Online Supplementary Materials

Materials

The OT-II transgenic mice (Jax Stock 004194) and the DBA1/J mice were from Jackson Laboratory (Bar Harbor, Maine). BALB/c and C57BL/6 mice were from Charles River Laboratories International, Inc. (Wilmington, MA). These mice were also used as wild-type controls were appropriate. H_4R-deficient mice on either the BALB/c or C57BL/6 background were from Lexicon Genetics (Woodlands Park, Tx) and generated as previously described [1]. Mice were housed in community cages on a 12-hour light cycle and fed mouse chow and water ad libitum. All procedures were performed according to the internationally accepted guidelines for the care and use of laboratory animals in research and were approved by the local Institutional Animal Care and Use Committee. JNJ 7777120 and JNJ 28307474 were synthesized as previously described [2, 3]. For all in vivo models the vehicle used was 20% hydroxypropyl-beta-cyclodextrin.

$CD11c^+$ cell Transfer

A standard protocol for $CD11c^+$ cell isolation with mCD11c isolation kit (Miltenyi Biotec, 130-052-001) was followed with no red blood cells lysis and 2 rounds of positive selection. Cells were isolated from spleens of Balb/C wild-type female mice and H_4R-deficient mice. After selection, cells were washed once with 5 ml ice cold PBS, resuspended in 0.5 ml PBS + 1% mouse serum, counted and adjusted to 3×10^6 cells/ml. CAIA model was run as given except that 0.1 ml/mouse of cell suspension was given by iv injection 2 h prior to antibody administration.

Collagen-induced arthritis in C57BL/6 mice

To induce arthritis in C57BL/6 H_4R deficient and wild-type animals the method was modified to included two CFA/collagen injection similar to that described previously [4]. Briefly, two CFA/chicken collagen (Chondrex, Inc., Redmond, WA) injections occurred on days 1 and 21 at the base of the tail, LPS (40 μg ip) was administered on day 31. After LPS boost animals were monitored for both disease onset and disease severity. In addition, starting at day 5 the wild-type mice were treated orally (by gavage) with either vehicle or 50 mg/kg JNJ 28307474 twice daily.

OT-II Th17 Model

An adoptive transfer model for Th17 cell development was previously described [5]. Briefly, wild-type or H_4R-deficient OT-II CD4+ T cells were isolated from the spleen and transferred to wild-type or H_4R-deficient C57BL/6 mice (intravenously, 10^5 cells per mouse), followed by immunization with ovalbumin peptide (sequence 323-339 (ISQAVHAAHAEINEAGR) in CFA; Life Technologies, Inc., Grand Island, NY) subcutaneously (at the base of the tail) at 100 μg/mouse the next day. Ten days after immunization, the inguinal lymph node was collected and a single cell suspension prepared. Cells were stained for CD4, intracellular IL-17 and the transgenic T-cell receptor and analyzed by FACS. Where indicated mice were treated with vehicle or JNJ 28307474 (50 mg/kg twice daily) starting the day after transfer of the OT-II cells just prior to the immunization.

Histological examination of disease severity

Paw tissue was prepared and histological analyses were performed as previously described [6]. Sections were scored in a blinded fashion for mean inflammation, pannus formation, cartilage damage, and bone damage, and the overall score was based on a set of three to four joints per animal. All parameters were scored on a 0–5 scale, as previously described [6]. A mean score for each animal was determined for each parameter, and these were averaged to determine group means. Mast cells were quantitated from 8 randomly selected high-power fields from 4 sections for each animal.

Paw Cytokine measurements

Briefly, disease hind paws were minced with surgical scissors and tissue was homogenized using a bead homogenizer. RNA was extracted using RNeasy Plus Mini Kit (QIAGEN, Germantown, MD) and analyzed by RT-PCR using primers obtained from Integrated DNA Technologies (Coralville, IA). Data is expressed as the ratio to the levels of a housekeeping gene.

IgG measurements

Serum collagen antibodies were quantitated using kits obtained from Chondrex, Inc. (Redmond, WA) as per the manufacturer's instructions.

