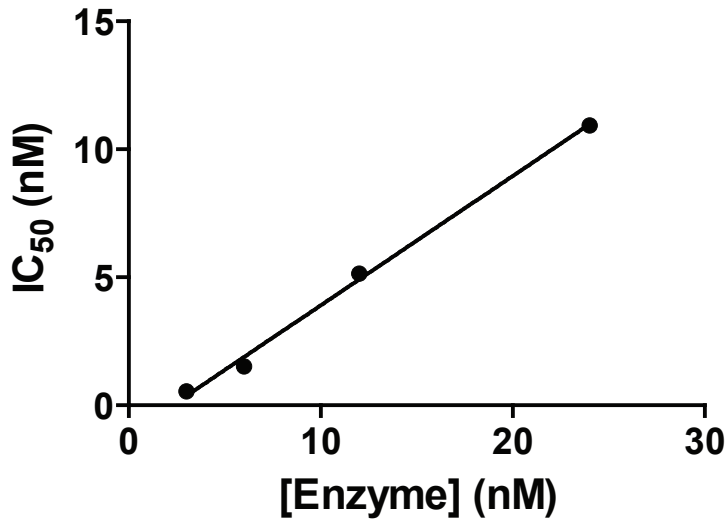

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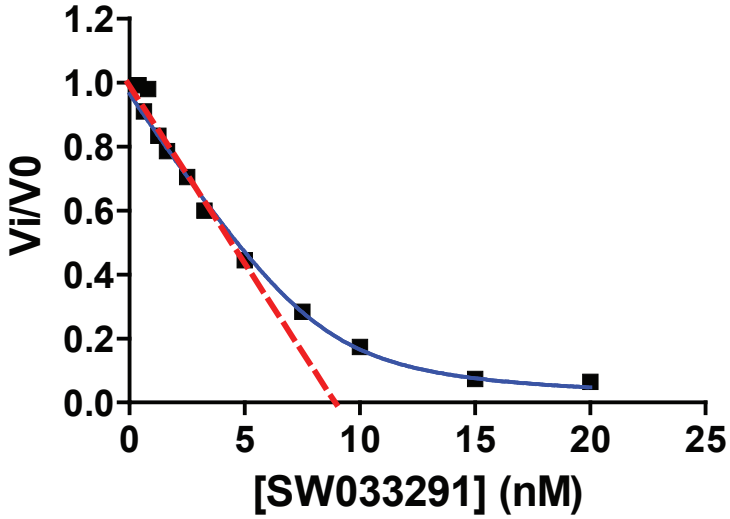

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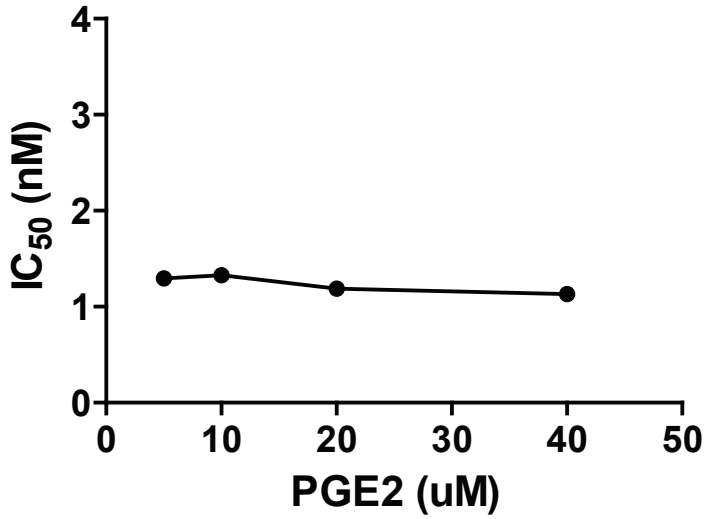
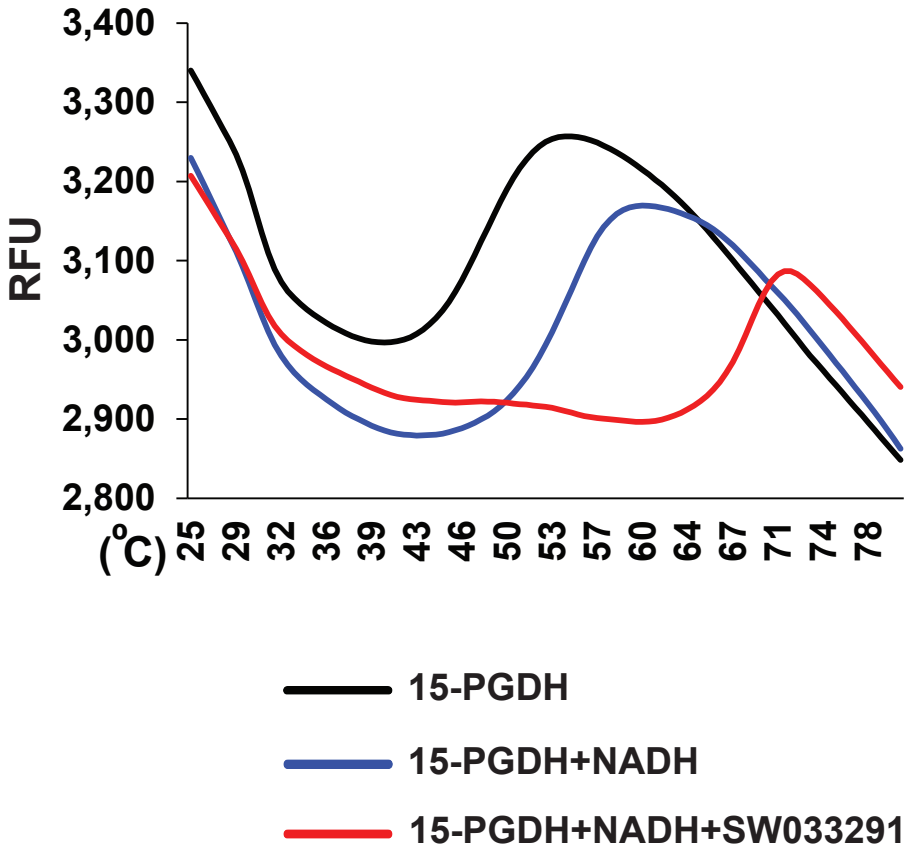
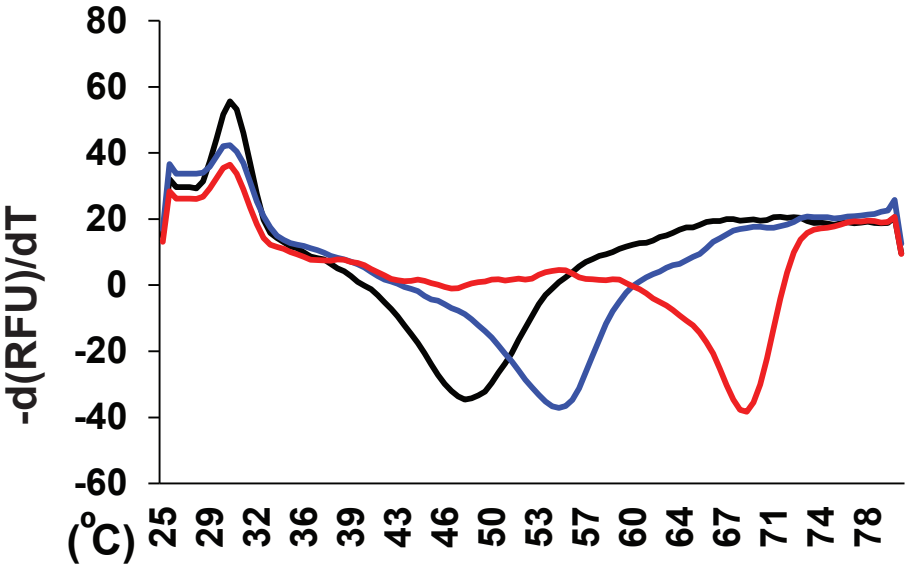


Fig. S1: SW033291 inhibition of 15-PGDH. (A) 15-PGDH enzyme activity in Vaco-503 cells after a 24 hour treatment with 2.5 µM SW033291 or DMSO control. N=2. (B, C) SW033291 inhibition of recombinant 15-PGDH enzyme activity. (B) Graphs plot percent inhibition (0-100%) of 15-PGDH enzyme activity on the Y-axis versus SW033291 concentration (log ([SW033291]nM) on the X-axis. 15-PGDH concentrations tested were: 3 nM  , 6 nM  , 12 nM  , and 24 nM  . Values are means of triplicate determinations. Most error bars (SEM) are smaller than symbol sizes. (C) Graphed is IC_{50} of SW033291 (Y-axis) (from data in Fig. S1B) versus reaction concentration of recombinant 15-PGDH (X-axis). (D) Graphed is relative initial reaction velocities V_i/V_0, on Y-axis, versus concentration of SW033291 on X-axis. Initial 15-PGDH reaction rates, were measured in reactions with 10 nM 15-PGDH enzyme and SW033291 concentrations increasing in 8 increments from 0 to 1nM, then in 7 increments from 1-20 nM. Fitting data to the Morrison equation yields Ki^{app}=0.10 nM for SW033291. Extrapolation (red line) of linear portion of curve to X intercept indicates 8.5 nM active 15-PGDH enzyme in the reactions. V_i determinations were calculated as averages of triplicate determinations. (E) Demonstrating the IC_{50} of SW033291 is independent of PGE2 concentration. Graphed is the SW033291 IC_{50} for inhibiting 15-PGDH (Y-axis) versus concentration of PGE2 substrate in the assay (X-axis). IC_{50} values were determined in parallel titrations of SW033291 inhibition of 15-PGDH enzyme activity in assays containing PGE2 at concentrations: 5 µM, 10 µM, 20 µM, and 40 µM. Plotted IC_{50} values are means of triplicate determinations.

S2A



S2B

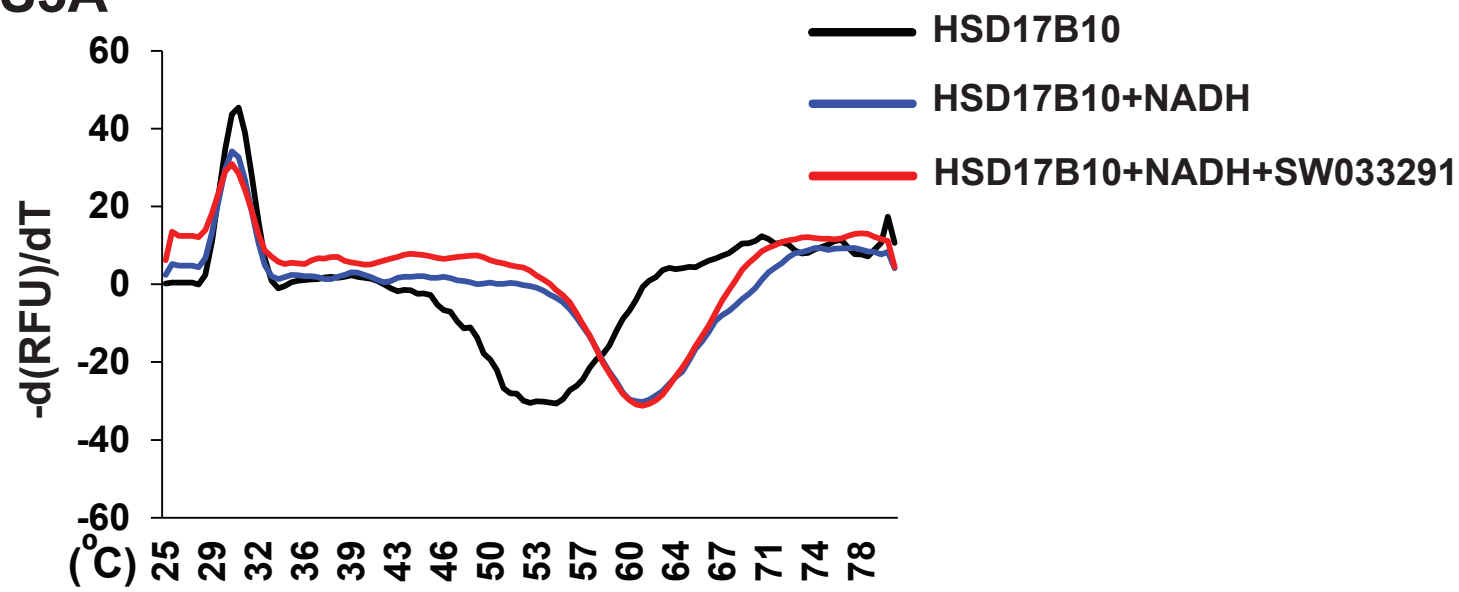


S2C

	15-PGDH	15-PGDH+NADH	15-PGDH+NADH +SW033291
T_m	49°C	54.5°C	68°C

Fig. S2: SW033291 shift of the thermal denaturation curve of 15-PGDH. Shown is effect on thermal denaturation of recombinant 15-PGDH protein by addition of the enzyme's cofactor NADH plus the enzyme inhibitor SW033291. (A) 15-PGDH thermal denaturation as monitored by differential scanning fluorimetry using SYPRO orange dye. Y-axis is Relative Fluorescence Units (RFU). X-axis is Temperature (°C). Conditions tested: 15-PGDH (black line); 15-PGDH + 100 µM NADH (blue line); 15-PGDH + 100 µM NADH + 20 µM SW033291 (red line). (B) Plotted is the negative first derivative of the melting curves, -d(RFU)/dT. Conditions tested: 15-PGDH (black line); 15-PGDH + 100 µM NADH (blue line); 15-PGDH + 100 µM NADH + 20 µM SW033291 (red line). (C) Listed are the melting temperatures T_m of 15-PGDH, as determined by the inflection points of the plots of –d(RFU)/dT for the three conditions tested. Shown is representative data from N=8 replicates with average melting point shift between (SW033291 + NADH) – (NADH) = 13.25° +/- 0.13° (SEM).

