Supplemental Material

PTPN2 links colonic and joint inflammation in experimental autoimmune arthritis

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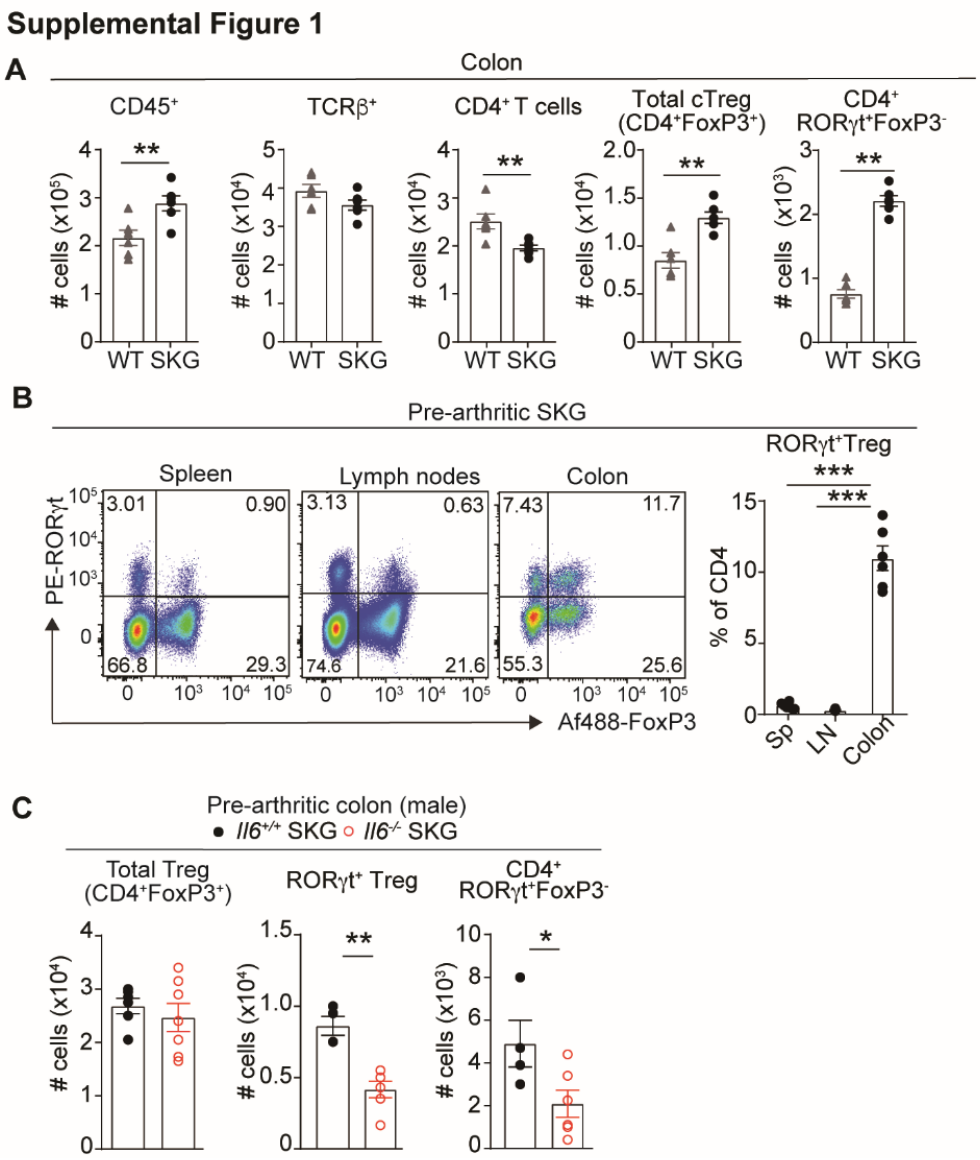
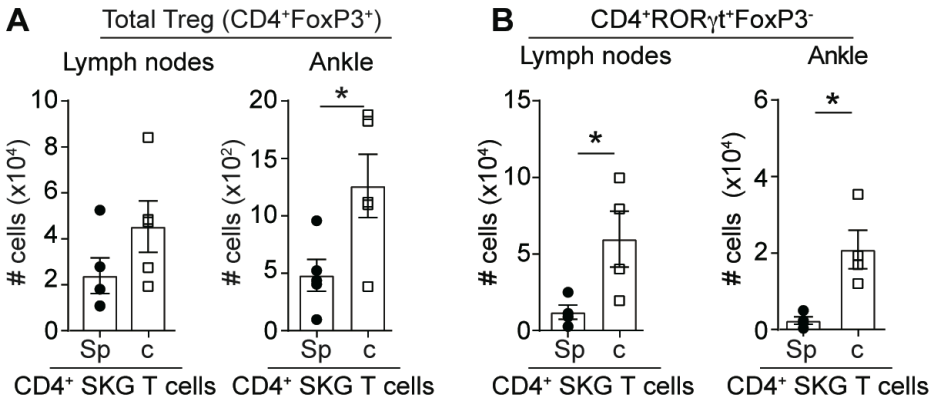
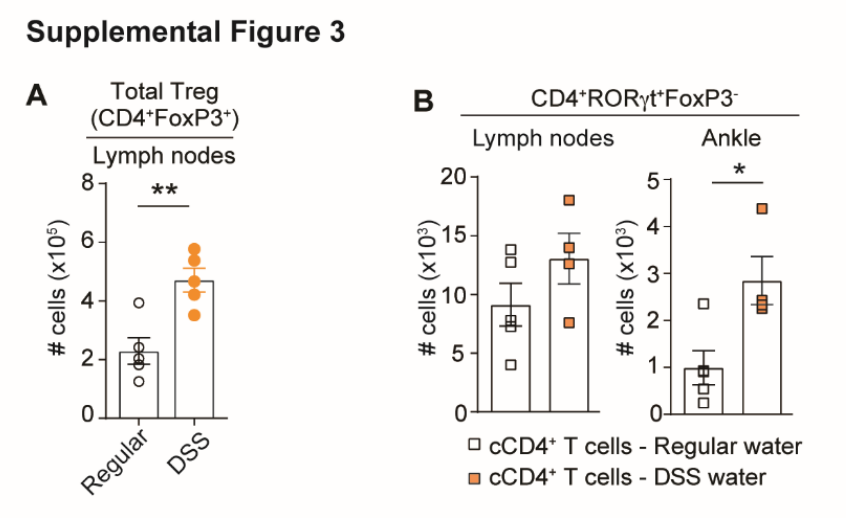