In Vitro and ex vivo IL-17 production

Blood was obtained from three healthy donors in collaboration with The Scripps Research Institute (San Diego, CA) and the procedures were approved by the Scripps Institutional Review Board. Peripheral blood mononuclear cells (PBMC) were isolated using Ficoll density gradient centrifugation and divided into 2-4 replicates. H_4R antagonist (1 μM) or vehicle (0.01% dimethylsulfoxide) was added and incubated at 37°C for 1h. PBMC were then transferred to plates pre-coated with anti-CD3 (1 μg/mL) and incubated with either 1 μg/mL anti-CD28, 10 ng/mL IL-1β and 50 ng/mL IL-23 in various combinations as given. Unstimulated PBMC were used as a negative control. The samples were incubated for 48 h at 37°C and then IL-17 levels were measured. Measurements were carried out using five different donors and the data was pooled for statistical analysis. For mouse Th17 cells, blood was collected 20 min after C57BL/6 wild-type mice and H_4R-deficient mice were given vehicle orally or wild-type mice were treated orally with 20 mg/kg JNJ 7777120. Blood was diluted 1:1.5 with RPMI media and then incubated for 18 h 37°C with 4 μg/mL mouse anti-CD3/CD28 and 50 ng/mL mouse IL-23. For both assays IL-17 levels were measured by ELISA.

Statistical Analysis

Details on the statistical analysis are given in each figure caption. The sample size used for the in vivo experiments was based on previous experience and pilot studies. In general, a Mann-Whitney test was used for all statistical comparisons between two groups. For three or more groups a one-way ANOVA with post-hoc Dunnett's test was used. Time course data was analyzed in several ways including a Wilcoxon ranked sum test or a Friedman test with a Dunn's

multiple comparison test for the overall time course and either a Mann-Whitney test or ANOVA to compare individual time points. For the CIA model the incidence of arthritis was analyzed by Fisher's Exact test to assess whether differences in the proportion of arthritis incidence between H_4R-deficient and wild-type mice was significant. Additionally, the incidence of arthritis in H_4R-deficient and wild-type mice was analyzed by a log-rank survival method. All statistical analysis was carried out using GraphPad Prism (San Diego, CA).

References

[1] Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung W-P. Histamine H_4 receptor mediates chemotaxis and calcium mobilization of mast cells. J Pharmacol Exp Ther. 2003;305(3):1212-21.

[2] Jablonowski JA, Grice CA, Chai W, Dvorak CA, Venable JD, Kwok AK, et al. The first potent and selective non-imidazole human histamine H_4 receptor antagonists. J Med Chem. 2003;46(19):3957-60.

[3] Arienti KL, Breitenbucher JG, Buzard DJ, Edwards JP, Hack MD, Khatuya H, et al., inventors; (USA). assignee. Preparation of benzimidazoles as histamine H_4 receptor modulators for the treatment of inflammatory diseases. US patent 2005070550. 2005 20050331.

[4] Inglis JJ, Simelyte E, McCann FE, Criado G, Williams RO. Protocol for the induction of arthritis in C57BL/6 mice. Nat Protoc. 2008;3(4):612-8.

[5] McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, Blumenschein WM, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol. 2009;10(3):314-24.

[6] Bendele AM, Chlipala ES, Scherrer J, Frazier J, Sennello G, Rich WJ, et al. Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models of rheumatoid arthritis. Arthritis Rheum. 2000;43(12):2649-59.

Supplementary Figure Captions

Figure S1. A) Mast cells in the synovial tissues were quantitated in wild-type and H_4R-deficient mice (n= 10 per group) in the CAIA model. Statistical comparison was conducted using a Mann-Whitney test. ***$p < 0.001$. B) Mast cells in the synovial tissues were quantitated in the CIA model in mice (n = 8 per group) treated with vehicle (Veh), 5 mg/kg, 20 mg/kg or 50 mg/kg JNJ 28307474. Statistical significance between each JNJ 28307474 group and vehicle was assessed by a one-way ANOVA with post-hoc Dunnett's test. **$p < 0.01$, ***$p < 0.001$.

Figure S2. (A) Wild-type mice (WT, n = 5), H_4R-deficient mice who received wild-type $CD11c^+$ cells (H4R KO w/ WT DC, n = 5) and H_4R-deficient mice who received H_4R-deficient $CD11c^+$ cells (H4R KO w/ KO DC, n = 4) were given collagen antibody cocktail and then challenged with LPS ip two days later. Starting the next day, mice were examined visually for the appearance of arthritis in the peripheral joints, and the severity of arthritis was graded on a scale of 0–4 for each paw. The mean and SEM for the sum of the severity scores are given in panel (B). Statistical significance was calculated for each time point of the severity score or comparing the AUC a one-way ANOVA with post-hoc Dunnett's test. *$p < 0.05$.