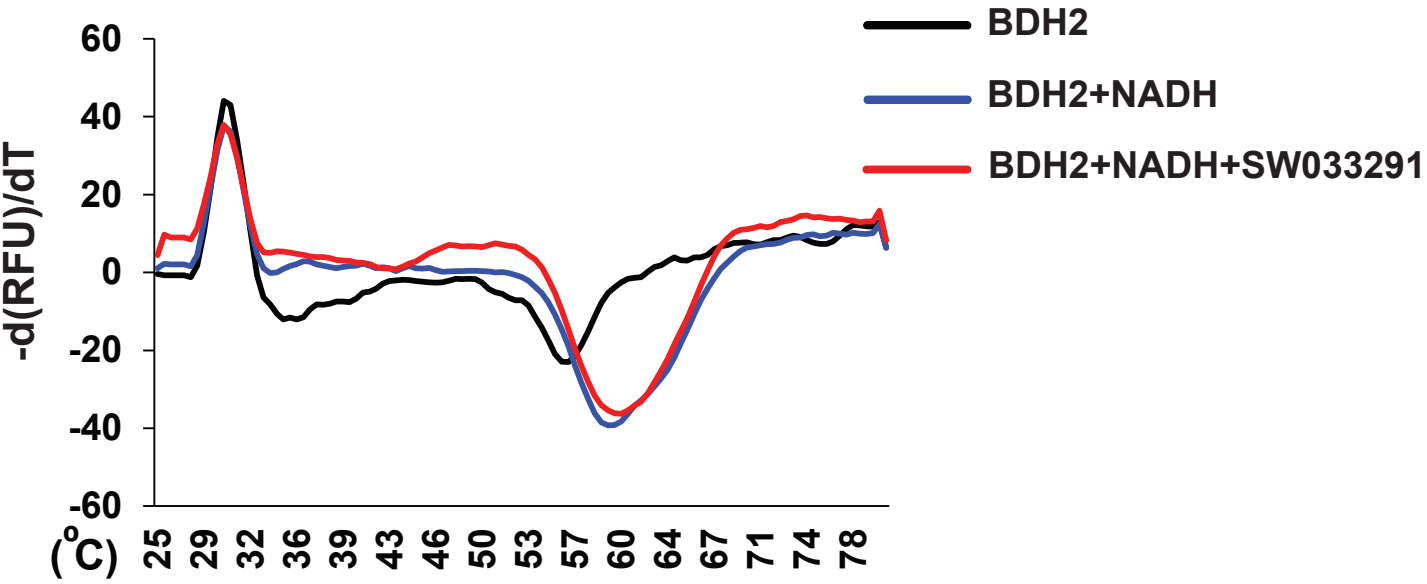
S3A



S3B

	HSD17B10	HSD17B10+NADH	HSD17B10+NADH +SW033291
T_m	54.5°C	61°C	61°C

S3C



S3D

	BDH2	BDH2+NADH	BDH2+NADH +SW033291
T_m	56°C	59°C	60°C

Fig. S3: Lack of SW033291 shift of the thermal denaturation curve of HSD17B10 and BDH2. HSD17B10 and BDH2 are short chain dehydrogenases closely related by structure to 15-PGDH. Shown is lack of effect of SW033291 on thermal denaturation of recombinant HSD17B10 and BDH2 proteins plus NADH. (A) HSD17B10 thermal denaturation as monitored by differential scanning fluorimetry using SYPRO orange dye. Plotted is the negative first derivative of the melting curves, -d(RFU)/dT. Conditions tested: HSD17B10 (black line); HSD17B10 + 100 µM NADH (blue line); HSD17B10 + 100 µM NADH + 20 µM SW033291 (red line). Shown is representative data from N=4 replicates. (B) Listed are the melting temperatures T_m of HSD17B10, as determined by the inflection points of the plots of –d(RFU)/dT for the three conditions tested. In contrast to the pronounced effect on thermal denaturation of 15-PGDH, SW033291 demonstrated no effect on thermal denaturation of HSD17B10, with average melting point shift between (SW033291 + NADH) – (NADH) = 0° +/- 0.2° (SEM) in N=4 replicates. (C) BDH2 thermal denaturation as monitored by differential scanning fluorimetry using SYPRO orange dye. Plotted is the negative first derivative of the melting curves, -d(RFU)/dT. Conditions tested: BDH2 (black line); BDH2 + 100 µM NADH (blue line); BDH2 + 100 µM NADH + 20 µM SW033291 (red line). Shown is representative data from N=4 replicates. (D) Listed are the melting temperatures T_m of BDH2, as determined by the inflection points of the plots of –d(RFU)/dT for the three conditions tested. In contrast to the pronounced effect on thermal denaturation of 15-PGDH, SW033291 demonstrated only barely perceptible effect on thermal denaturation of BDH2, with average melting point shift between (SW033291 + NADH) – (NADH) = 0.75° +/- 0.1° (SEM) in N=4 replicates.

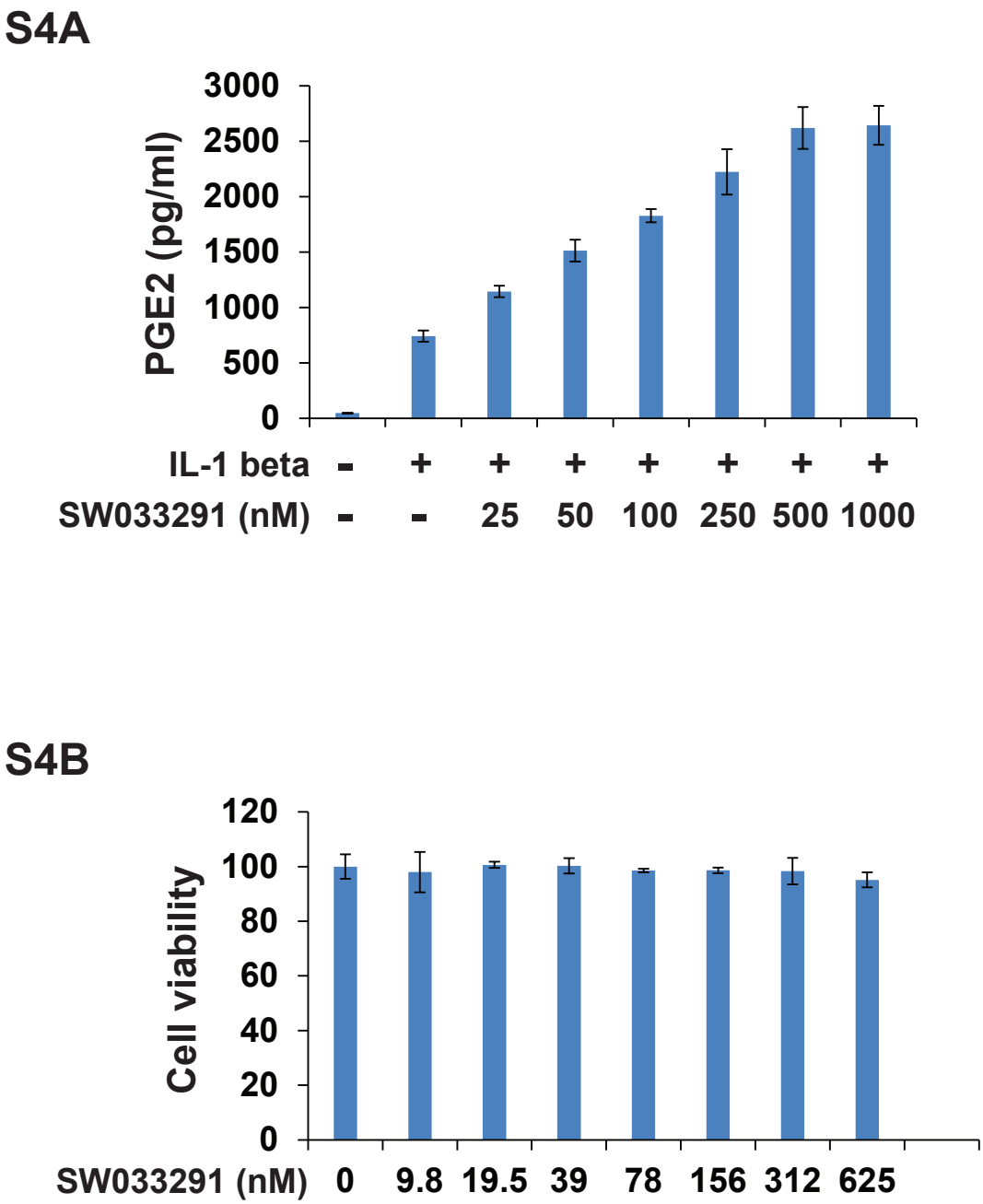


Fig. S4: SW033291 induction of PGE2 in A549 cells. (A) PGE2 levels in medium of A549 cells following IL-1 beta stimulation and treatment with increasing concentrations of SW033291. Y-axis records PGE2 values and X-axis records treatment conditions. Graphed are means of 4 independent determinations +/- SEM. In this assay SW033291 shows EC50 between 50-100 nM, and maximum activity at 500 nM. (B) SW033291 effect on A549 cell viability. Cell viability was assessed by CellTiter-Glo® assay (Promega, Madison, WI) and plotted as percentage of baseline value. SW033291 doses that induce production of PGE2 show no effect on A549 cell viability. Graphed are means of 4 independent determinations +/- SEM.

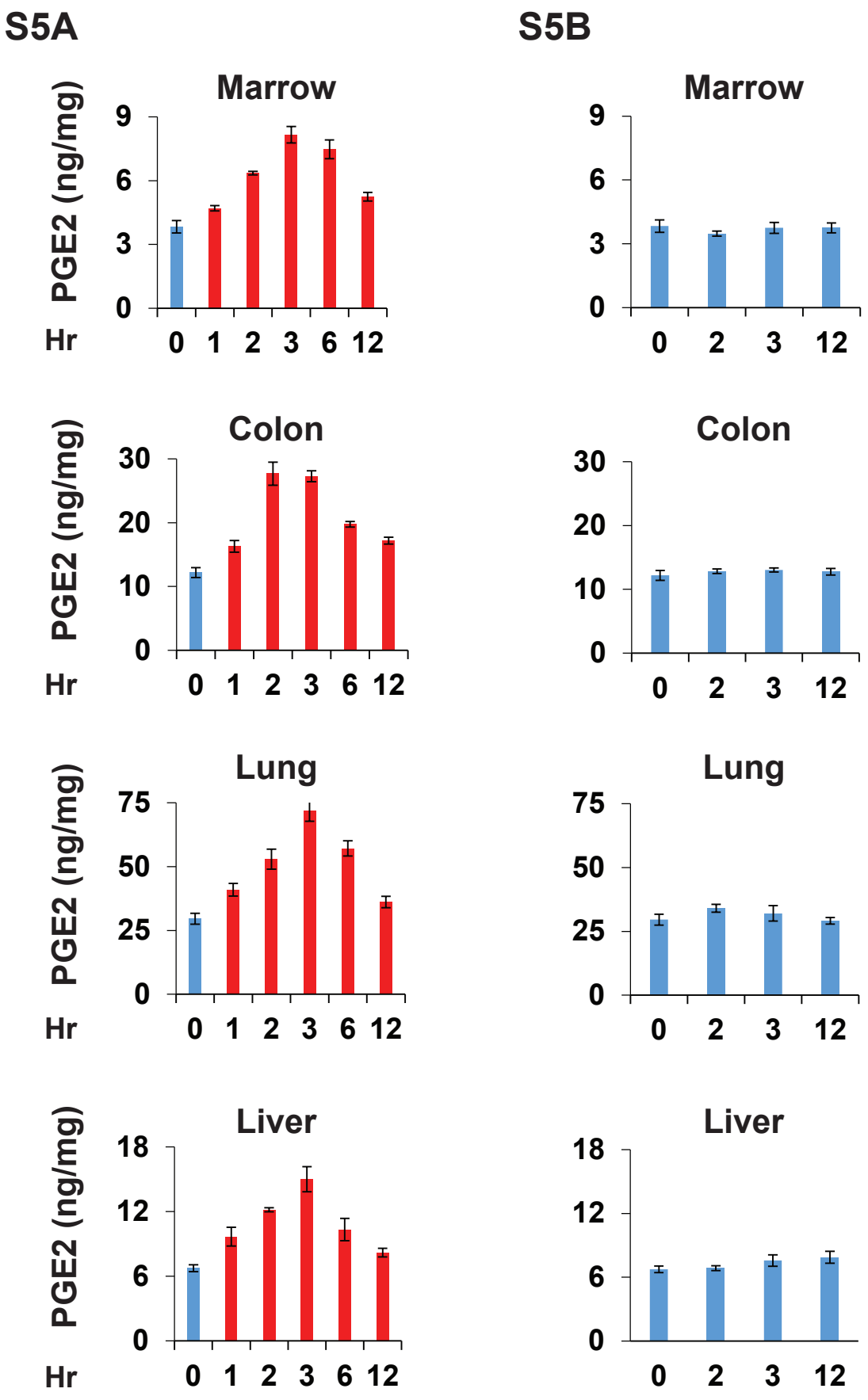


Fig. S5: SW033291 induction of PGE2 in mouse tissues. (A) PGE2 levels in bone marrow, colon, lung, and liver of mice from 0 to 12 hours post injection with SW033291 10 mg/kg IP. (B) PGE2 levels in bone marrow, colon, lung, and liver of mice from 0 to 12 hours post injection with vehicle control IP. Graphed are means from 6 mice +/- SEM.