Supplemental Figure 1. Additional T cell counts in mice assessed in Figure 1. (**A**) Number of leukocytes (CD45$^+$), total T cells (TCRβ$^+$), CD4$^+$ T cells (TCRβ$^+$CD4$^+$), total Tregs (TCRβ$^+$CD4$^+$FoxP3$^+$) and RORγt$^+$FoxP3$^-$CD4$^+$ T cells (CD45$^+$TCRβ$^+$CD4$^+$RORγt$^+$ FoxP3$^-$) in colon isolated from mice in **Figure 1A**. (**B**) Representative flow cytometric staining for FoxP3- and RORγt-expressing CD4$^+$ T cells in spleen, lymph nodes and colons of pre-arthritic SKG mice (n=7). (**C**) Number of total Tregs (CD4$^+$FoxP3$^+$), RORγt$^+$ Tregs (CD4$^+$FoxP3$^+$RORγt$^+$) and RORγt$^+$FoxP3$^-$ CD4$^+$ T cells (CD4$^+$FoxP3$^-$RORγt$^+$) in colons of pre-arthritic male *Il6*$^{+/+}$ (n=6) and *Il6*$^{-/-}$ (n=7) SKG mice. Each symbol in **A-C** represents an individual mouse. Graphs show mean ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$ by unpaired t-test (**A** and **C**) or 1-way ANOVA (**B**).

Supplementary figure 2

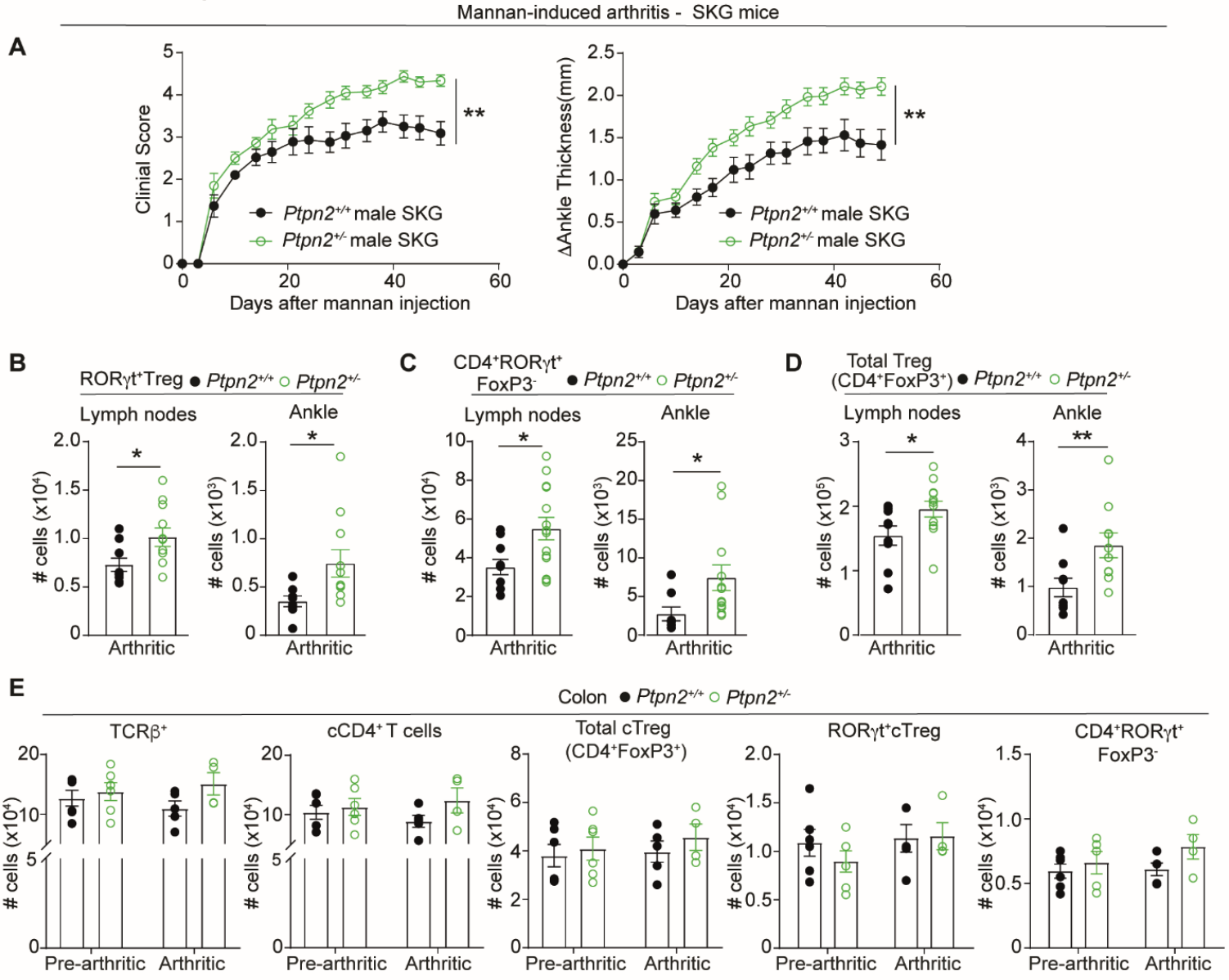


Supplemental Figure 2. Total Tregs and RORγt$^+$FoxP3$^-$CD4$^+$ T cell counts in lymph nodes and ankles of arthritic Rag2-KO mice. (**A-B**) Flow cytometric analysis of **Figure 1D-F**. (**A**) Total Tregs (TCRβ$^+$CD4$^+$FoxP3$^+$) and (**B**) RORγt$^+$FoxP3$^-$ CD4$^+$ T cells (CD45$^+$TCRβ$^+$CD4$^+$RORγt$^+$FoxP3$^-$) cells in lymph nodes and ankles of arthritic Rag2-KO mice. Compiled data from 2 independent experiments are shown. Each symbol represents an individual mouse. Graphs show mean ± SEM. *P < 0.05 by unpaired t-test.