Figure S3. mRNA levels in paw tissue homogenates were measured by RT-PRC in the CIA model in mice treated with vehicle or 50 mg/kg JNJ 28307474 (H4R Antag). Data are presented as expression relative to a housekeeping gene. Statistical significance (p-values) was calculated using a Mann-Whitney test.

Figure S4. Collagen II specific IgG levels were measured in the serum in the CIA model in mice treated with vehicle or 50 mg/kg JNJ 28307474 (H4R Antag). Statistical significance (p-values) was calculated using a Mann-Whitney test.

Figure S1

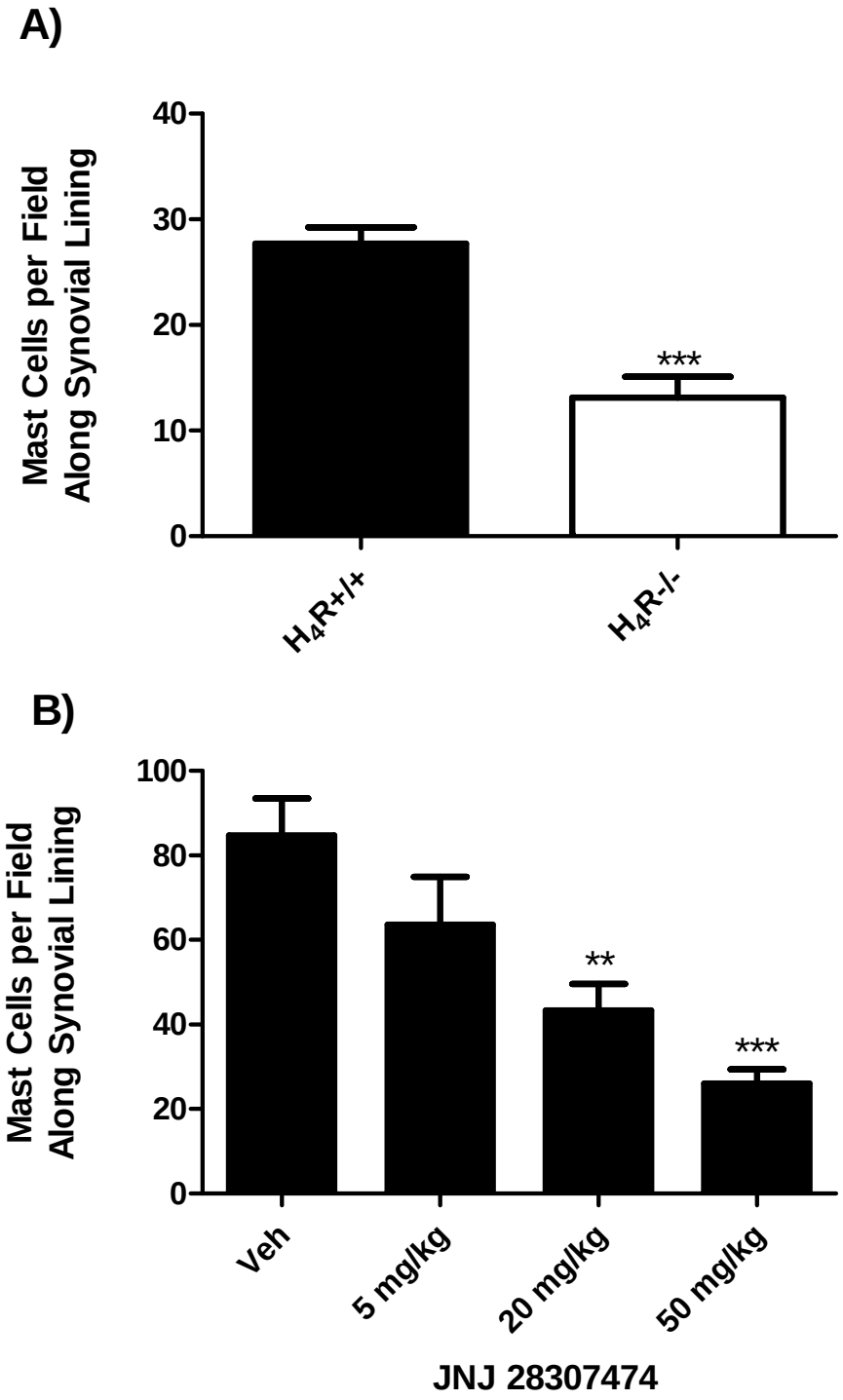

A)
Mast Cells per Field Along Synovial Lining
40
30
20
10
0
***
H4R+/+
H4R-/-
B)
Mast Cells per Field Along Synovial Lining
100
80
60
40
20
0
**
***
Veh
5 mg/kg
20 mg/kg
50 mg/kg
JNJ 28307474

