GPR101 mediates the pro-resolving actions of RvD5$_{n-3\ DPA}$ in arthritis and infections

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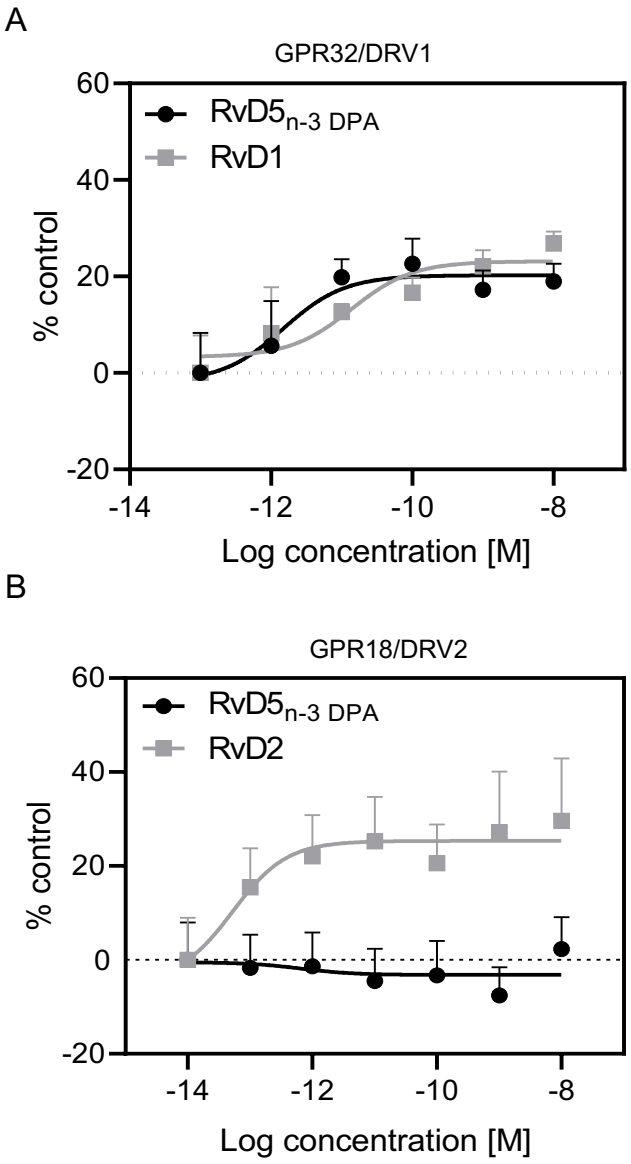
Keywords:

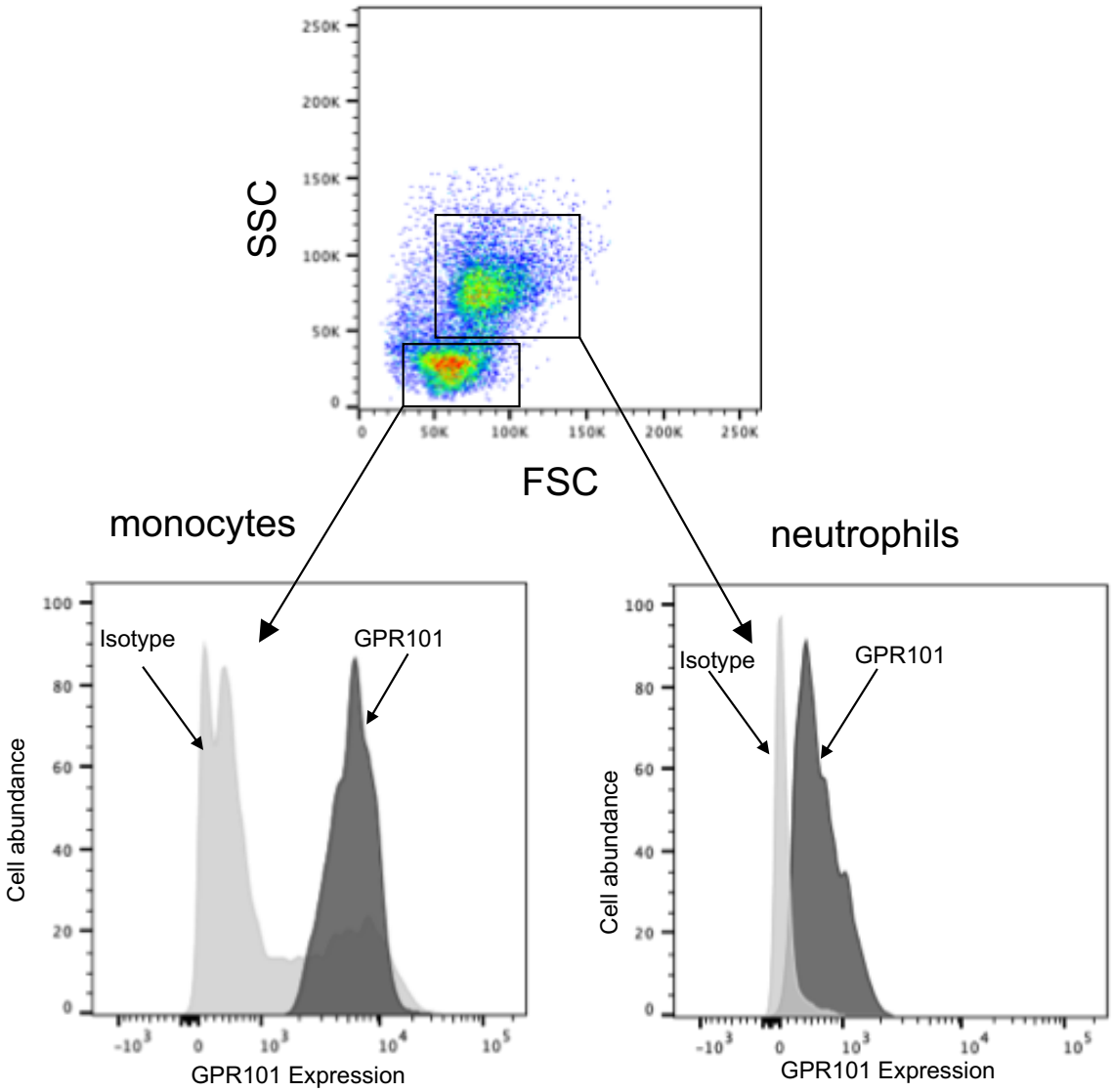
lipid mediators, resolvins, specialized pro-resolving mediators, rheumatoid arthritis, G-protein coupled receptor, omega-3