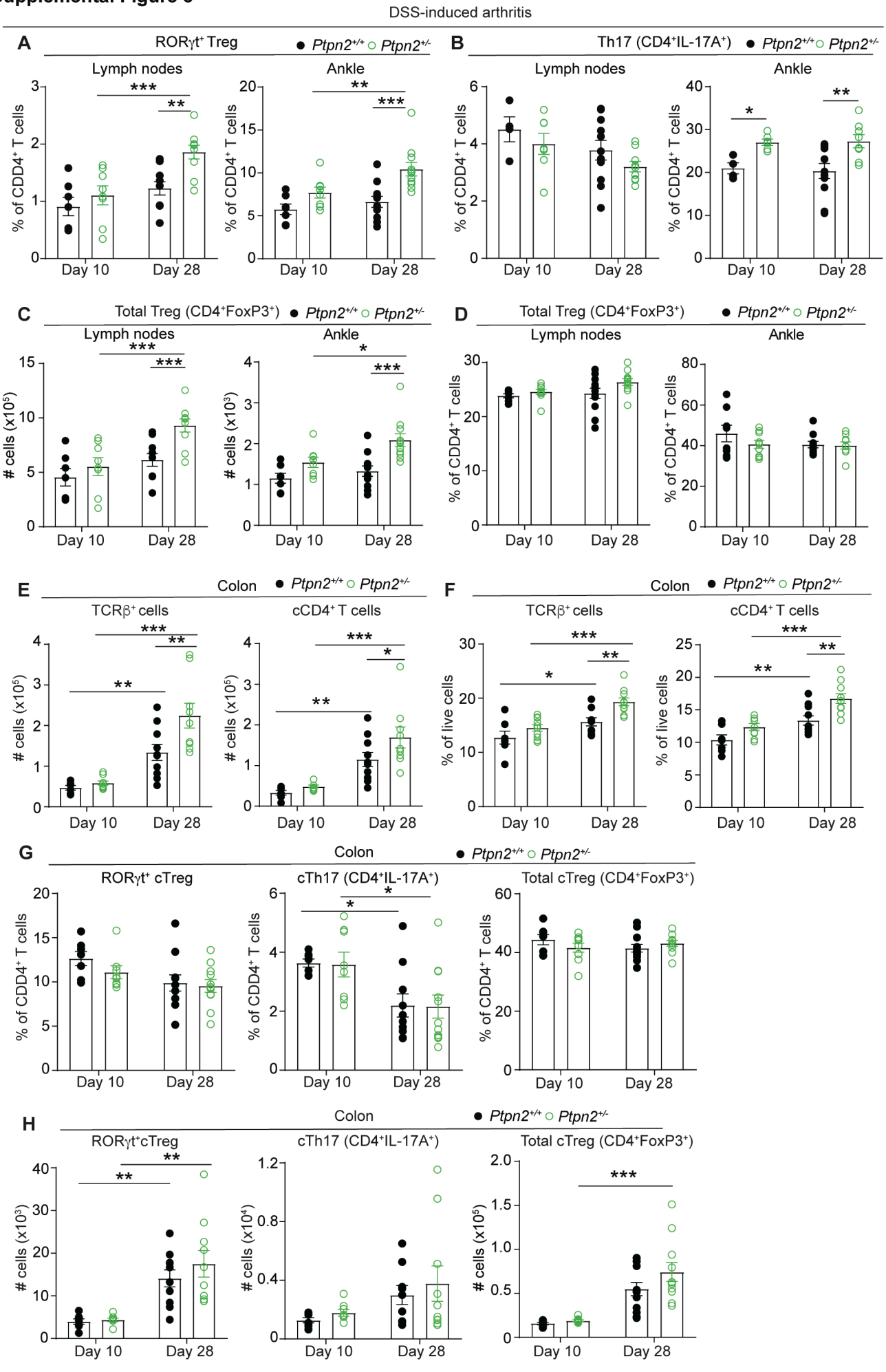
Supplemental Figure 3. Total Tregs and RORγt$^+$FoxP3$^-$CD4$^+$ T cell counts in lymph nodes and ankles of DSS-induced arthritic mice. (**A**) Number of total Tregs (CD45.1$^+$CD4$^+$FoxP3$^+$) in lymph nodes isolated from mice in **Figure 2A-E**. (**B**) Flow cytometric analysis of RORγt$^+$FoxP3$^-$CD4$^+$ T cells (CD4$^+$RORγt$^+$FoxP3$^-$) in lymph nodes and ankles of arthritic Rag2-KO mice in **Figure 2F-H**. Compiled data from 2 independent experiments are shown in **A**. Each symbol in **A** and **B** represents an individual mouse. Graphs show mean ± SEM. *P < 0.05, **P < 0.01 by unpaired t-test (**A** and **B**).