Figure S2

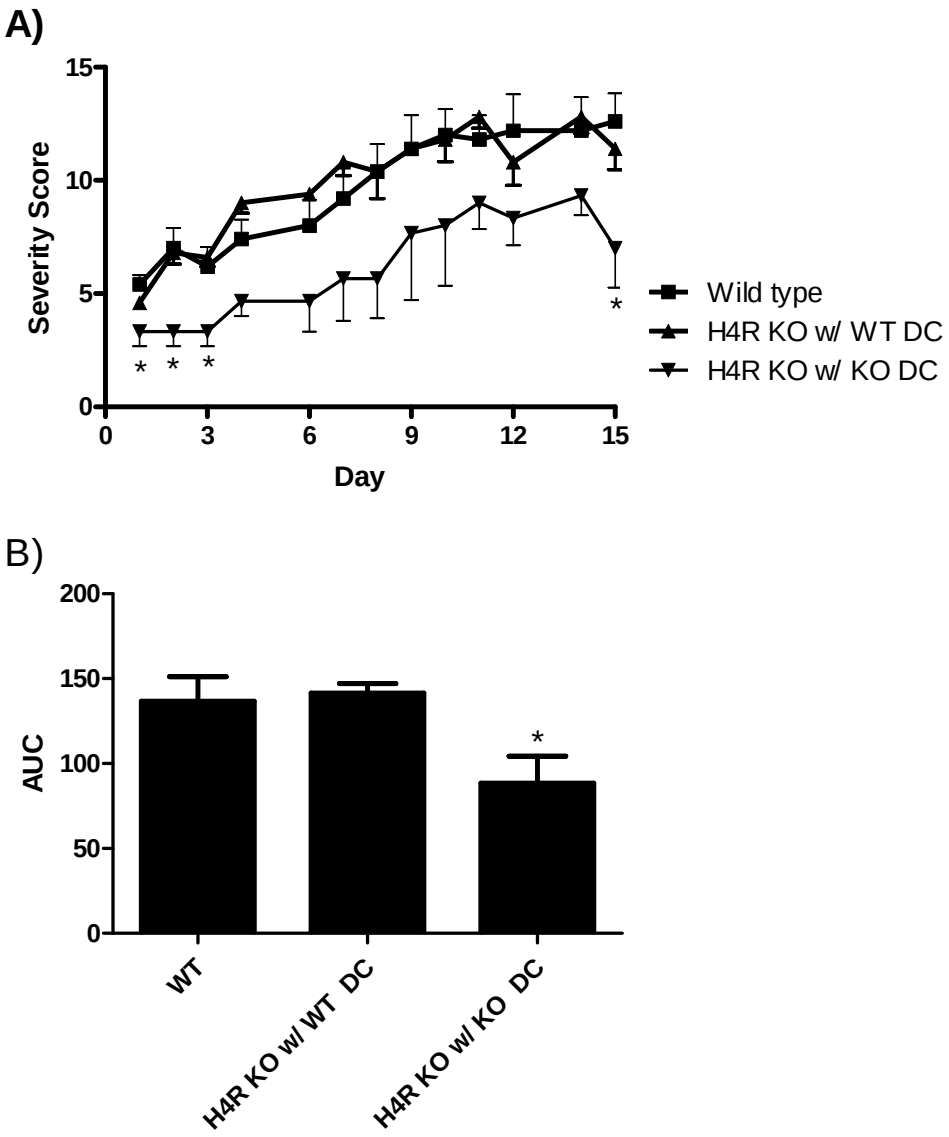

A)
Severity Score
15
10
5
0
0
3
6
9
12
15
Day
*
*
*
*
Wild type
H4R KO w/ WT DC
H4R KO w/ KO DC
B)
AUC
200
150
100
50
0
*
WT
H4R KO w/ WT DC
H4R KO w/ KO DC