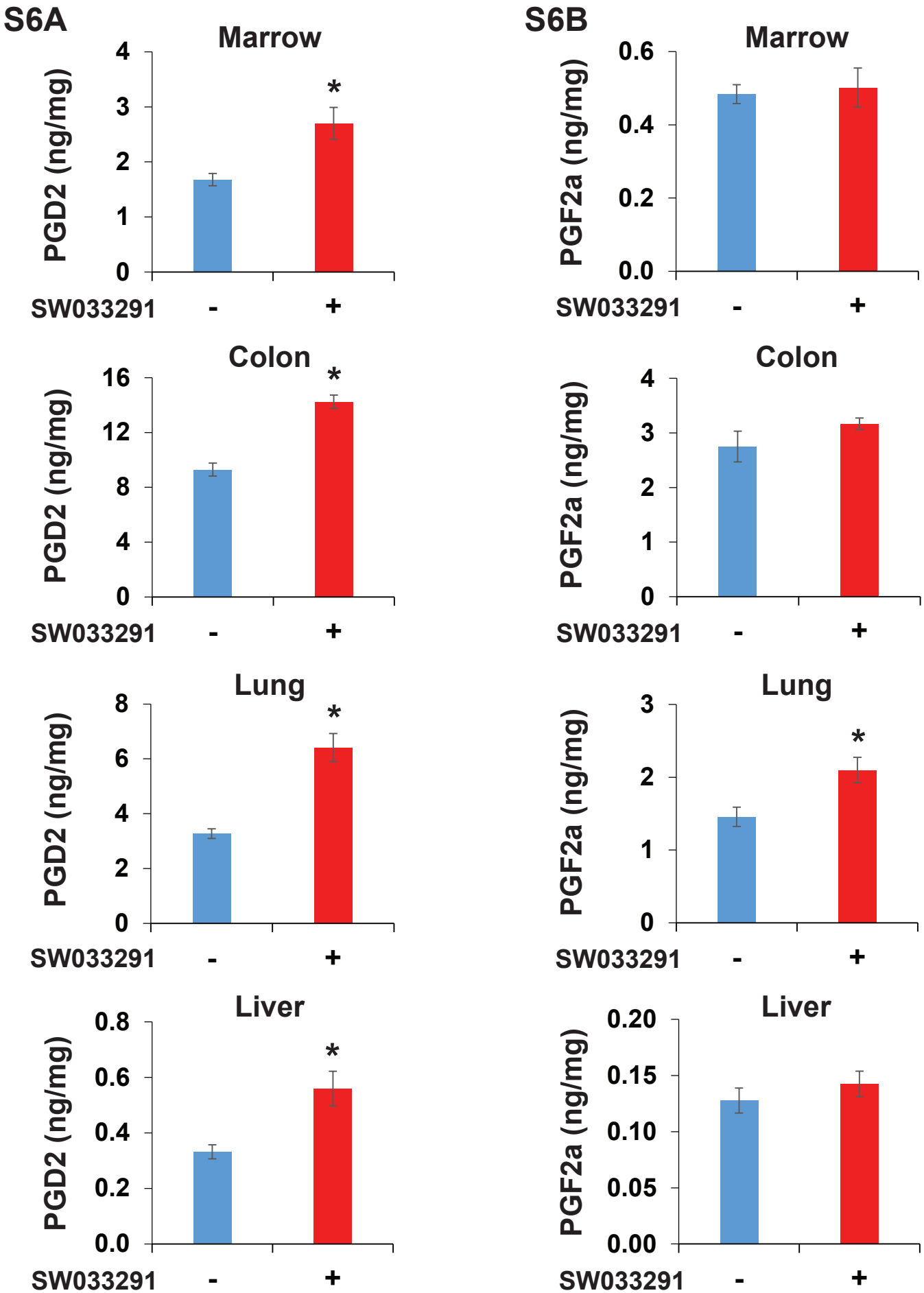


Fig. S6: SW033291 induction of PGD2 and PGF2a in mouse tissues. (A, B) PGD2 and PGF2a levels in bone marrow, colon, lung, and liver of mice were assayed at 3 hours post injection with SW033291 10 mg/kg IP. Graphed are means from 6 mice +/- SEM. (A) PGD2. * Indicates P<0.01, Student's t-test. (B) PGF2a. * Indicates P<0.02, Student's t-test.

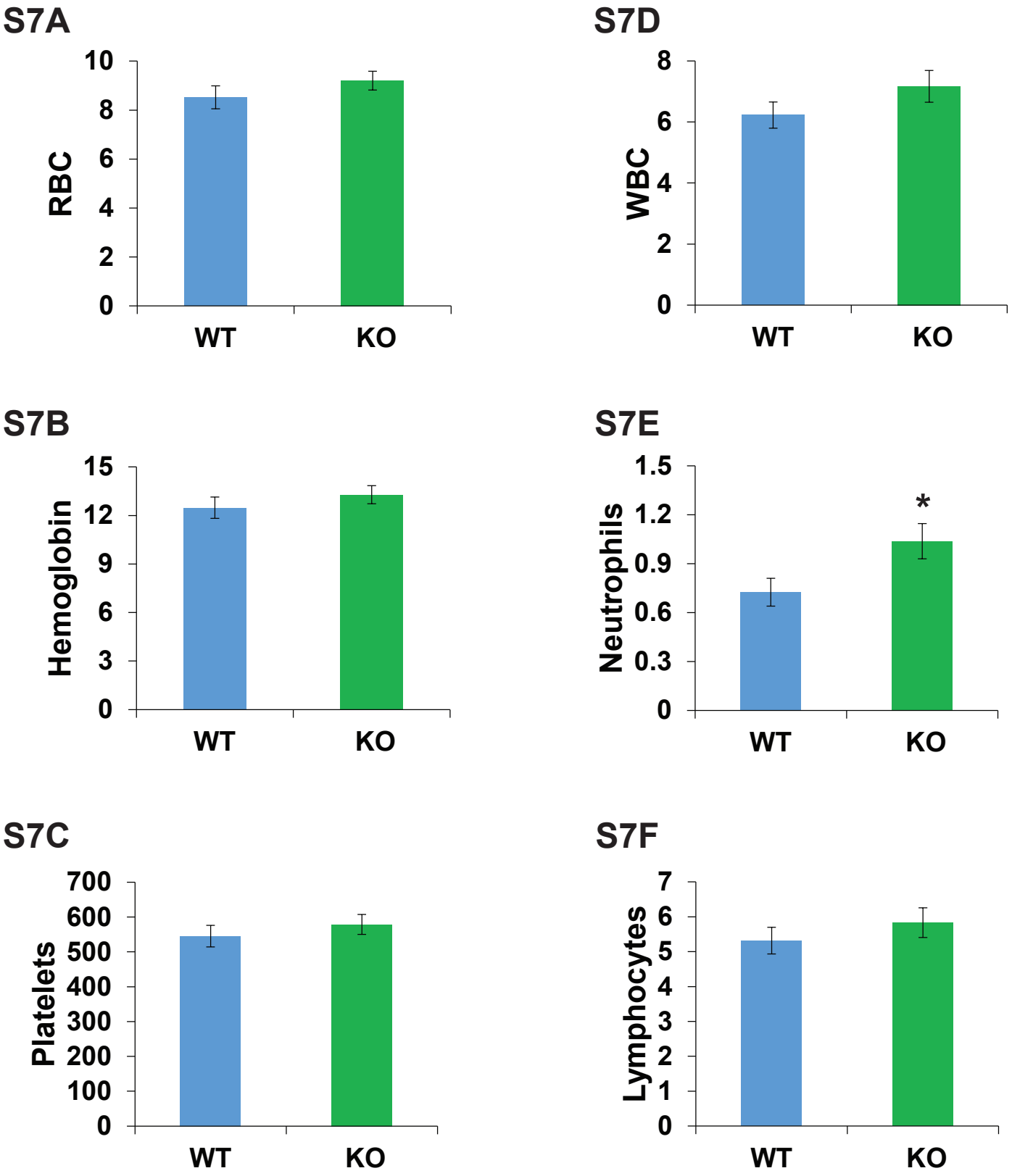


Fig. S7: Comparison of peripheral blood counts from 15-PGDH wild-type (WT) and knockout (KO) mice. Graphed are means from 16 mice +/- SEM. (A) Red Blood Cell Counts (RBC) (Y-axis scale: 10^6 cells/µl). (B) Hemoglobin (Y-axis scale: g/dL). (C) Platelet Counts (Y-axis scale: 10^3 cells/µl). (D) White Blood Cell Counts (WBC) (Y-axis scale: 10^3 cells/µl). (E) Neutrophil Counts (Y-axis scale: 10^3 cells/µl). * Indicates P=0.031, Student's t-test. (F) Lymphocyte Counts (Y-axis scale: 10^3 cells/µl).

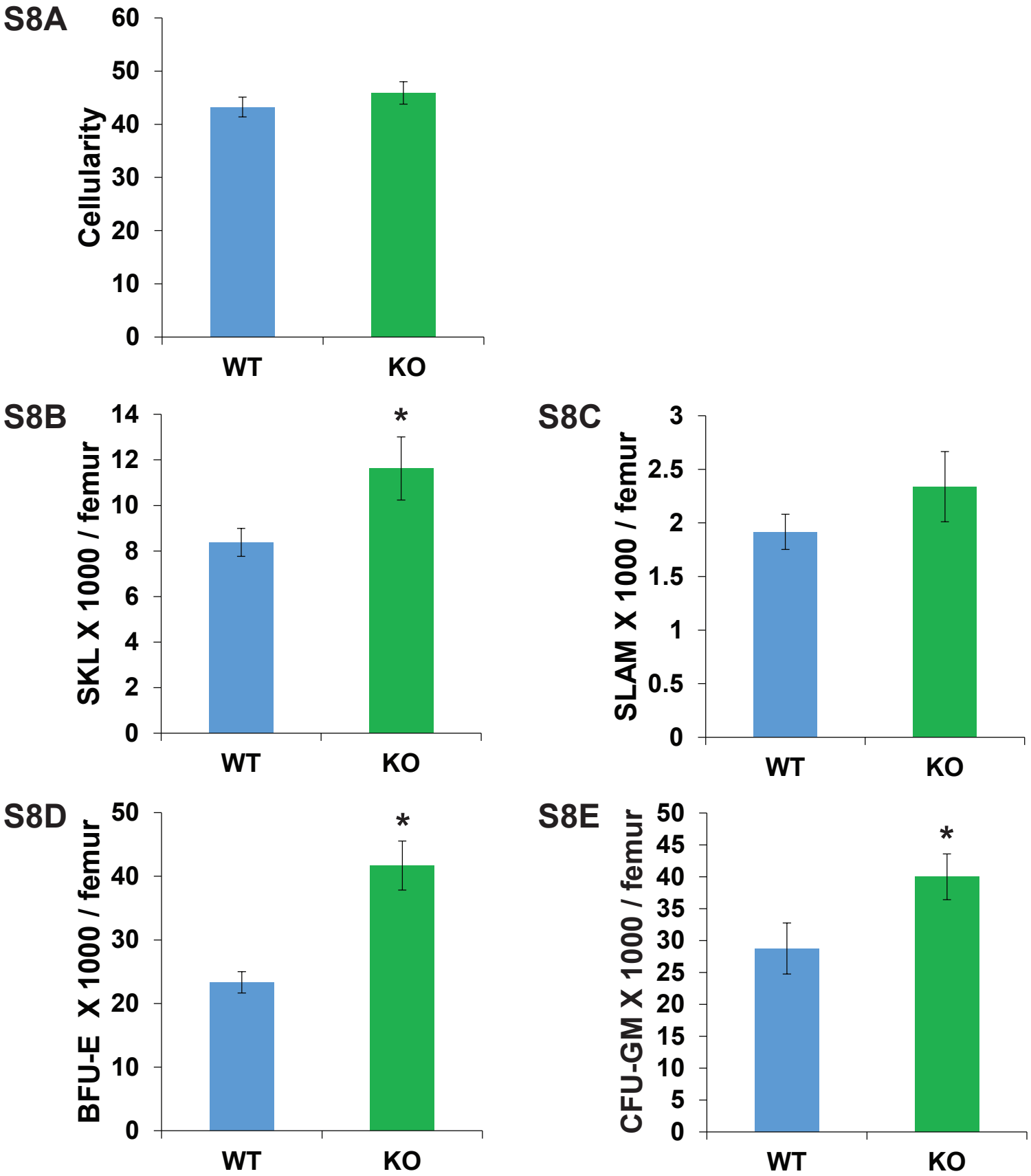


Fig. S8: Comparison of bone marrow from 15-PGDH wild-type (WT) and knockout (KO) mice. (A) Bone marrow cellularity, graphed as number of bone marrow cells per mouse (Y-axis, scale: 10^6 cells/mouse). Graphed are means +/- SEM. N=9 mice per data point. (B) SKL cells per femur (Y-axis scale: 10^3 cells/femur). Graphed are means +/- SEM. N=16 mice per data point. * Indicates P<0.04, Student's t-test. (C) SLAM cells per femur (Y-axis scale: 10^3 cells/femur). Graphed are means +/- SEM. N=13 mice per data point. (D, E) Numbers of BFU-E and CFU-GM colonies (Y-axis) formed on day 14 per femur from 20,000 bone marrow cells plated into methylcellulose with cytokines. Data are averages from 4 mice, each plated in duplicate determinations and graphed as means +/- SEM. (D) BFU-E. * Indicates P=$5.6X10^{-4}$, Student's t-test. (E) CFU-GM. * Indicates P=0.027, Student's t-test.