Supplemental Figure 4



Supplemental Figure 4. *Ptpn2* haploinsufficiency promotes mannan-induced arthritis in male SKG mice. (**A-D**) Male *Ptpn2*$^{+/+}$ (n=9) and *Ptpn2*$^{+/-}$ (n=15) SKG mice were injected intraperitoneally with mannan and arthritis development was monitored by clinical scoring and measurement of ankle swelling. Mice were analyzed at day 49. (**A**) Clinical score and change in ankle thickness. (**B**) Number of RORγt^{+} Tregs (CD4^{+} FoxP3^{+}RORγt^{+}) in lymph nodes and ankles. (**C**) Number of RORγt^{+}FoxP3^{-} CD4^{+} T cells in lymph nodes and ankles. (**D**) Number of total Tregs (CD4^{+}FoxP3^{+}) in lymph nodes and ankles. (**E**) Number of T cells (TCRβ^{+}), CD4^{+} T cells (TCRβ^{+}CD4^{+}), total Tregs (CD4^{+}FoxP3^{+}), RORγt^{+} Tregs (CD4^{+}FoxP3^{+}RORγt^{+}) and RORγt^{+}FoxP3^{-}CD4^{+} T cells in the colon of pre-arthritic or 10-days post mannan SKG mice (*Ptpn2*$^{+/+}$ (n=6) and *Ptpn2*$^{+/-}$ (n=6) pre-arthritic SKG mice; *Ptpn2*$^{+/+}$ (n=5) and *Ptpn2*$^{+/-}$ (n=4) SKG mice 10-days post mannan mice). Compiled data from 4 independent experiments are shown in **A-E**. Each symbol in **B-E** represents an individual mouse. Clinical score and swelling ankles were quantified using the area under the curve. Graphs show mean ± SEM. *P < 0.05, **P < 0.01 by Mann-Whitney U test (**A**), unpaired t-test (**B-D**) or 2-way ANOVA (**E**).

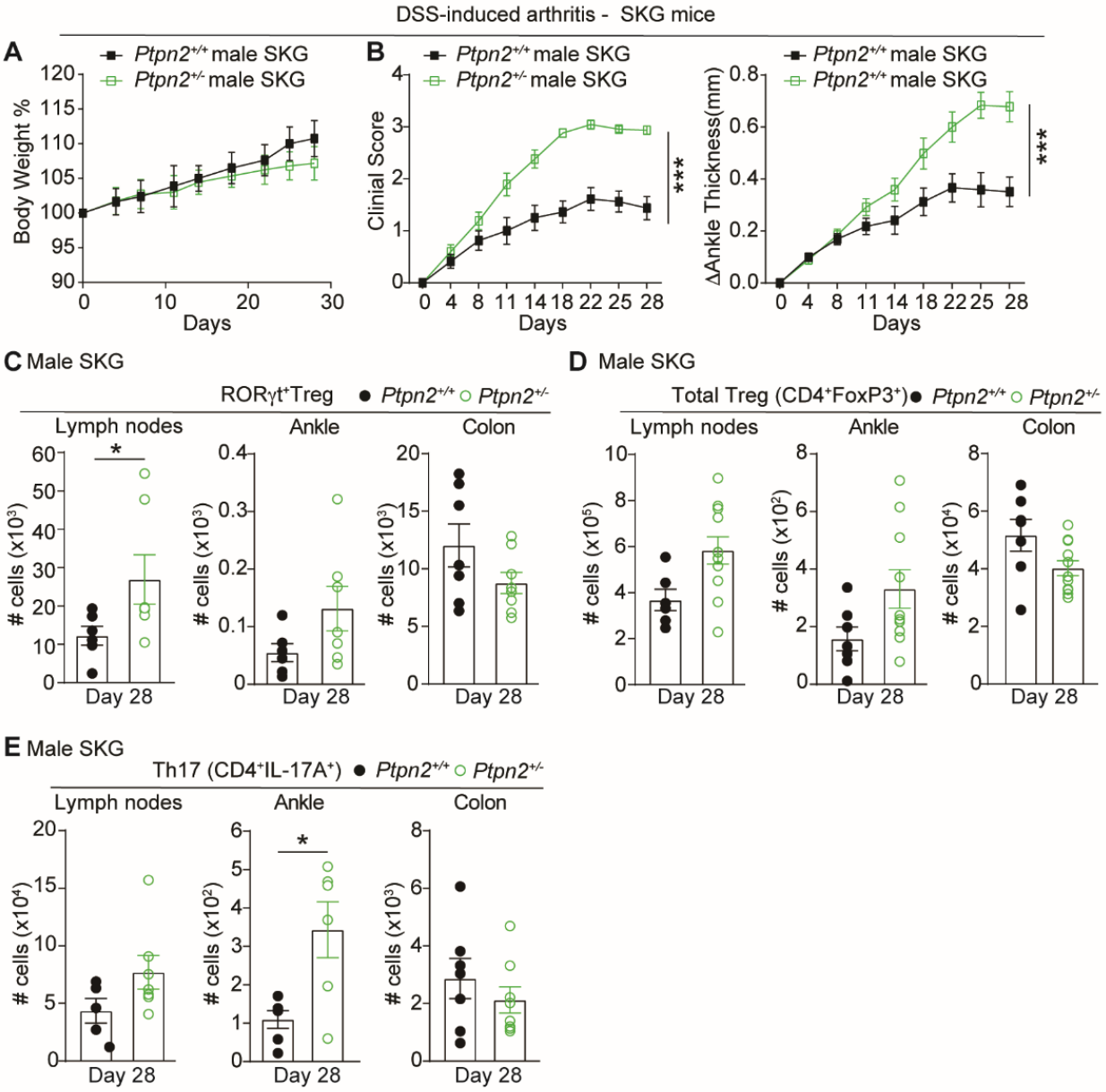
Supplemental Figure 5



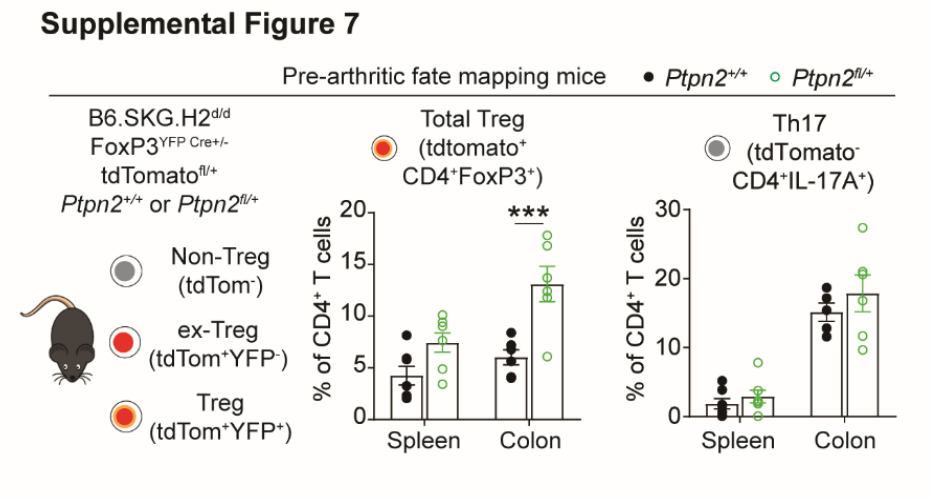
Supplemental Figure 5. Frequency and number of total Tregs, RORγt-expressing Tregs, Th17, T cells and CD4$^+$ T cells in SKG mice with DSS-induced arthritis.