Figure S3

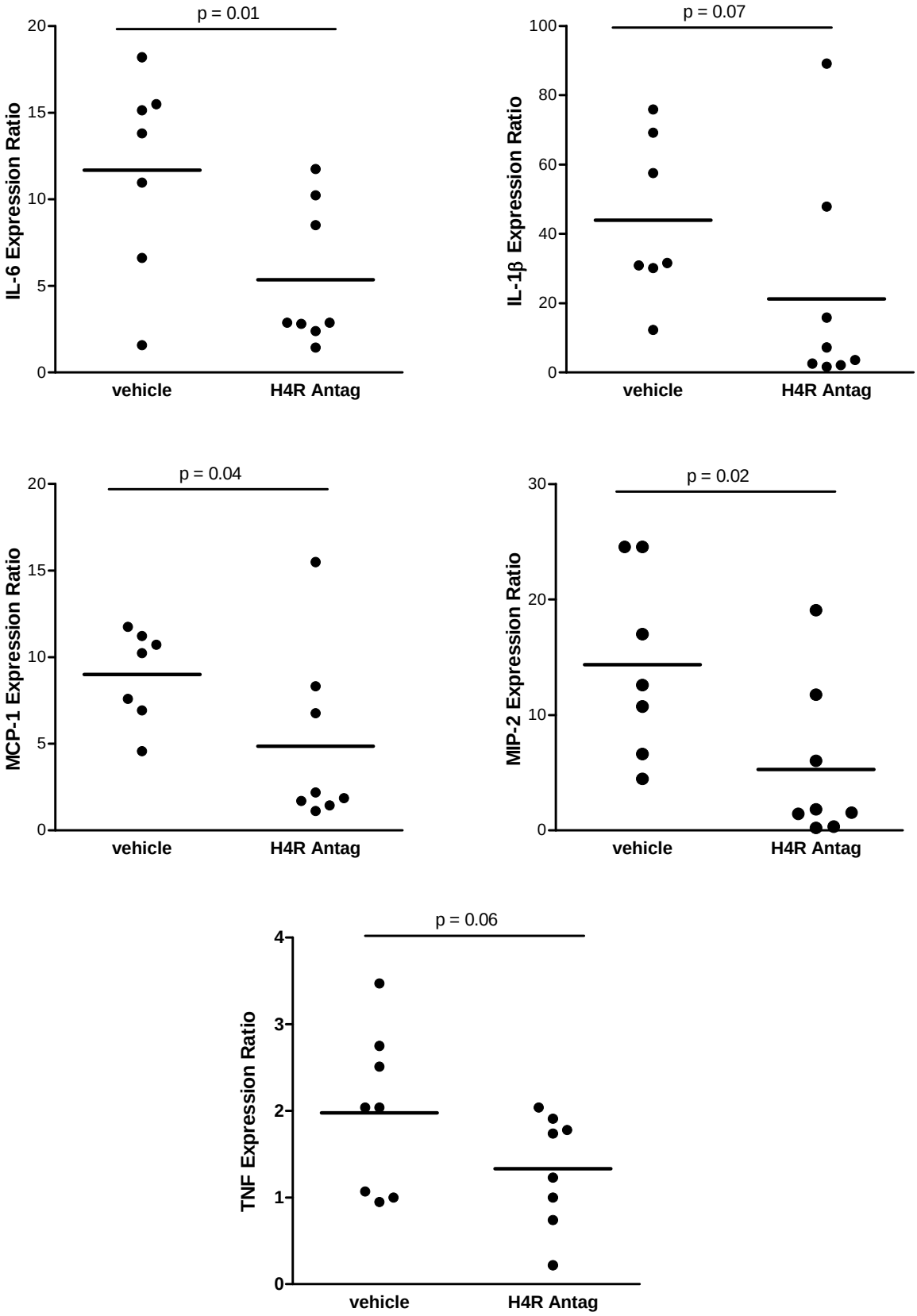
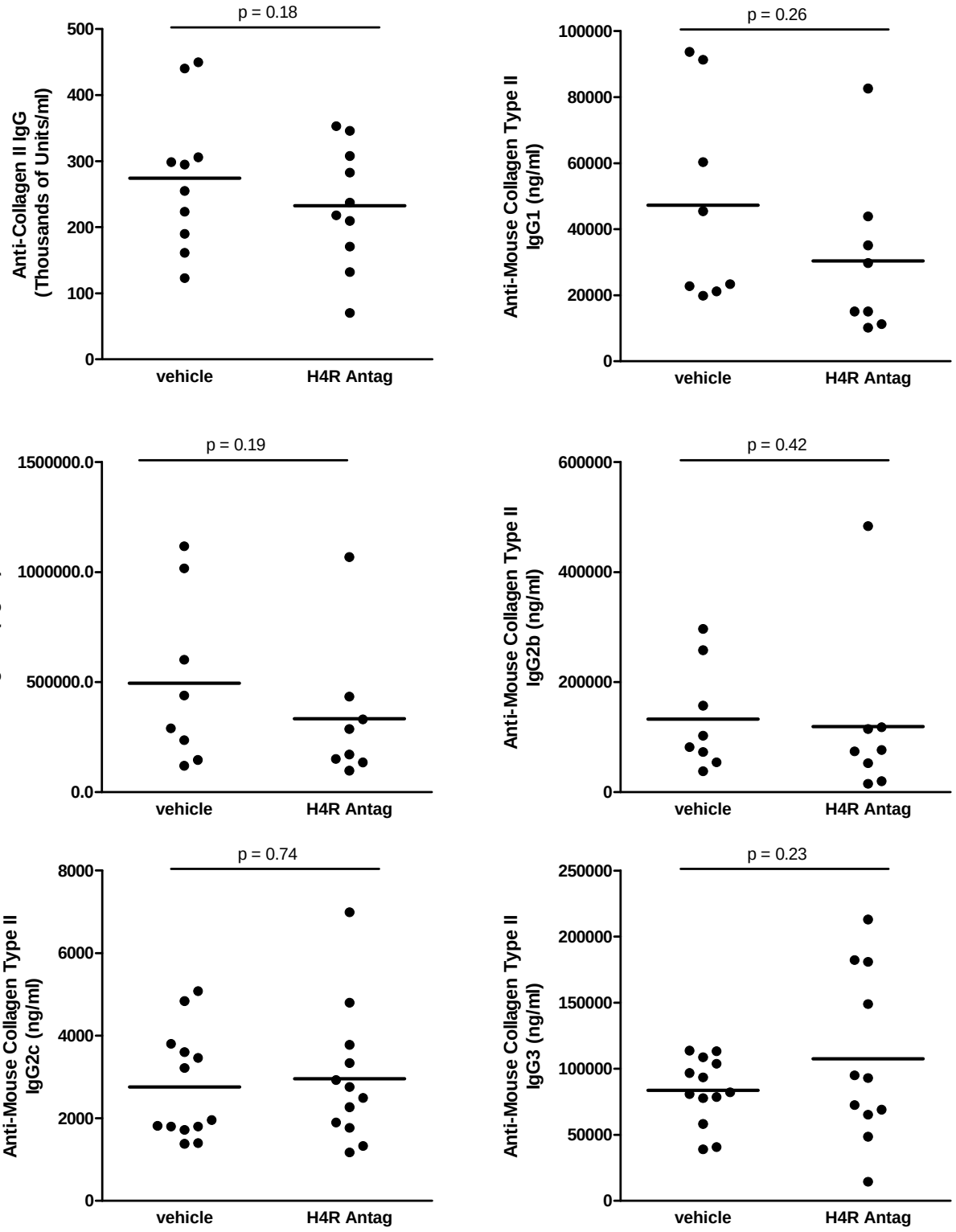