Supplemental Figures

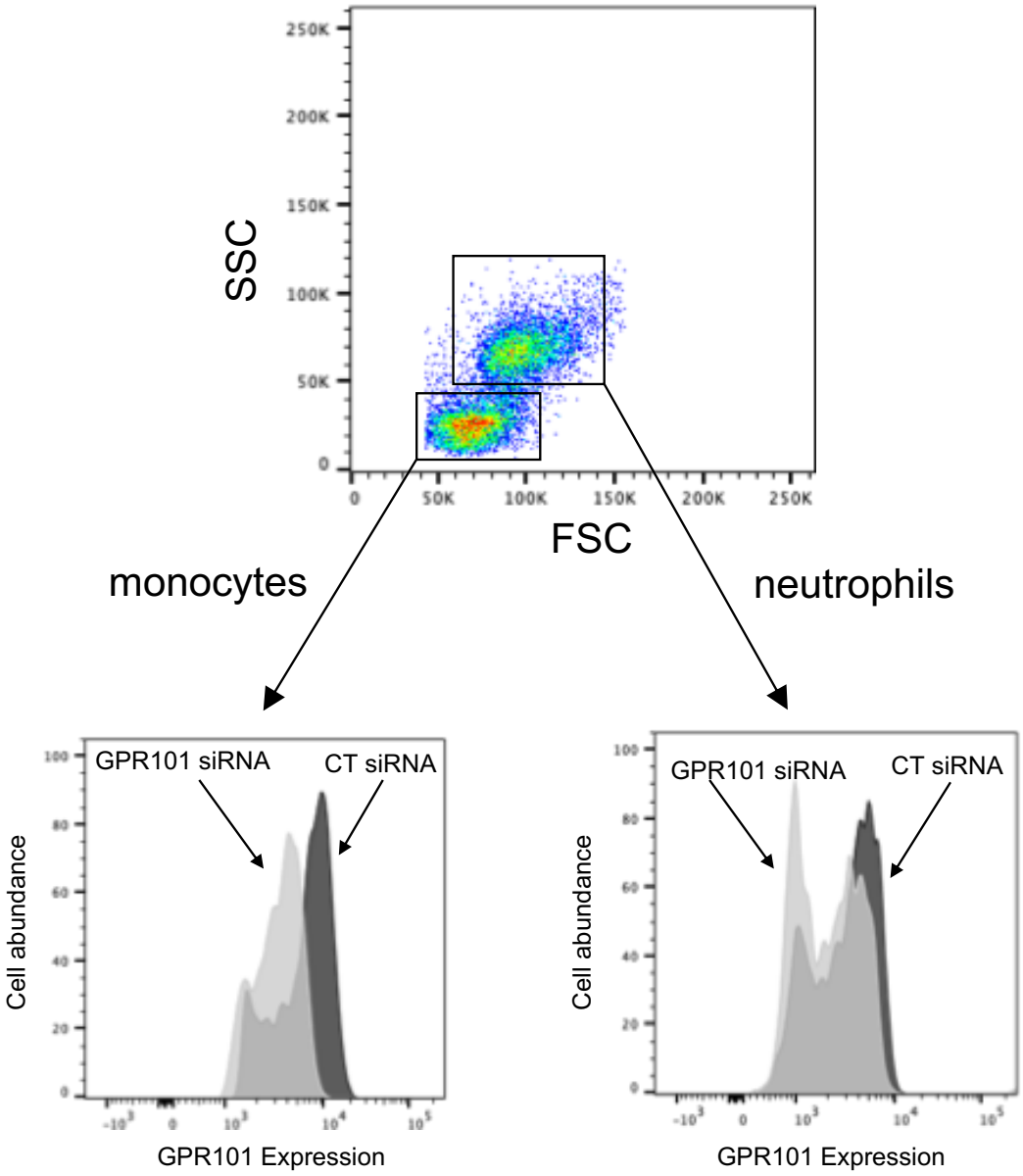


Supplemental Figure 1: Interaction of $RvD5_{n\text{-}3\ DPA}$ with GPR32/DRV1 and GPR18/DRV2. (A) CHO cells expressing GPR32 coupled with the β-arrestin luminescent reporter system were incubated with the indicated concentrations of RvD1 (EC_{50} ~1.4 x 10^{-11} M), $RvD5_{n\text{-}3\ DPA}$ (EC_{50} ~1.5 x 10^{-12} M), or vehicle (Cell Plating Reagent containing 0.01% ethanol) and receptor activation was measured as an increase in luminescence signal. (B) CHO cells expressing