(**A-H**) Cells isolated from each tissue 10 days or 28 days after start of 0.5% DSS water treatment of mice shown in **Figure 3**. (**A**) Frequency of RORγt$^+$ Tregs (CD4$^+$FoxP3$^+$ RORγt$^+$) in lymph nodes and ankles. (**B**) Frequency of Th17 (CD4$^+$IL-17A$^+$FoxP3$^-$) cells in lymph nodes and ankles. (**C-D**) Number (**C**) and frequency (**D**) of total Tregs (CD4$^+$ FoxP3$^+$) in lymph nodes and ankles. (**E-F**) Number (**E**) and frequency (**F**) of T cells (TCRβ$^+$) and CD4$^+$ T cells (TCRβ$^+$CD4$^+$) in colon. (**G-H**) Frequency (**G**) and number (**H**) of RORγt$^+$ cTregs (CD4$^+$RORγt$^+$FoxP3$^+$), cTh17 (CD4$^+$IL-17A$^+$FoxP3$^-$) and total cTregs (CD4$^+$ FoxP3$^+$) in colon. Compiled data from 6 independent experiments are shown in **A-H**.Each symbol represents an individual mouse. Graphs show mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.005 by 2-way ANOVA (**A-H**).

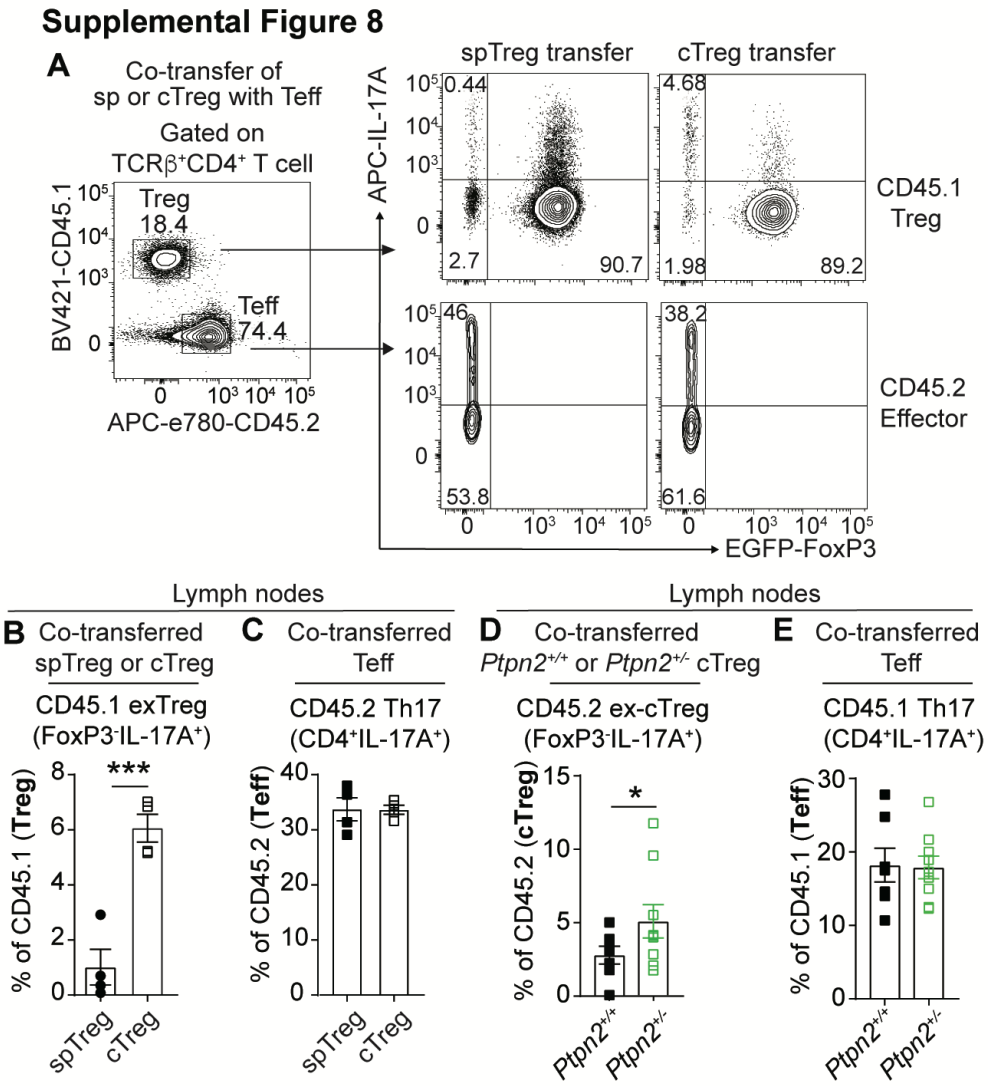
Supplemental Figure 6



Supplemental Figure 6. *Ptpn2* haploinsufficiency enhances susceptibility to DSS-induced arthritis in male SKG mice. (**A-E**) Male *Ptpn2*$^{+/+}$ (n=8) and *Ptpn2*$^{+/-}$ (n=11) SKG mice received 0.5% DSS in their drinking water as in **Figure 3A**. (**A**) Change in body weight. (**B**) Clinical score and change in ankle thickness. (**C-E**) Number of RORγt^{+} Tregs (**C**; CD4^{+}FoxP3^{+}RORγt^{+}), total Tregs (**D**; CD4^{+}FoxP3^{+}) and Th17 (**E**; CD4^{+}IL-17A^{+}FoxP3^{-}) in lymph nodes, ankles, and colon. Compiled data from 7 independent experiments are shown in **A-E**. Each symbol in **C-E** represents an individual mouse. Arthritis severity was quantified using the area under the curve. Graphs show mean ± SEM. **P* < 0.05, ****P* < 0.001 by Mann-Whitney *U* test (**B**), or unpaired t-test (**C-E**).