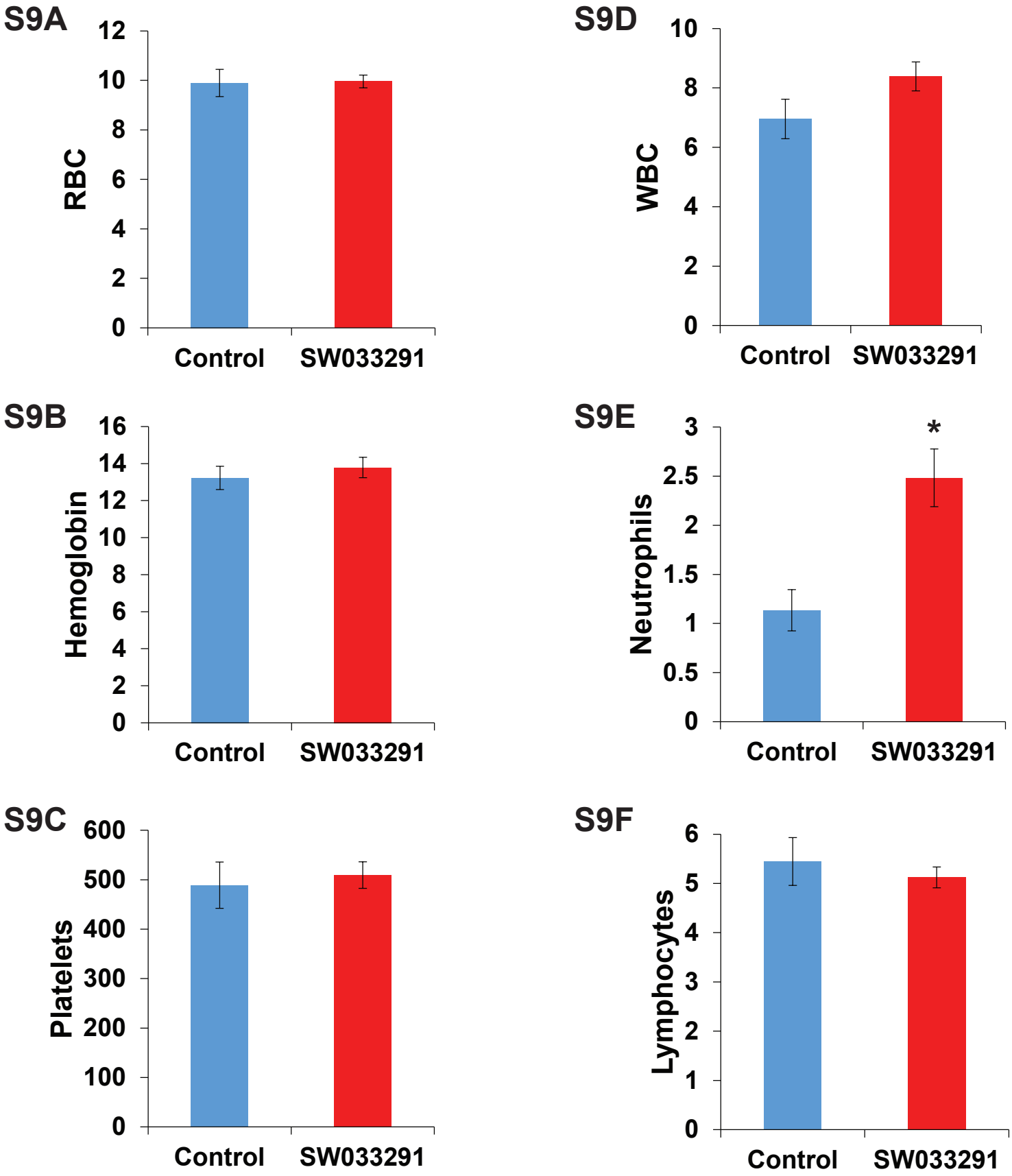
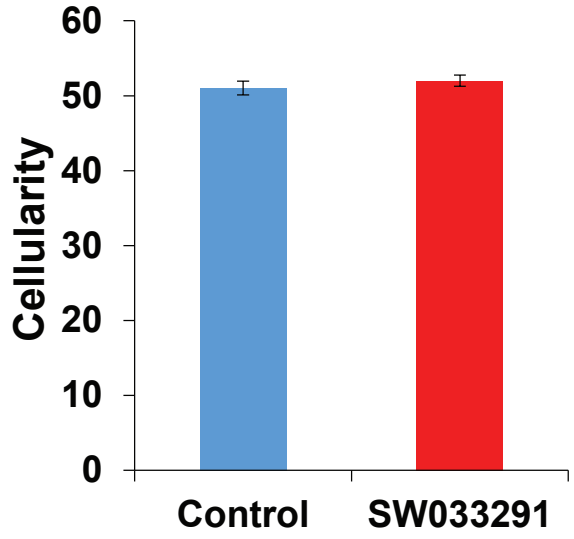


Fig. S9: SW033291 effect on peripheral blood counts. Mice were treated with SW033291 10 mg/kg, or vehicle control, IP twice daily for 5 doses, and blood drawn at 6 hours after the fifth dose. Data are average values determined from 6 SW033291-treated and 9 control mice and graphed as means +/- SEM. (A-F) Comparison of peripheral blood counts. (A) Red Blood Cell Counts (RBC) (Y-axis scale: 10^6 cells/µl. (B) Hemoglobin (Y-axis scale gm/dl). (C) Platelet Counts (Y-axis scale: 10^3 cells/µl). (D) White Blood Cell Counts (WBC) (Y-axis scale: 10^3 cells/µl). (E) Neutrophils (Y-axis scale: 10^3 cells/µl). * Indicates $P<0.003$, Student's t-test. (F) Lymphocytes (Y-axis scale: 10^3 cells/µl).

S10A



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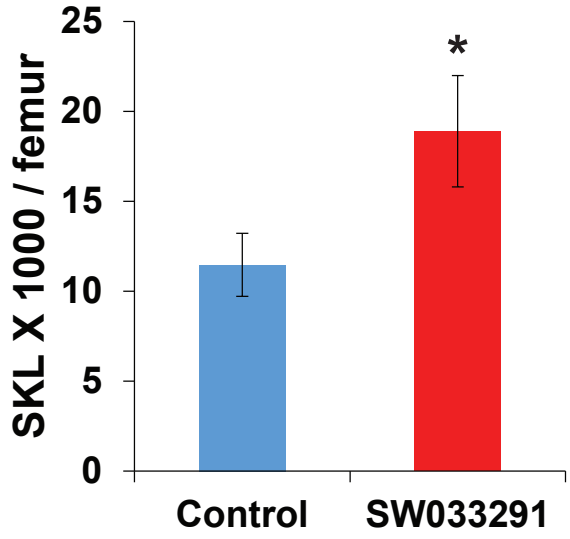

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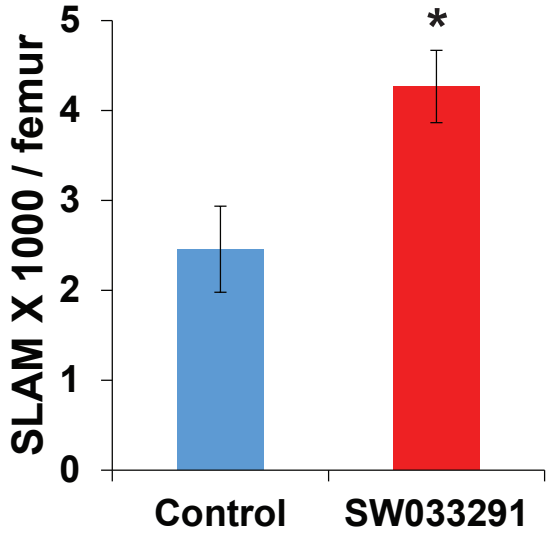

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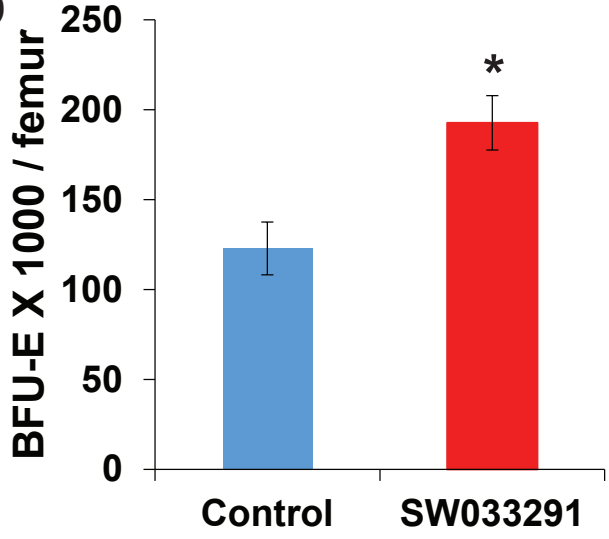

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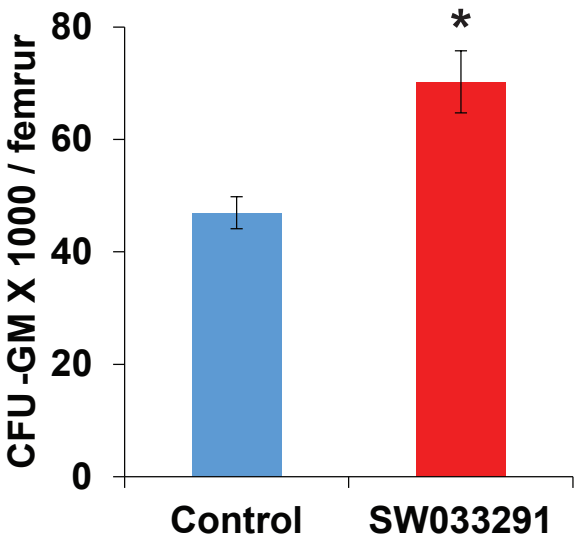
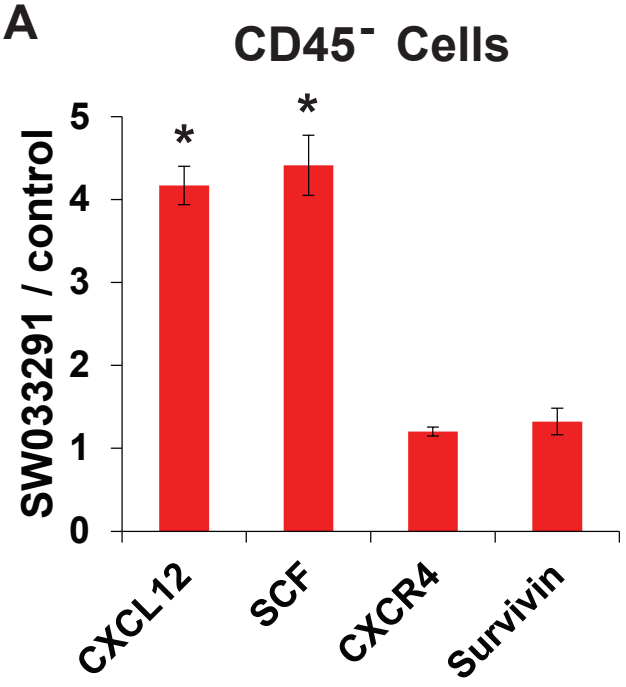


Fig. S10: SW033291 effect on bone marrow. Mice were treated with SW033291 10 mg/kg, or vehicle control, IP twice daily for 5 doses, and bone marrow harvested at 6 hours after the fifth dose. (A-C) SW033291 effect on bone marrow populations. Data are average values determined from 6 SW033291-treated and 9 control mice (A, C), or 9 SW033291-treated and 12 control mice (B), and are graphed as means +/- SEM. (A) Bone marrow cellularity, graphed as number of bone marrow cells per mouse (Y-axis scale: 10^6 cells/mouse). (B) SKL cells per femur (Y-axis scale: 10^3 cells/femur). * Indicates P=0.039, Student's t-test. (C) SLAM cells per femur (Y-axis scale: 10^3 cells/femur). * Indicates P=0.019, Student's t-test. (D, E) SW033291 effect on bone marrow colony forming activity. Y-axis graphs comparison of hematopoietic colony forming units generated per femur on day 14 after plating bone marrow cells into methylcellulose with cytokines. Data are averages from 4 mice, each plated in duplicate determinations, and graphed as means +/- SEM. (D) BFU-E. * Indicates P<0.016, Student's t-test. (E) CFU-GM. * Indicates P<0.008, Student's t-test.