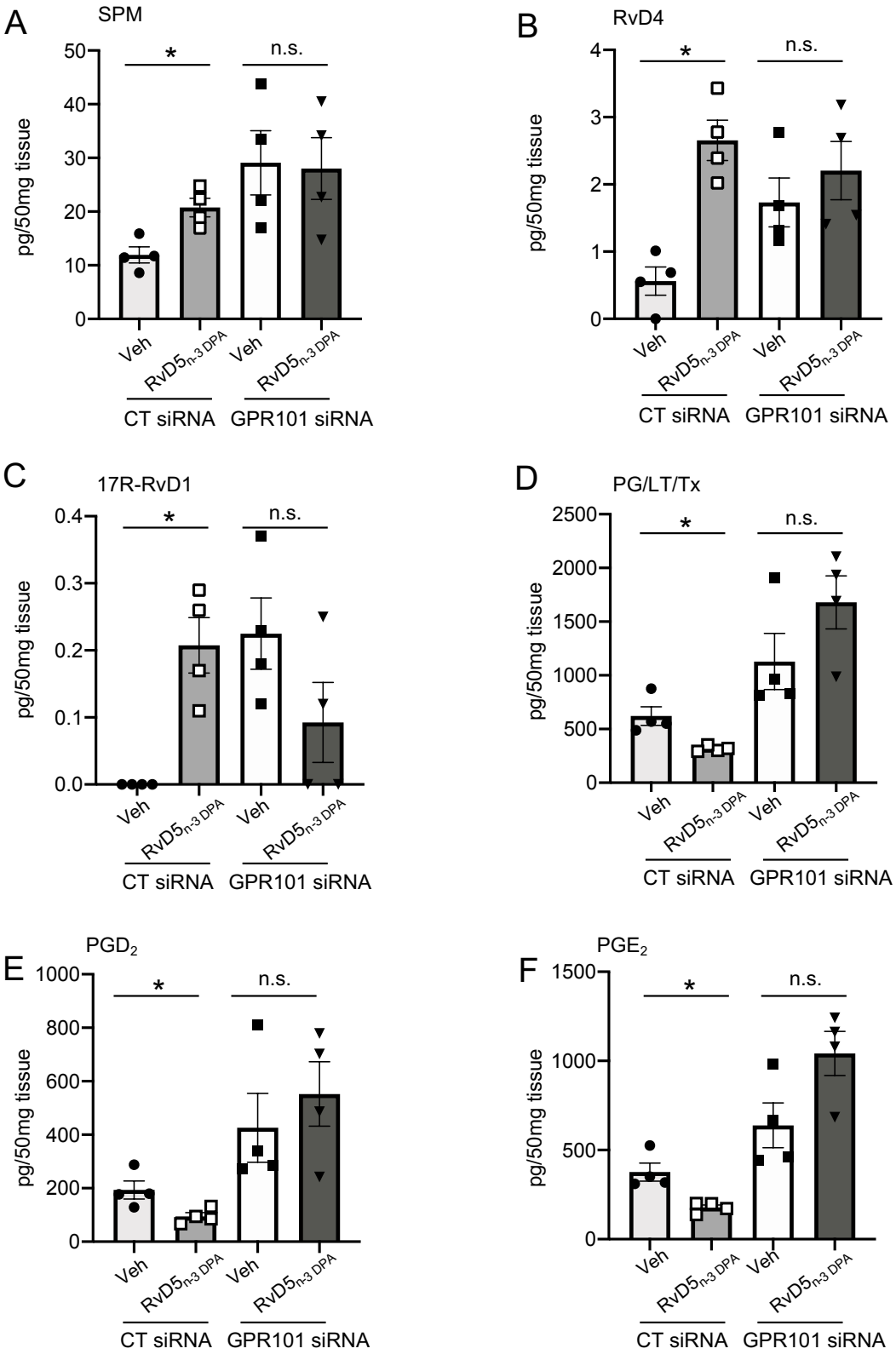
GPR18 coupled with the β-arrestin luminescent reporter system were incubated with the indicated concentrations of RvD2 (EC_{50} ~5.3 x 10^{-14} M), $RvD5_{n\text{-}3\ DPA}$ (no response), or vehicle (Cell Plating Reagent containing 0.01% ethanol) and receptor activation was measured as an increase in luminescence signal. Results are shown as mean ± SEM (n = 3 in two independent experiments).



Supplemental Figure 2: Expression of GPR101 in mouse leukocytes. Peripheral blood was collected and the expression of GPR101 was determined in neutrophils and monocytes using flow cytometry. Results are representative of n = 4 mice per group from two distinct experiments.



Supplemental Figure 3: Administration of siRNA targeting GPR101 reduces receptor expression on mouse circulating neutrophils and monocytes. Mice were administered 9 µg of siRNA to mouse GPR101 or a scrambled control sequence (CT siRNA). After 72 h blood was collected and the expression of GPR101 was determined on neutrophils and monocytes. Results are representative of n = 4 mice per group from two distinct experiments.