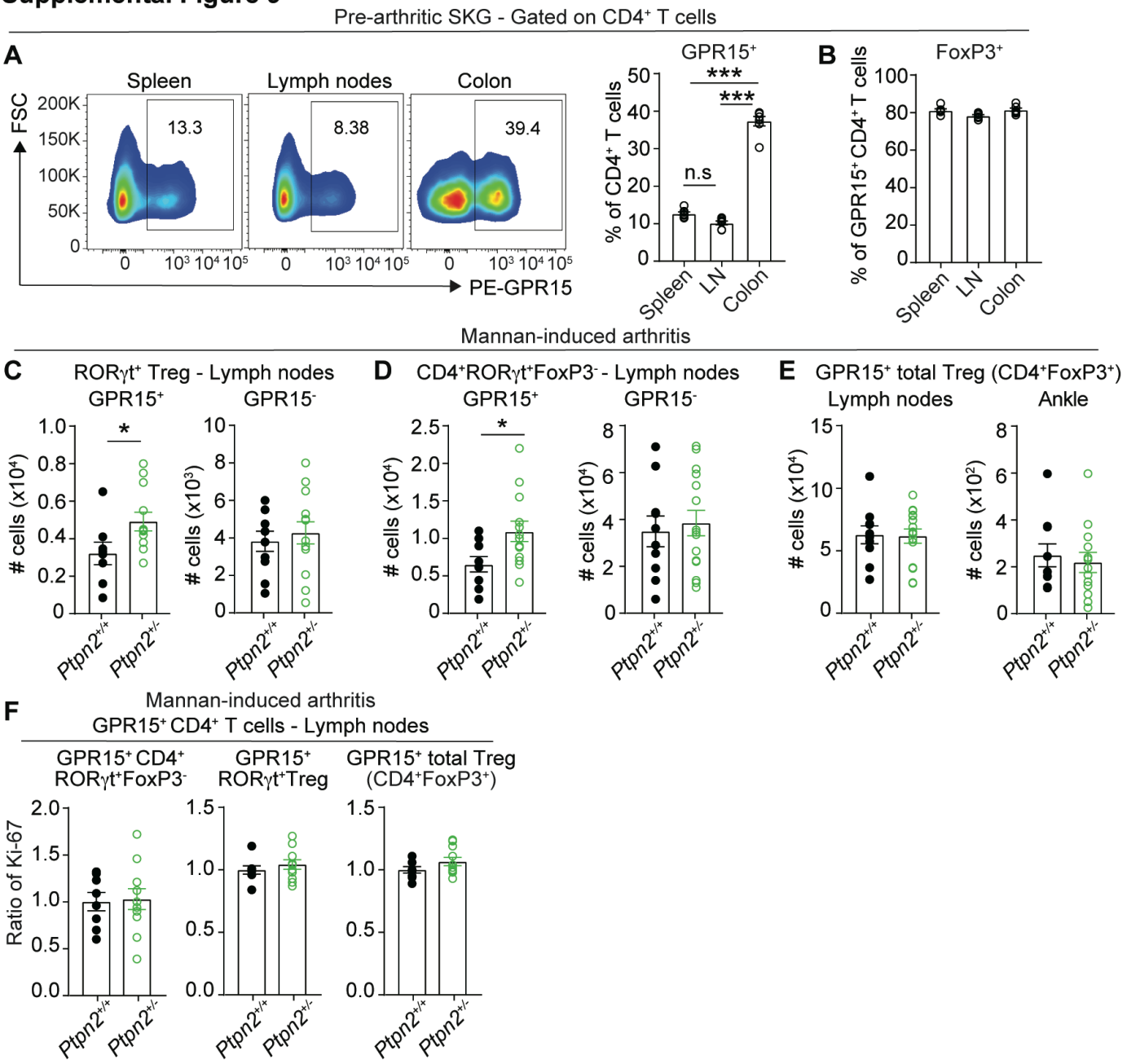


Supplemental Figure 7. Frequency of total Tregs and Th17 in pre-arthritic fate-mapping SKG mice. Frequency of total Tregs ($CD4^+tdTomato^+FoxP3^{YFP+}$) and tdTomato- Th17 ($CD4^+tdTomato^-IL\text{-}17A^+FoxP3^{YFP-}$) in spleen and colon isolated from mice shown in **Figure 4A**. Compiled data from 2 independent experiments are shown. Each symbol represents an individual mouse. Graphs show mean ± SEM**.** ***$P < 0.001$ by unpaired t-test.