S11A



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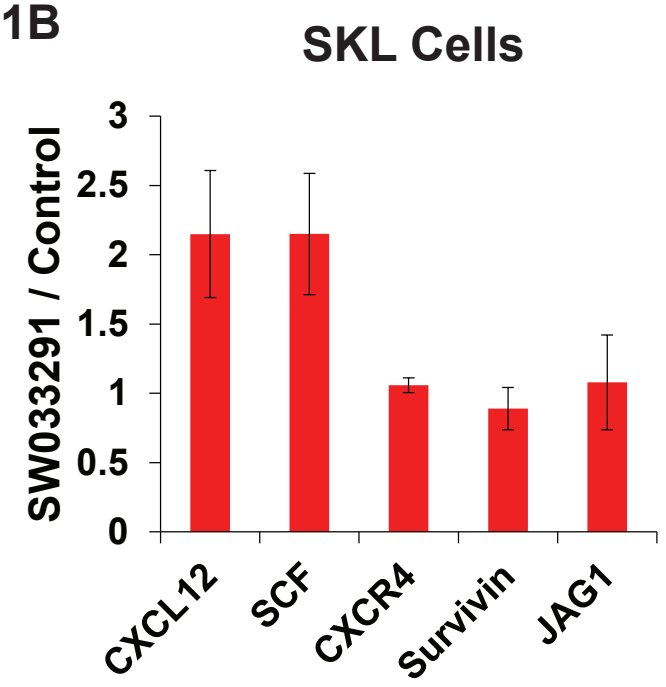
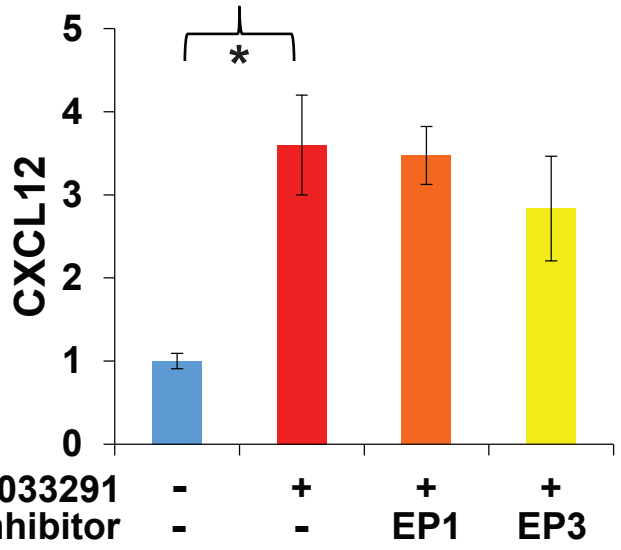


S11C



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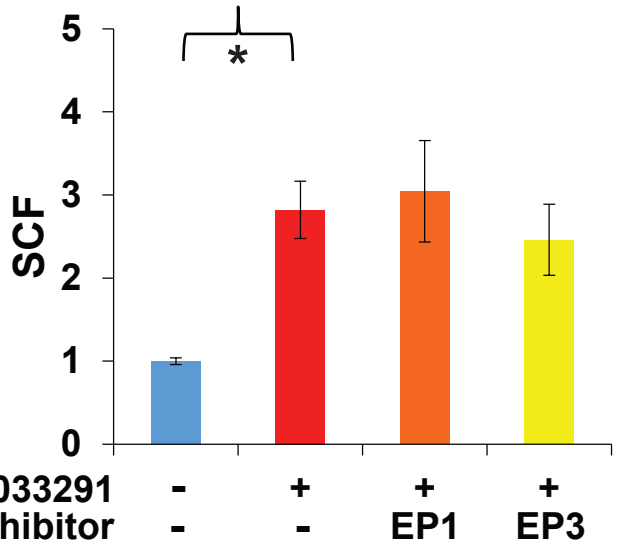

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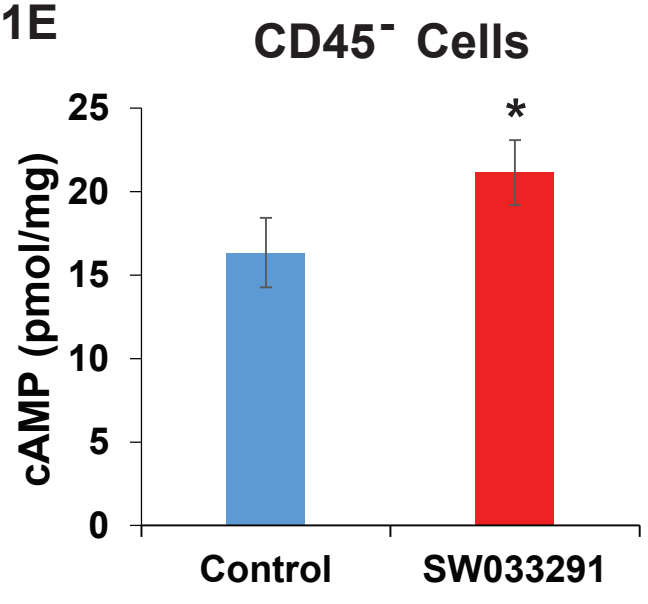


Fig. S11: SW033291 effect on bone marrow cells. (A, B) SW033291 induced gene induction in bone marrow cells from SW033291 versus vehicle treated animals. Gene expression levels measured by real-time PCR are plotted (Y-axis) as fold change in SW033291 versus vehicle treated groups. Data are graphed as means +/- SEM. N=7 pools of 3 mice per pool for CXCL12 and SCF assays, and 3 pools of 3 mice per pool for other gene assays. (A) Bone marrow $CD45^-$ cells. * Indicates $P<4.0x10^{-6}$, Student's t-test. (B) Bone marrow SKL cells. (C, D) Gene induction in $CD45^-$ bone marrow cells from mice treated with (+) or without (-) SW033291 in combination with (+) or without (-) EP1 antagonist SC-51322 (EP1) or EP3 antagonist L-798,106 (EP3). N=3 pools of 3 mice each for EP1 and EP3 inhibitor. (C) Relative CXCL12 expression. * Indicates P<0.013 by Student's t-test. (D) Relative SCF expression. * Indicates P<0.007 by Student's t-test. (E) SW033291 induction of cyclic AMP in $CD45^-$ cells. Graphed on Y-axis are cyclic AMP levels (cAMP, pmol/mg protein) in $CD45^-$ cells from mice treated with SW033291 or vehicle control. Each value is the mean of N=6 mice all assayed in triplicate and graphed as means +/- SEM. * Indicates P=0.002 by Student's t-test.

S12

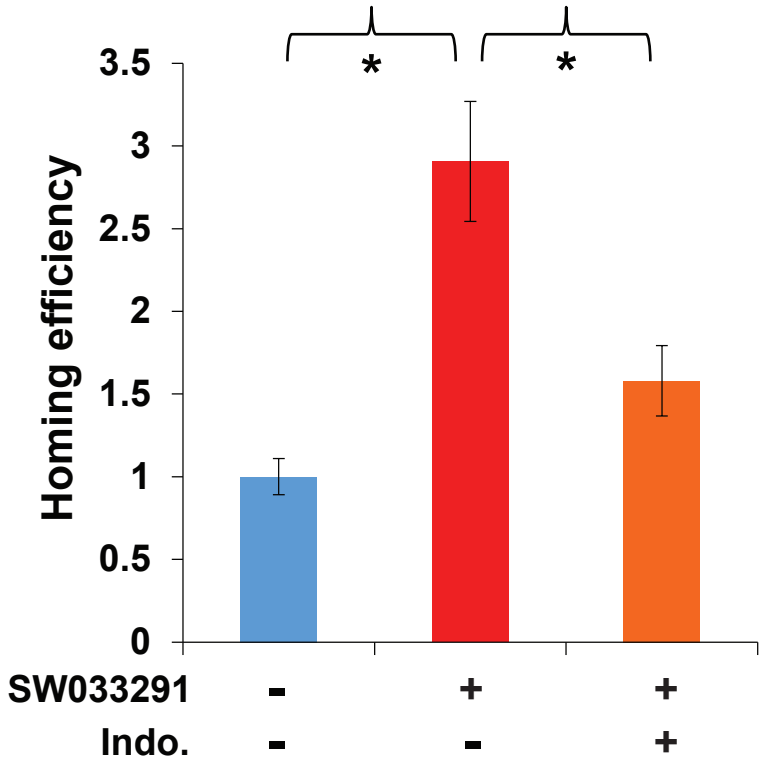


Fig. S12: Homing of CFSE labeled donor marrow cells to bone marrow of SW033291-treated versus vehicle-treated irradiated recipient mice. Y-axis graphs ratio of % CFSE labeled cells in marrow of SW033291-treated / vehicle-treated mice at 16 hours post donor cell infusion (homing efficiency). N=9 mice per data point, with data graphed as means +/- SEM. Graphs show comparison of recipient mice treated with (+) or without (-) SW033291 in combination with (+) or without (-) indomethacin (Indo). * Indicates $P<0.003$ versus SW033291 only treated mice, Student's t-test.

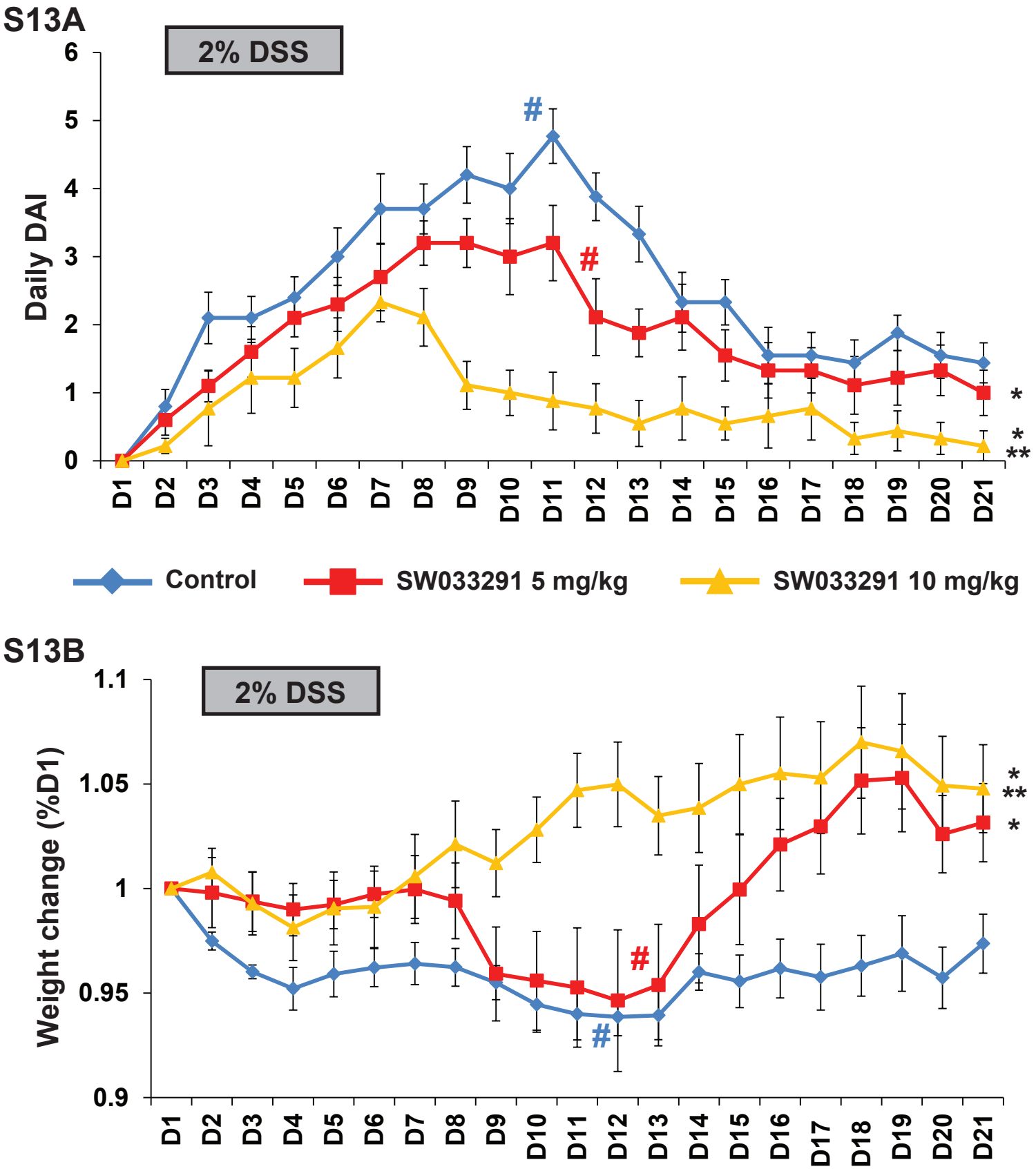


Fig. S13: SW033291 effect on disease activity and daily weights of DSS treated mice. Shown are observations over study days 1-21 (D1-D21) of DSS treated (day 1 through 7) mice comparing: control mice (N=10) (blue), mice treated at 5 mg/kg of SW033291 twice daily (N=10) (red), and mice treated at 10 mg/kg of SW033291 twice daily (N=9) (gold), graphed as daily means +/- SEM. (A) Graphical plots of daily disease activity index (DAI). * Indicates $P<5X10^{-6}$ versus control, paired Student's t-test. ** Indicates $P<5x10^{-6}$ versus mice treated at 5 mg/kg SW033291, paired Student's t-test. # Indicates death of one mouse. (B) Plots of daily weights, graphed as percent of day 1 value. * Indicates $P<3X10^{-6}$ versus control mice, paired Student's t-test. ** Indicates $P<0.0004$ versus mice treated at 5 mg/kg SW033291, paired Student's t-test. # Indicates death of one mouse.