A
SPM
pg/50mg tissue
50
40
30
20
10
0
*
n.s.
Veh
RvD5n-3 DPA
Veh
RvD5n-3 DPA
CT siRNA
GPR101 siRNA
B
RvD4
pg/50mg tissue
4
3
2
1
0
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n.s.
Veh
RvD5n-3 DPA
Veh
RvD5n-3 DPA
CT siRNA
GPR101 siRNA
C
17R-RvD1
pg/50mg tissue
0.4
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Veh
RvD5n-3 DPA
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CT siRNA
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2500
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Veh
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RvD5n-3 DPA
CT siRNA
GPR101 siRNA
E
PGD2
pg/50mg tissue
1000
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400
200
0
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n.s.
Veh
RvD5n-3 DPA
Veh
RvD5n-3 DPA
CT siRNA
GPR101 siRNA
F
PGE2
pg/50mg tissue
1500
1000
500
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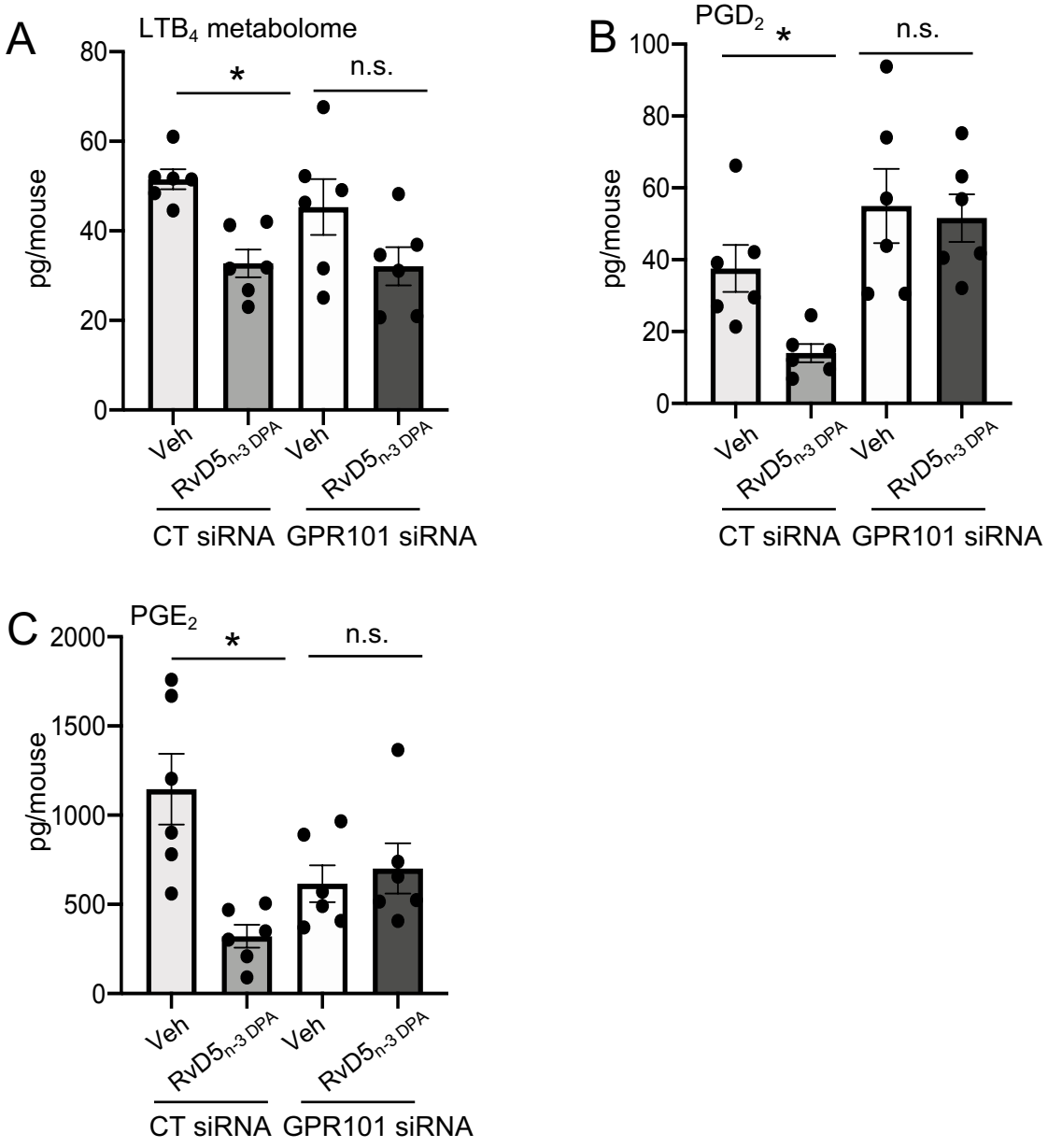
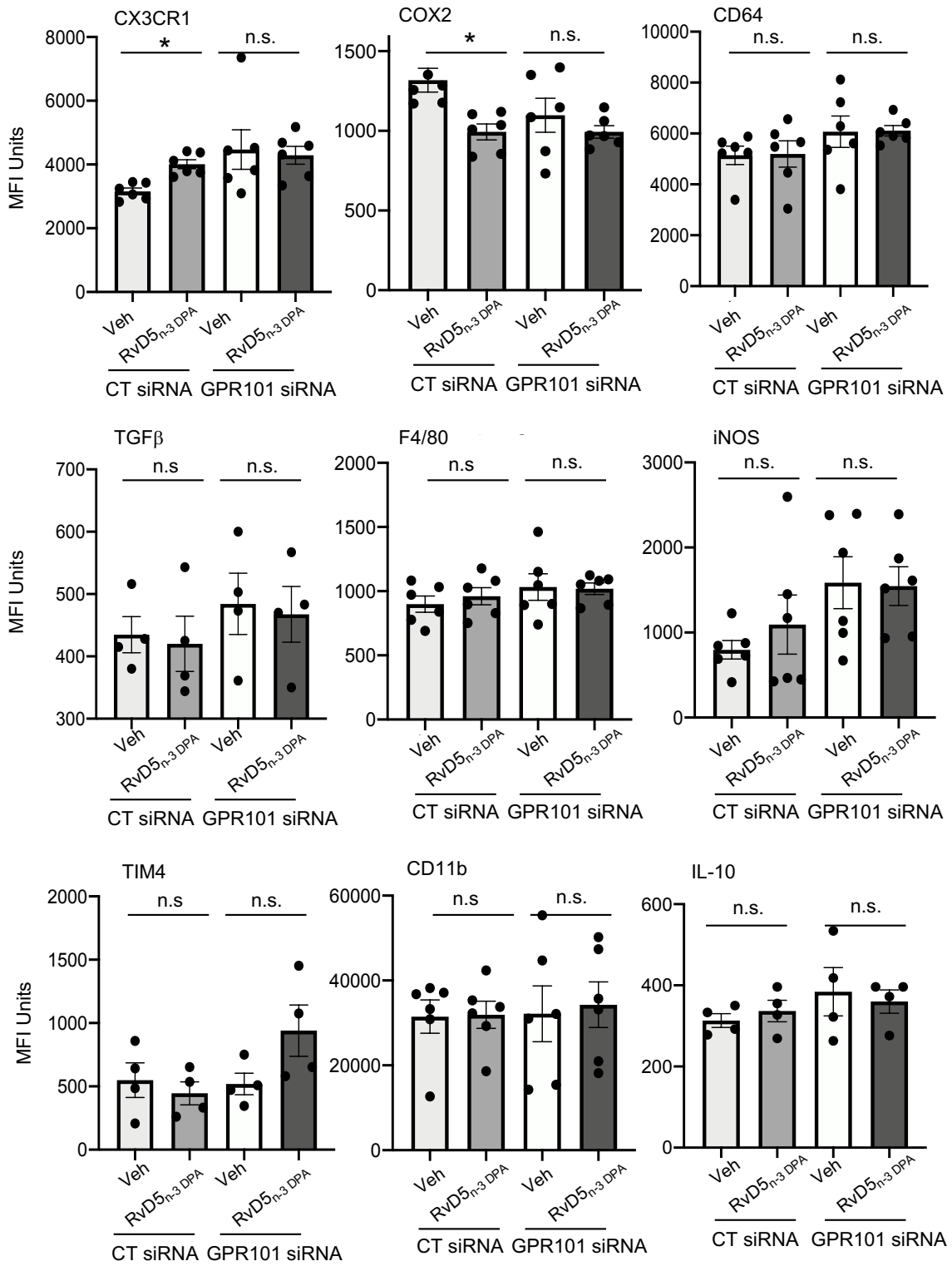
n.s.
Veh
RvD5n-3 DPA
Veh
RvD5n-3 DPA
CT siRNA
GPR101 siRNA