p = 0.01
IL-6 Expression Ratio
20
15
10
5
0
vehicle
H4R Antag
p = 0.07
IL-1β Expression Ratio
100
80
60
40
20
0
vehicle
H4R Antag
p = 0.04
MCP-1 Expression Ratio
20
15
10
5
0
vehicle
H4R Antag
p = 0.02
MIP-2 Expression Ratio
30
20
10
0
vehicle
H4R Antag
p = 0.06
TNF Expression Ratio
4
3
2
1
0
vehicle
H4R Antag

Figure S4


p = 0.18
Anti-Collagen II IgG (Thousands of Units/ml)
500
400
300
200
100
0
vehicle
H4R Antag
p = 0.26
Anti-Mouse Collagen Type II IgG1 (ng/ml)
100000
80000
60000
40000
20000
0
vehicle
H4R Antag
p = 0.19
Anti-Mouse Collagen Type II IgG2a (ng/ml)
1500000.0
1000000.0
500000.0
0.0
vehicle
H4R Antag
p = 0.42
Anti-Mouse Collagen Type II IgG2b (ng/ml)
600000
400000
200000
0
vehicle
H4R Antag
p = 0.74
Anti-Mouse Collagen Type II IgG2c (ng/ml)
8000
6000
4000
2000
0
vehicle
H4R Antag
p = 0.23
Anti-Mouse Collagen Type II IgG3 (ng/ml)
250000
200000
150000
100000
50000
0
vehicle
H4R Antag