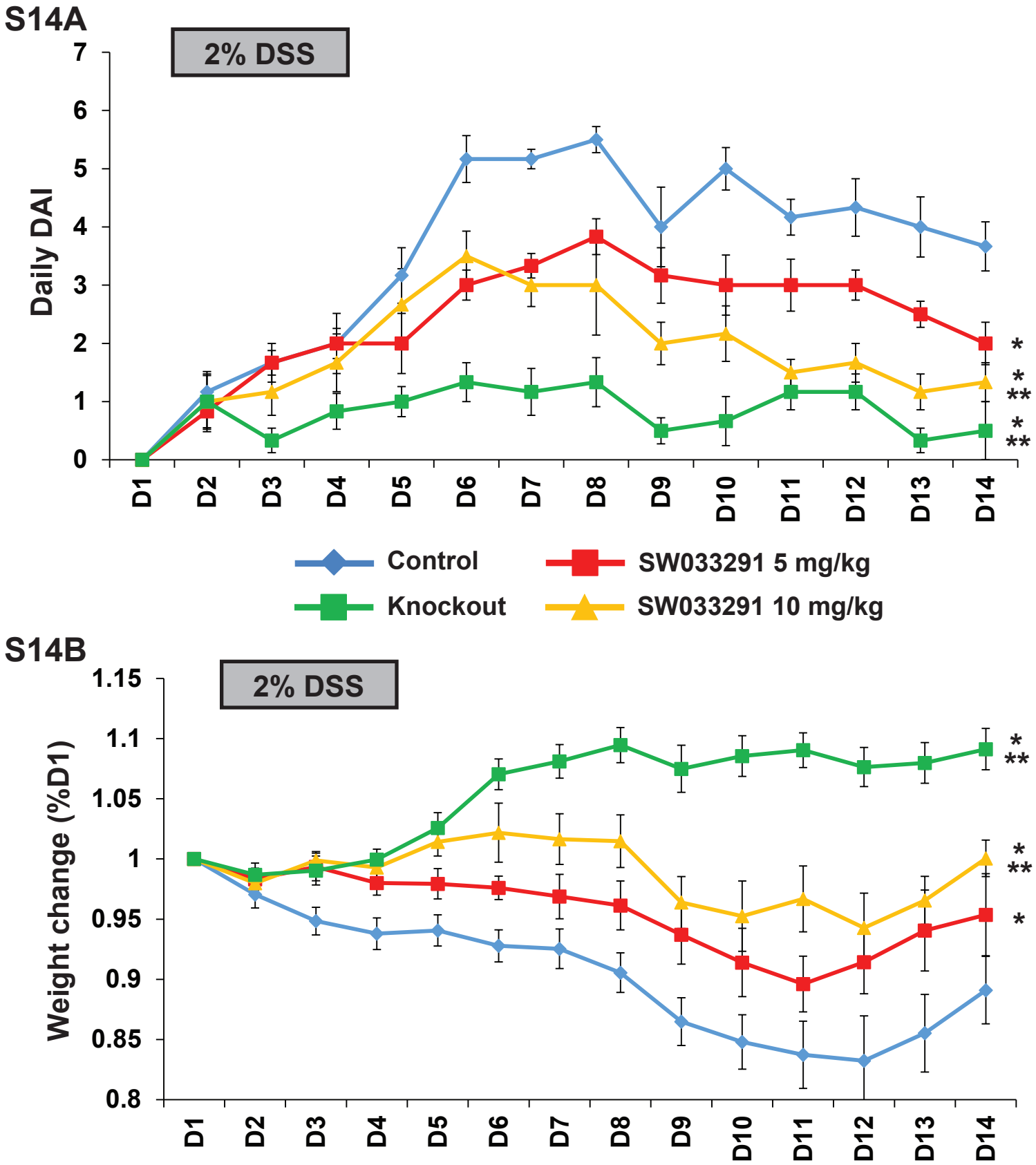
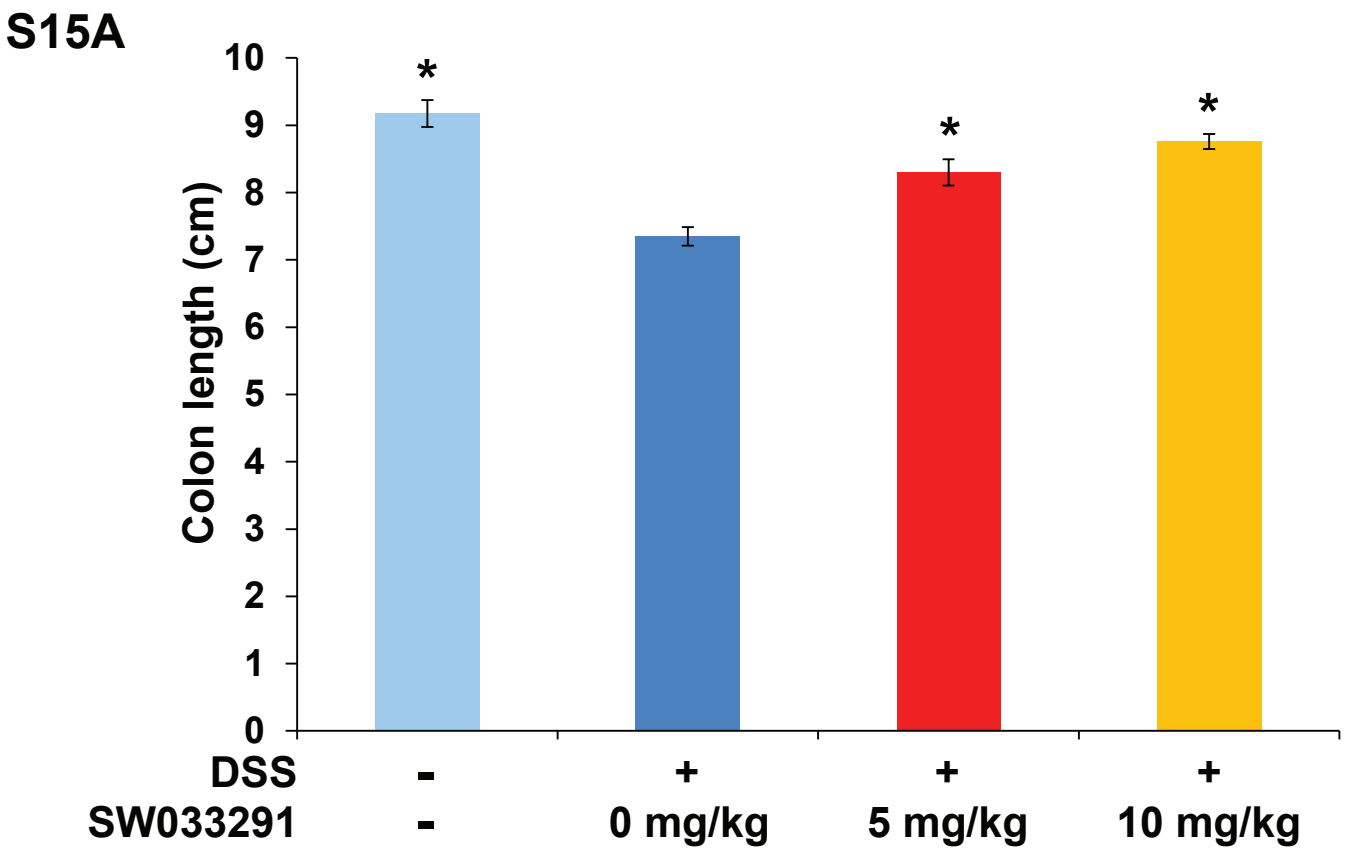


Fig. S14: 15-PGDH inhibition effect on disease activity and daily weights of DSS treated mice. Shown are observation over study days 1-14 (D1-D14) of DSS treated (day 1 through 7) mice comparing: control 15-PGDH wild-type mice (blue), 15-PGDH wild-type mice treated at 5 mg/kg of SW033291 twice daily (red), 15-PGDH wild-type mice treated at 10 mg/kg of SW033291 twice daily (gold), and 15-PGDH knockout mice (green), graphed as daily means +/- SEM. N=6 for each data point. (A) Graphical plots of daily disease activity index (DAI). * Indicates $P<0.00011$ versus control by paired Student's t-test comparison across the time course. ** Indicates $P<0.013$ versus mice treated at 5 mg/kg SW033291 by paired Student's t-test. (B) Plots of daily weights, graphed as percent of day 1 value. * Indicates $P<4X10^{-5}$ versus control mice, by paired Student's t-test. ** Indicates $P<0.0002$ versus mice treated at 5 mg/kg SW033291, by paired Student's t-test.



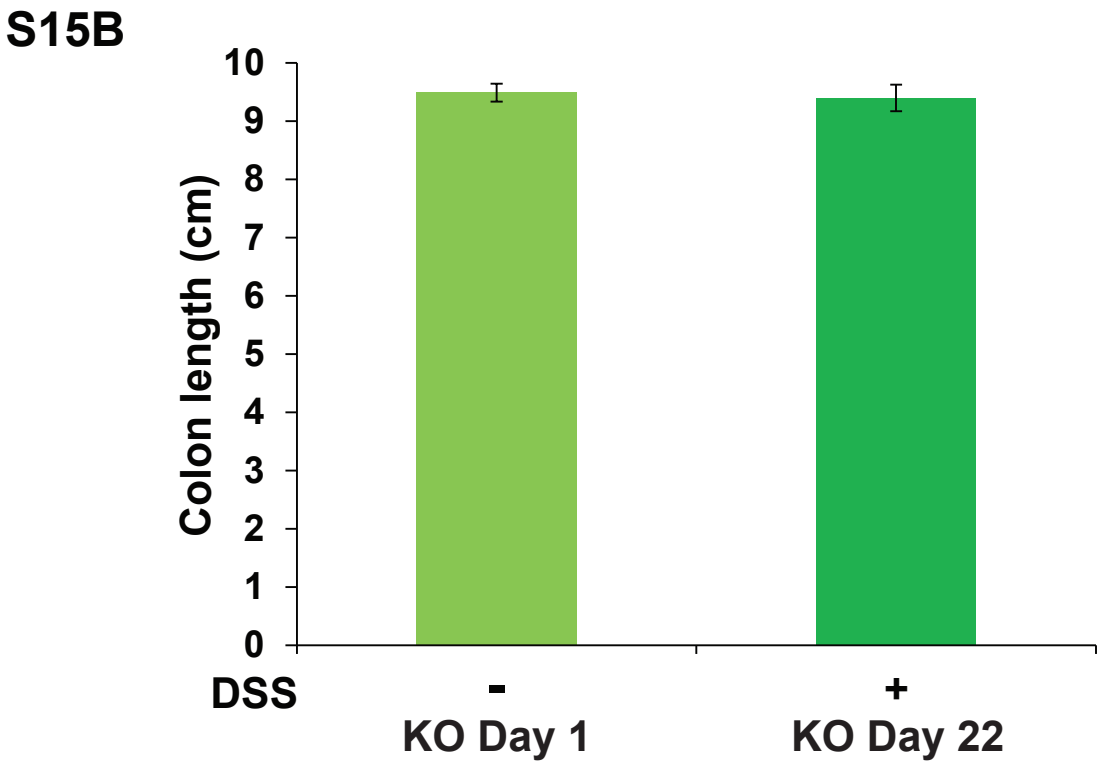
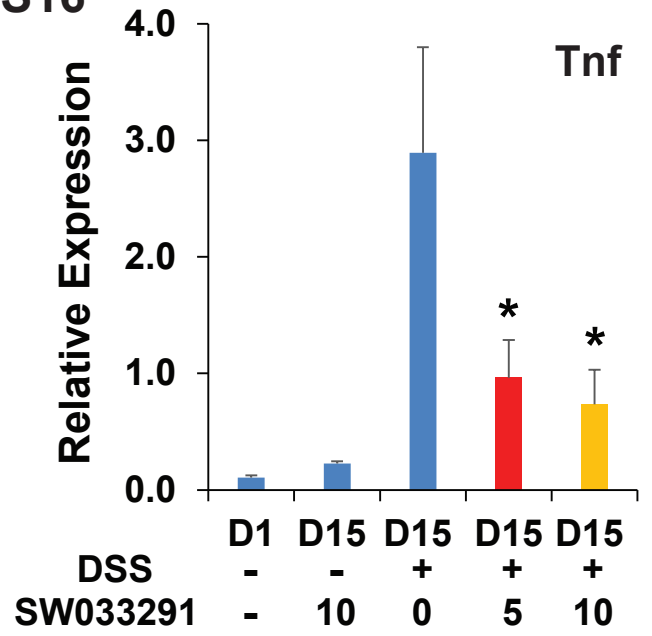


Fig. S15: 15-PGDH inhibition effect on colon shortening of DSS treated mice. Comparison of average lengths of excised colons from mice treated with DSS from study days 1-7. (A) SW033291 protection from colon shortening. Graphed as means +/- SEM are average lengths of: baseline day 1 colons from untreated mice (N=8), day 22 colons from mice treated with DSS plus vehicle control (N=9), day 22 colons from mice treated with DSS plus SW033291 at 5 mg/kg IP twice daily (N=9), and day 22 colons from mice treated with DSS plus SW033291 at 10 mg/kg IP twice daily (N=9). * Indicates $P<0.002$ compared to mice treated with DSS plus vehicle control, Student's t-test. (B) 15-PGDH knockout mice show protection from colon shortening. Graphed as means +/- SEM are baseline lengths of 15-PGDH knockout (KO) mice (day 1) (N=9) compared to DSS treated (day 22) mice (N=5).