Supplemental Figure 4: Reduction of GPR101 expression limits the ability of $RvD5_{n\text{-}3\ DPA}$ to regulate intestinal eicosanoid and SPM concentrations. Mice were administered 9 µg of siRNA to mouse GPR101 or a scrambled control sequence (CT siRNA). After 24 h and 72 h mice were administered arthritogenic serum. Mice were then treated with $RvD5_{n\text{-}3\ DPA}$ (150ng/mouse) or vehicle (72h and 96h after siRNA administration), small intestines were collected on day 7 post serum administration and lipid mediator profiles determined using LC-MS/MS based lipid mediator profiling. Concentrations of (A) pro-resolving mediators (sum of DHA, n-3 DPA, EPA and AA derived specialized pro-resolving mediators- SPM), (B) RvD4, (C)17R-RvD1, (D) sum of prostaglandins (PG), Leukotrienes (LT) and Thromboxane (Tx), (E) PGD_2, (F) PGE_2. Results are representative of n = 4 mice per group. * $P < 0.05$ *versus* vehicle group using Kruskal-Wallis test with Dunn's post hoc multiple comparisons test.



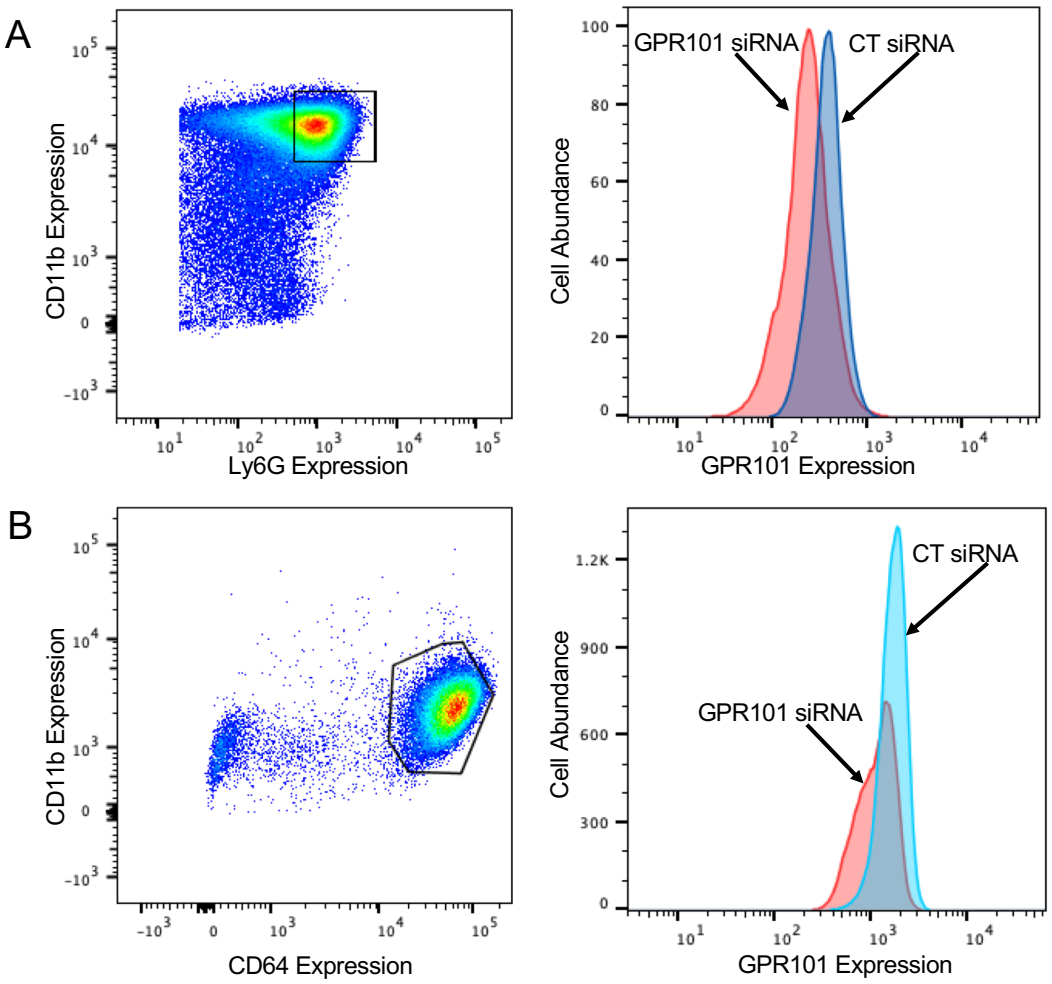
Supplemental Figure 5: Knockdown of GPR101 limits the ability of $RvD5_{n\text{-}3\ DPA}$ to regulate exudate prostaglandin and leukotriene concentrations. Mice were administered 9 μg of siRNA to mouse GPR101 or a scrambled control sequence (CT siRNA). After 72 h they were administered $RvD5_{n\text{-}3\ DPA}$ (100ng/mouse) or vehicle control (PBS containing 0.1 % ethanol) then inoculated via intraperitoneal injection with 10^5 c.f.u. *E. coli*. After 14h exudates were collected