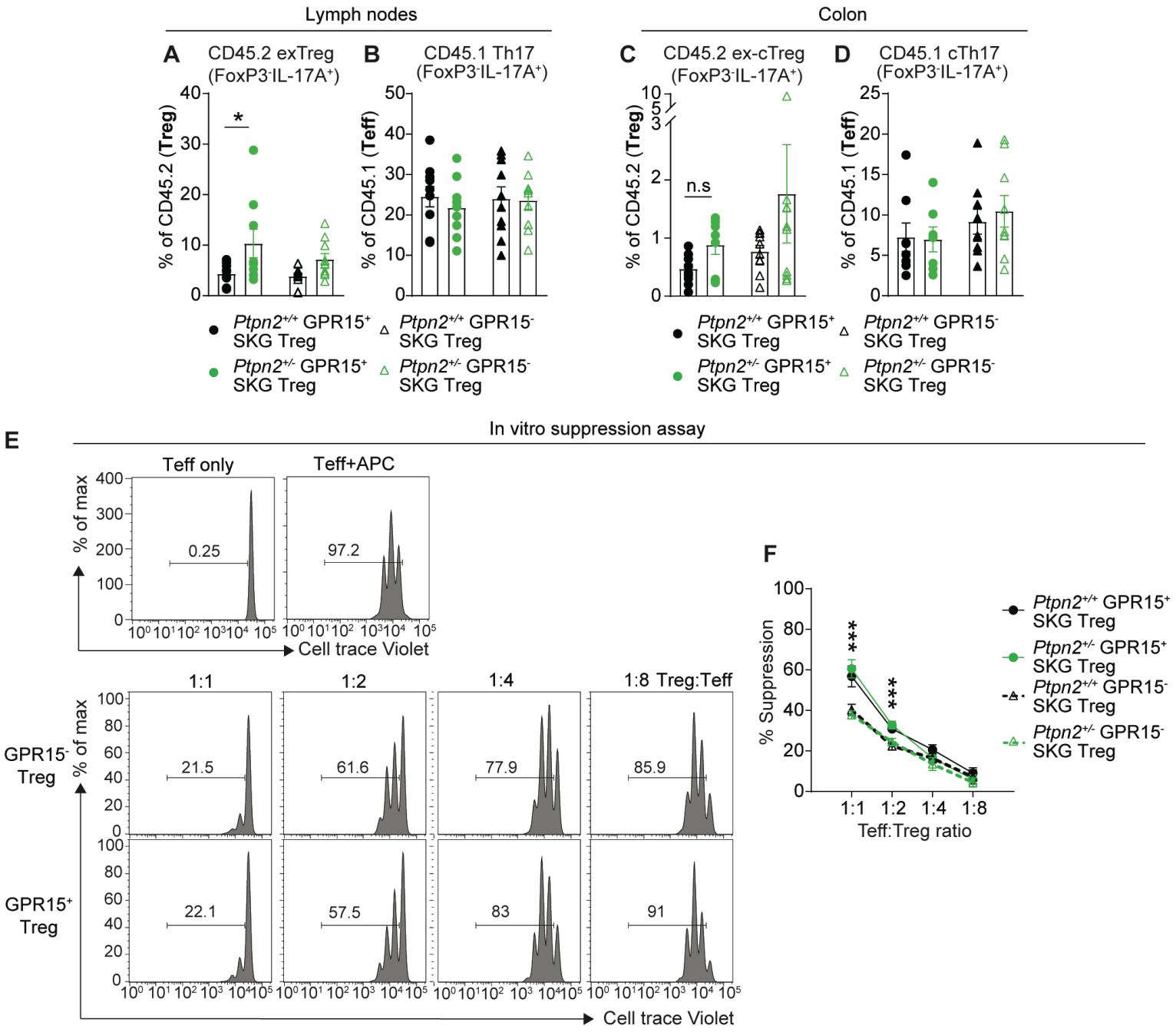
Supplemental Figure 8. SKG cTregs are more arthritogenic than SKG spTregs after transfer into Rag2-KO mice. (**A-C**) Analysis of transferred Tregs and Teff by flow cytometry from recipient Rag2-KO mice in **Figure 4D-E** receiving either SKG splenic (sp) or colonic (c) Tregs. (**A**) Representative gating strategy. (**B**) Frequency of exTregs ($CD45.1^+CD4^+IL\text{-}17^+FoxP3^-$) in lymph nodes of recipient mice. (**C**) Frequency of Th17 ($CD4^+IL17A^+FoxP3^-$) among transferred CD45.2 SKG $CD4^+CD25^-$ Teffs in lymph nodes of recipient mice. (**D-E**) Analysis of transferred Tregs and Teff by flow cytometry from recipient Rag2-KO mice in **Figure 4H-I** receiving cTregs from *Ptpn2*$^{+/+}$ or *Ptpn2*$^{+/-}$ SKG mice. (**D**) Frequency of *Ptpn2*$^{+/+}$ and *Ptpn2*$^{+/-}$ ex-cTreg ($CD45.2^+CD4^+IL\text{-}17^+FoxP3^-$) cells in lymph nodes of recipient mice. (**E**) Frequency of Th17 cells ($CD4^+IL17A^+FoxP3^-$) among transferred CD45.1 SKG $CD4^+CD25^-$ *Ptpn2*$^{+/+}$ (WT) Teffs in lymph nodes of recipient mice. Compiled data from 2 independent experiments are shown in **B-E**. Each symbol in **B-E** represents an individual mouse. Graphs show mean ± SEM. *$P < 0.05$, ***$P < 0.001$ by unpaired t-test.