S16


Tnf
Relative Expression
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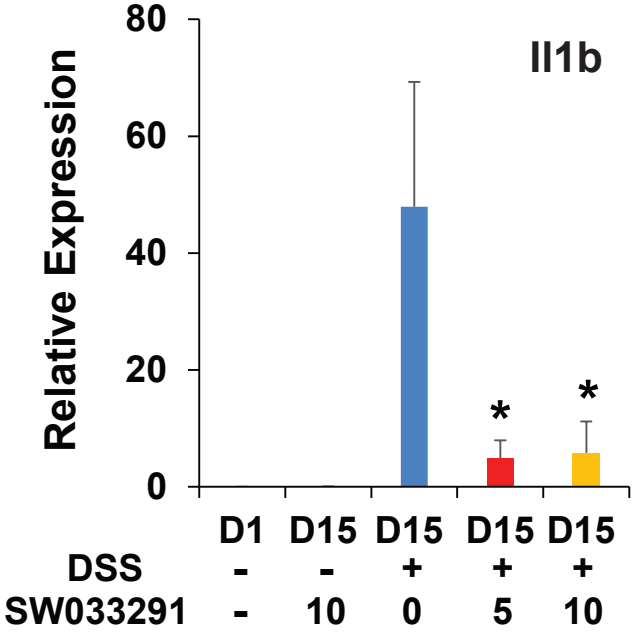
3.0
2.0
1.0
0.0
D1 D15 D15 D15 D15
DSS - - + + +
SW033291 - 10 0 5 10


Il1b
Relative Expression
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60
40
20
0
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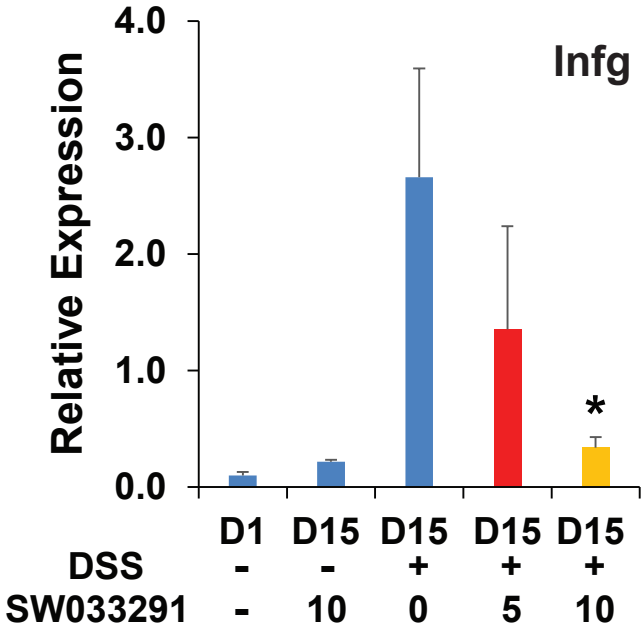
SW033291 - 10 0 5 10


Infg
Relative Expression
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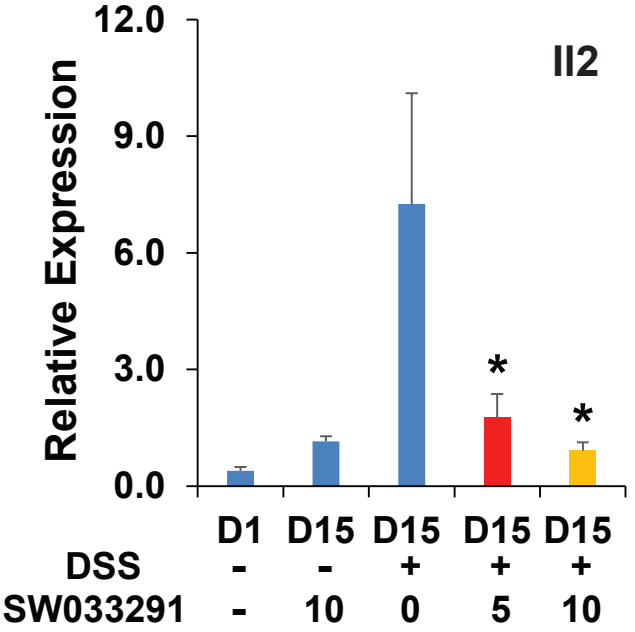
3.0
2.0
1.0
0.0
D1 D15 D15 D15 D15
DSS - - + + +
SW033291 - 10 0 5 10


Il2
Relative Expression
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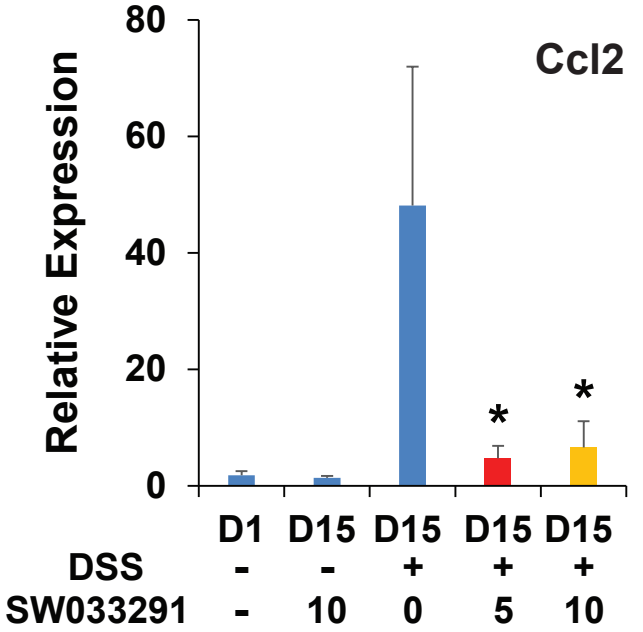
6.0
3.0
0.0
D1 D15 D15 D15 D15
DSS - - + + +
SW033291 - 10 0 5 10


Ccl2
Relative Expression
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60
40
20
0
D1 D15 D15 D15 D15
DSS - - + + +
SW033291 - 10 0 5 10

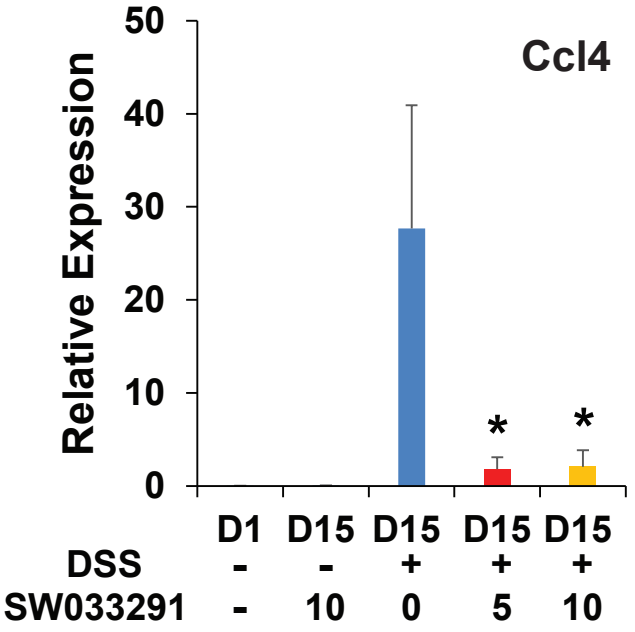

Ccl4
Relative Expression
50
40
30
20
10
0
D1 D15 D15 D15 D15
DSS - - + + +
SW033291 - 10 0 5 10

Fig. S16: SW033291 effect on DSS induction of inflammatory cytokines. Graphed in arbitrary units as means +/- SEM are relative levels of mRNA expression for inflammatory cytokines shown assessed by real-time PCR on day 15 (D15) for DSS treated (days 1-7) (+) versus untreated control (-) mice that were also treated twice daily IP with SW033291 at 5 mg/kg (5), 10 mg/kg (10), or vehicle control (-). Statistical comparisons to day 15 vehicle control mice are shown for day 15 mice treated with SW033291 at 5 mg/kg and at 10 mg/kg. * On all graphs, indicates $P<0.05$ (Student's t-test) for comparison to day 15 vehicle control mice. For reference, basal expression levels for non-DSS treated mice with and without SW033291 are also shown. Day 1, N=12. Day 15, DSS (-), SW033291 10 mg/kg, N=12. Day 15, DSS (+), SW033291 0 mg/kg, N=6. Day 15, DSS (+), SW033291 5 mg/kg, N=11. Day 15, DSS (+), SW033291 10 mg/kg, N=10.

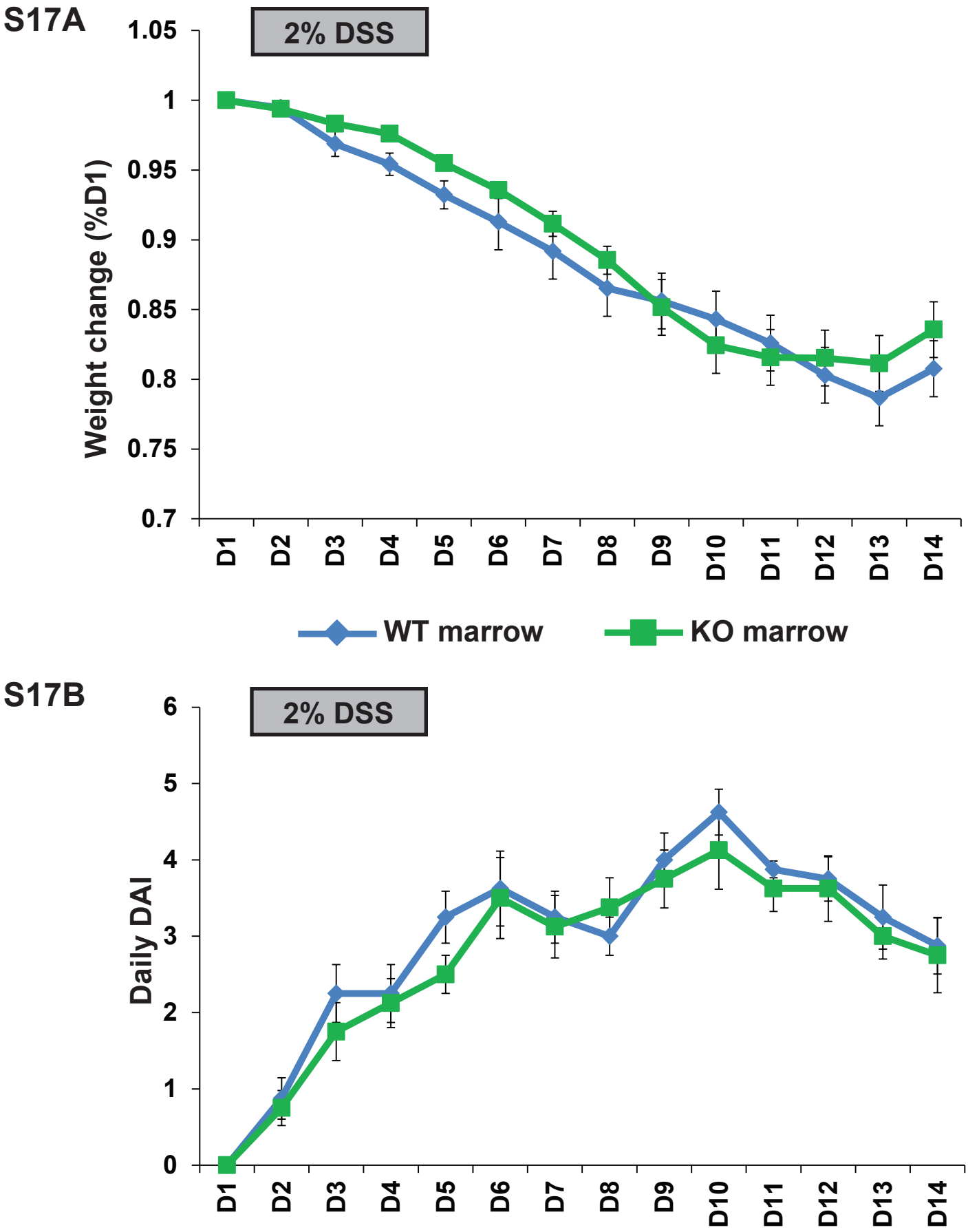


Fig. S17: DSS induced colitis activity and weight loss in chimeric mice. 15-PGDH wild-type FVB mice received bone marrow transplants from FVB donor mice that were either 15-PGDH wild-type (WT marrow) (N=8) or 15-PGDH knockout (KO marrow) (N=8). Chimeric mice were DSS treated on days 1-7. (A) Plots of daily weights, graphed as percent of day 1 value. (B) Graphical plots of daily disease activity index (DAI). All data is graphed as means +/- SEM.