and concentrations of (A) the Leukotriene B_4 metabolome (LTB_4, 5S, 12S-diHETE and 20-OH-LTB_4), (B) PGD_2, (C) PGE_2 were determined using LC-MS/MS based lipid mediator profiling. Results are representative of n = 6 mice per group from two distinct experiments. * $P < 0.05$ *versus* vehicle group using Kruskal-Wallis test with Dunn's post hoc multiple comparisons test.



Supplemental Figure 6: Knockdown of GPR101 limits the ability of $RvD5_{n\text{-}3\ DPA}$ to regulate exudate monocyte-derived macrophage phenotype during infectious inflammation. Mice

were administered 9 μg of siRNA to mouse GPR101 or a scrambled control sequence (CT siRNA) after 72 h they were administered $RvD5_{n\text{-}3\ DPA}$ (100ng/mouse) or vehicle control (PBS containing 0.1 % ethanol) then inoculated with 10^5 c.f.u. *E. coli*. After 14h exudates were collected and the expression of macrophage lineage markers was determined using fluorescently labelled antibodies and flow cytometry. Results are representative of n = 4-6 mice per group from two distinct experiments. *P < 0.05 *versus* vehicle group using Kruskal-Wallis test with Dunn's post hoc multiple comparisons test.



Supplemental Figure 7: Administration of siRNA targeting GPR101 reduces receptor expression on mouse peritoneal neutrophils and macrophages during *E. coli* infections. Mice were administered 9 µg of siRNA to mouse GPR101 or a scrambled control sequence (CT siRNA). After 72 h they were challenged with *E. coli* (10^5 c.f.u./mouse) and exudates collected after 4h. GPR101 Expression was determined on (A) neutrophils and (B) macrophages using flow cytometry and fluorescently labelled antibodies. Results are representative of n = 4 mice per group.