Supplemental Figure 9



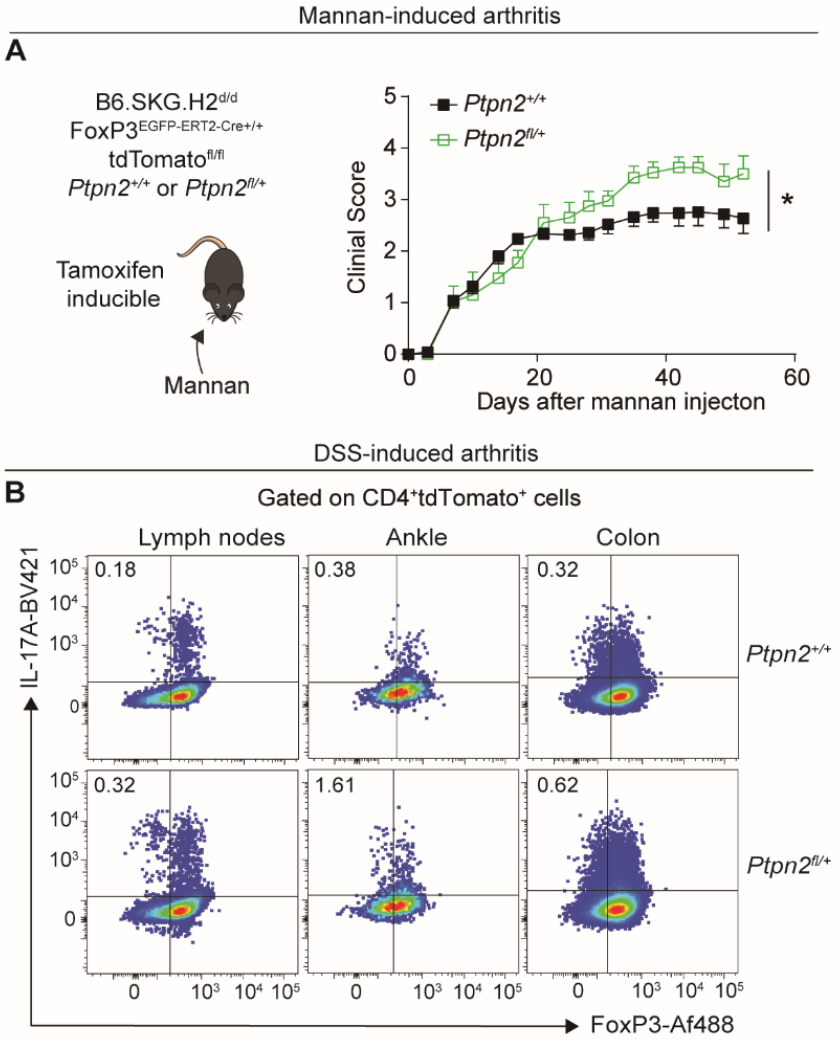
Supplemental Figure 9. Enrichment of GPR15⁺ CD4⁺ T cells correlates with enhanced arthritis in *Ptpn2* haploinsufficient SKG mice. (**A**) Representative gating (*left*) and frequency (*right*) of GPR15⁺ expressing cells among CD4⁺ T cells in spleen (Sp), lymph nodes (LN) and colon of pre-arthritic SKG mice (n=7). (**B**) Frequency of FoxP3⁺ cells among GPR15⁺ CD4⁺ T cells in 9 week-old pre-arthritic SKG mice (n=7). (**C-F**) Cells isolated from *Ptpn2*$^{+/+}$ (n=9) and *Ptpn2*$^{+/-}$ (n=13) SKG mice with mannan-induced arthritis from **Supplemental Figure 4**. Mice were analyzed at day 49 after mannan injection. (**C**) Number of GPR15⁺ and GPR15⁻ RORγt⁺ Tregs (CD4⁺FoxP3⁺RORγt⁺) in lymph nodes. (**D**) Number of GPR15⁺ and GPR15⁻ RORγt⁺FoxP3⁻CD4 T cells (CD4⁺FoxP3⁻RORγt⁺ GPR15⁺ or GPR15⁻) in lymph nodes. (**E**) Number of GPR15⁺ total Tregs (CD4⁺FoxP3⁺GPR15⁺) in lymph nodes and ankles. (**F**) Ki-67 MFI ratio in GPR15-expressing RORγt⁺FoxP3⁻CD4⁺ T cells (CD4⁺FoxP3⁻ RORγt⁺GPR15⁺), RORγt⁺ Tregs (CD4⁺RORγt⁺FoxP3⁺GPR15⁺) and total Tregs (CD4⁺FoxP3⁺GPR15⁺) in lymph nodes. Compiled data from at least 2 independent experiments are shown. Each symbol in **A-F** shows individual mice. Graphs show mean ± SEM. **P* < 0.05, ****P* < 0.005 by 1-way ANOVA (**A**) or unpaired t-test (**C-D**).

Supplemental Figure 10



Supplemental Figure 10. *Ptpn2* haploinsufficiency causes increased conversion of GRP15⁺ SKG Tregs to IL-17⁺ exTregs during arthritis. (**A-D**) Frequency of IL-17 producing exTregs (CD45.2⁺CD4⁺IL-17A⁺FoxP3⁻) and Th17 (CD45.1⁺CD4⁺IL-17A⁺FoxP3⁻) in lymph nodes (**A-B**) and colon (**C-D**) of Rag2-KO recipient mice shown in **Figure 5E-H**. (**E-F**) Percent suppression of naïve CD4⁺ T cell proliferation in vitro by GPR15⁺ and GPR15⁻ Tregs isolated from either *Ptpn2*$^{+/+}$ (n=3) or *Ptpn2*$^{+/-}$ (n=3) FoxP3^{EGFP} SKG mice. (**E**) Representative gating strategy for analysis of in vitro suppression assay by flow cytometry in (**F**). Compiled data from 3 independent experiments are shown. Each symbol in **A-D** represents an individual mouse. Graphs show mean ± SEM. *$P < 0.05$, ***$P < 0.001$ by 2-way ANOVA (**A**) unpaired t-test (**F**). Non-significant (n.s).

Supplemental Figure 11



Supplemental Figure 11. Inducible Treg-specific *Ptpn2* haploinsufficiency aggravates severity of DSS-induced arthritis. (**A**) Clinical score of female B6.SKG.H2$^{d/d}$ FoxP3$^{EGFP\text{-}ERT2\text{-}Cre+/+}$ tdTomato$^{fl/fl}$ *Ptpn2*$^{+/+}$ (n=5) or *Ptpn2*$^{fl/+}$ (n=4) inducible Treg fate-mapping mice after intraperitoneal injection of mannan. Treg fate-mapping mice were administered 100 μL tamoxifen (20 mg/ml) via i.p. injection for 5 consecutive days to induce Cre expression. After the last treatment with tamoxifen, mice were rested for 2 weeks before induction of arthritis by mannan injection. (**B**) Representative flow cytometric gating of IL-17 producing cells among tdTomato^{+} CD4^{+} T cells isolated from B6.SKG.H2$^{d/d}$ FoxP3$^{EGFP\text{-}ERT2\text{-}Cre+/+}$ tdTomato$^{fl/fl}$ *Ptpn2*$^{+/+}$ or *Ptpn2*$^{fl/+}$ inducible Treg fate-mapping mice shown in **Figure 6**. Clinical score was quantified using the area under the curve. Graphs shows mean ± SEM. **P* < 0.05 by Mann-Whitney *U* test (**A**).