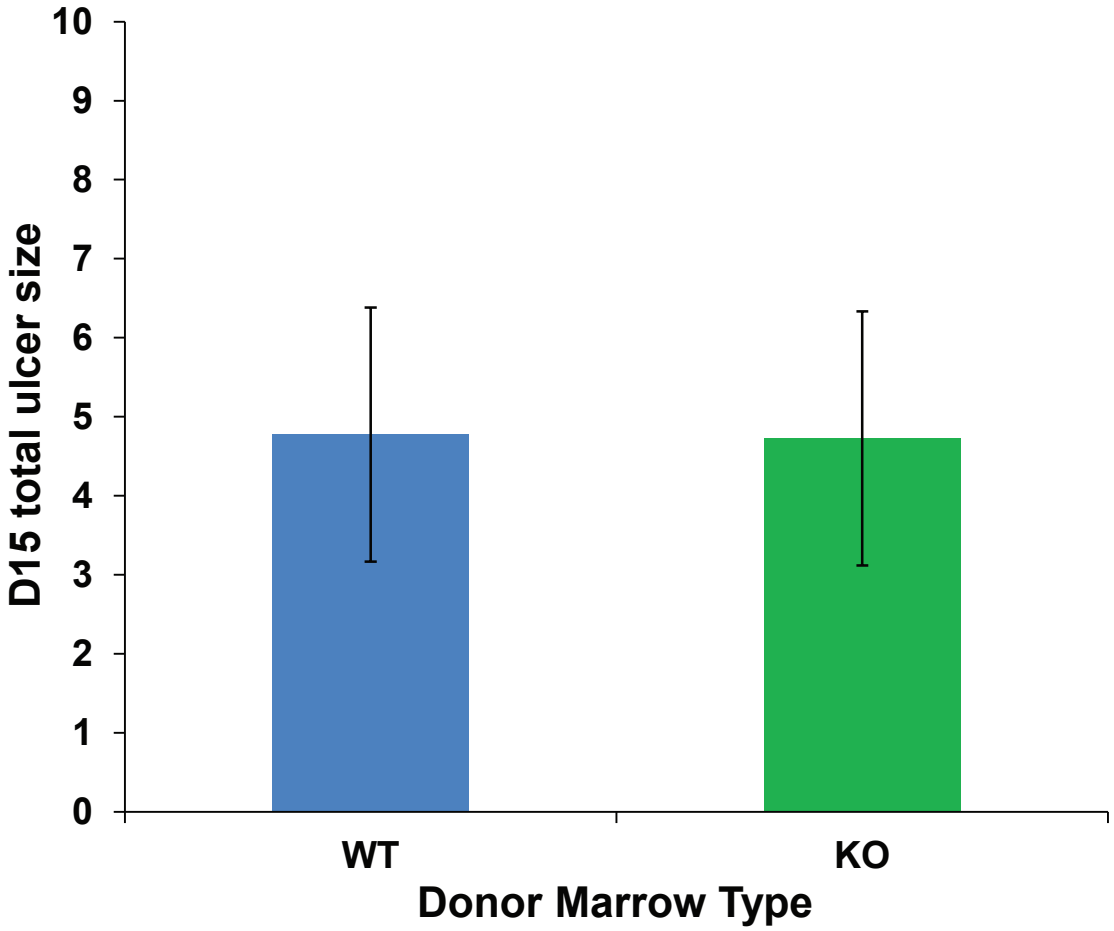
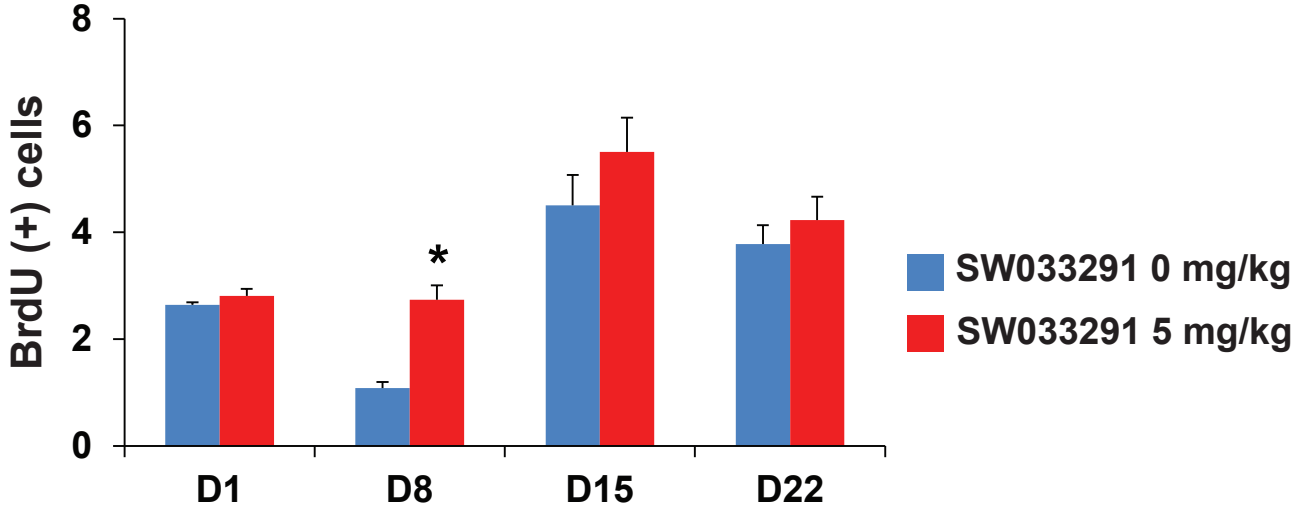


Fig. S18: DSS induced colon ulceration in chimeric mice. 15-PGDH wild-type FVB mice received bone marrow transplants from FVB donor mice that were either 15-PGDH wild-type (WT) (N=8) or 15-PGDH knockout (KO) (N=8). Chimeric mice were DSS treated on days 1-7. Graph shows mean percent of total colon length covered by ulcerated mucosa on study day 15 as determined by histomorphometric assessment and graphed as means +/- SEM.

S19A



S19B

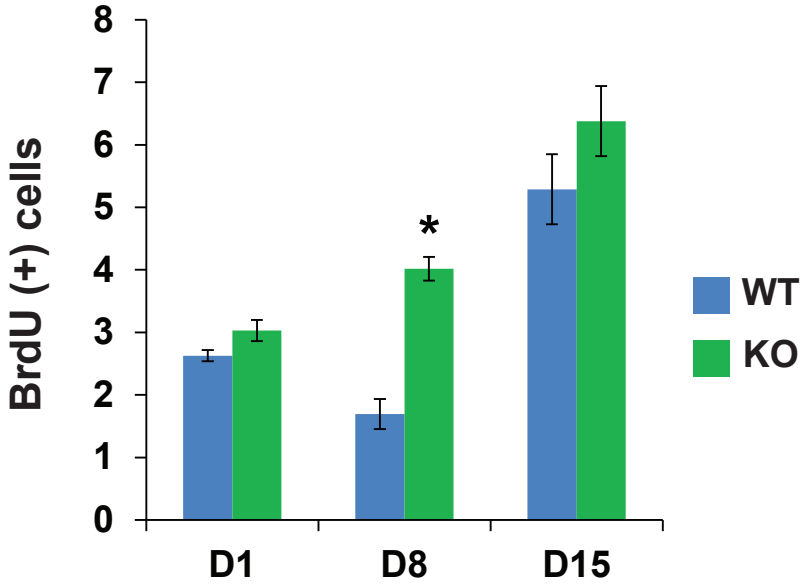


Fig. S19: 15-PGDH inhibition potentiates BrdU incorporation in colonic crypts. Time course of change in BrdU incorporation in DSS treated mice. (A) Graphed are means +/- SEM of average number of BrdU positive cells per crypt in mice treated with SW033291 5 mg/kg IP twice daily or with vehicle control (SW033291 0 mg/kg). N=6 for each data point. * Indicates P<0.0003 (by Student's t-test) for day 8 (D8) comparison of SW033291-treated versus vehicle control treated mice. (B) Graphed are means +/- SEM of average number of BrdU positive cells per crypt in DSS-treated 15-PGDH wild-type (WT) versus 15-PGDH knockout (KO) mice. N=6 to 8 mice for each data point. * Indicates $P<3X10^{-5}$ (by Student's t-test) for day 8 (D8) comparison of wild-type and knockout mice.

S20

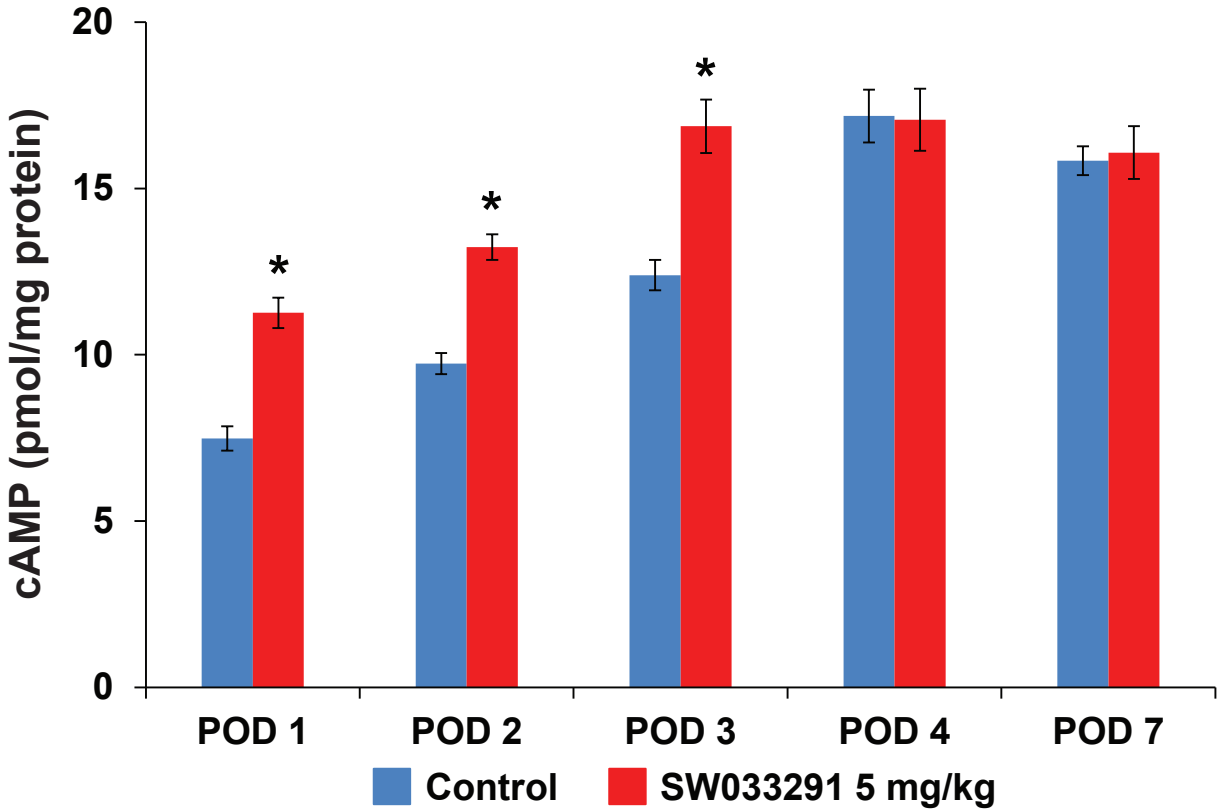


Fig. S20: SW033291 induction of cyclic AMP in regenerating livers. Graphed on Y-axis are cyclic AMP levels (cAMP, pmol/mg protein) in regenerating liver tissue of mice injected twice daily IP with vehicle control or SW033291 5 mg/kg on post-operative days 1-7 (POD 1-7). Each value is the mean of N=5 mice all assayed in duplicate and graphed as means +/- SEM. * Indicates $P<0.002$ by Student's t-test.

Table S1. Survival Following Serial Rounds of Bone Marrow Transplantation

Founder mice were transplanted with 1 million bone marrow cells along with twice daily treatment with SW033291 (10 mg/kg IP) or vehicle control. Successive rounds of serial bone marrow transplantation were then performed using founder mice as bone marrow donors to establish first round descendants, then using first round mice as donors to establish second round descendants, and finally using second round mice as donors to establish third round descendants. Survival in each cohort is indicated as (# of mice surviving at 8 weeks post-transplant) / (# of mice bone marrow transplant recipient mice).

Founder Mouse Treatment	First Round Survival	Second Round Survival	Third Round Survival
Vehicle	**5/5**	**5/5**	**4/5**
SW033291	**5/5**	**5/5**	**4/5**