

SUPPLEMENTARY TABLE 1

Gene name and nucleotide composition for primers used in real-time PCR. Cycling parameters are described in references.

| Gene | Primer Sequences |
|--------------------|--|
| <i>Hprt</i> (1) | For: 5'-AGCCTAAGATGAGCGCAAGT-3' Rev: 5'-TTACTAGGCAGATGGCCACA-3' |
| <i>Stat1</i> * | For: 5'-CCTGCAACTGAAGGAACAGA-3' Rev: 5'-TCACCACGACAGGAAGAGAG-3' |
| <i>Stat6</i> (2) | For: 5'-GACTATGGTAGAGGACAGTTGCCTAA-3' Rev: 5'-GGTTCTCCAGGAGAAGCTTGGT-3' |
| <i>Il4</i> (3) | For: 5'-CCACGGATGCGACAAAATC-3' Rev: 5'-TGTTCTTCGTTGCTGTGAGGAC-3' |
| <i>Il5</i> (4) | For: 5'-TGACAAGCAATGAGACGATGAGG-3' Rev: 5'-ACCCCCACGGACAGTTTGATTC-3' |
| <i>Il13</i> (5) | For: 5'-GCAACATCACACAAGACCAGA-3' Rev: 5'-GTCAGGGAATCCAGGGCTAC-3' |
| <i>Gata3</i> (6) | For: 5'-AGTTCGCGCAGGATGTCC-3' Rev: 5'-AGAACCGGCCCTTATCAA-3' |
| <i>Il1b</i> (7) | For: 5'-CAACCAACAAGTGATATTCTCCATG-3' Rev: 5'-GATCCACACTCTCCAGCTGCA-3' |
| <i>Il12p40</i> (3) | For: 5'-AAAGGCTGGGTATCGGTGG-3' Rev: 5'-ACTGGCTGTGCTGGA ACTCC-3' |
| <i>Tnfa</i> (8) | For: 5'-AAGGGAGAGTGGTCAGTTGCC-3' |

Tbet (6) Rev: 5'-CCTCAGGGAAGAGTCTGGAAAGG-3'
For: 5'-TTCTCTGTTTGGCTGGCTGTT-3'
Rev: 5'-GGATTCTGGGGTTTACTTCTT-3'

Inos (9) For: 5'-CACCAAGCTGAACTTGAGCGA-3'
Rev: 5'-CCATAGGAAAAGACTGCACCGA-3'

Arg1 (9) For: 5'-CAGAAGAATGGAAGAGTCAG-3'
Rev: 5'-CAGATATGCAGGGAGTCACC-3'

Chi3l3 [Ym1] (10) For: 5'-CATGAGCAAGACTTGCGTGAC-3'
Rev: 5'-CATGAGCAAGACTTGCGTGAC-3'

Retnla [Fizz1] (10) For: 5'-TCCCAGTGAATACTGATGAGA-3'
Rev: 5'-CCACTCTGGATCTCCCAAGA-3'

Mmp12 (9) For: 5'-TCAATTGGAATATGACCCCCTG-3'
Rev: 5'-ACCAGCAAGCACCCCTTCACTAC-3'

Il4ra (11) For: 5'-GAGTGAGTGGAGTCCTAGCATC-3'
Rev: 5'-GCTGAAGTAACAGAACAGGC-3'

Il13ra1 (11) For: 5'-GCAGCCTGGAGAAAAGTCGTCAAT-3'
Rev: 5'-ACAGCGTCGGCAAGAACACCA-3'

Il13ra2 (12) For: 5'-TGCAGATGATGGAATTTGGA -3'
Rev: 5'-GCTCAATGTGGGTTTCAGGTT-3'

Ifng (13) For: 5' -AGCTCATCCGGTGGTCCAC-3 '
Rev: 5'-AAAATTCAAATAGTGCTGGCAGAA-3'

Cxcl9 For: 5'-TCGAGGAACCCTAGTGATAA-3'
Rev: 5'-ACTGTTTGAGGTCTTTGAGG-3'

Cxcl10 For: 5'-GTCTGAGTGGGACTCAAGG-3'

Irf1 Rev: 5'-TGATTTCAAGCTTCCCTATG-3'
For: 5'- AGAGAGAAAGTCCAAGTCCAGC -3'
Rev: 5'- GTCCATCAGAGAAAGTGTCCG -3'

Ifnb For: 5'- TACGTCTCCTGGATGAACTC -3'
Rev: 5'- TCTTCAAGTGGAGAGCAGTT -3'

Socs1 (14) For: 5'-ACCTTCTTGGTGCGCGAC -3'
Rev: 5'- AAGCCATCTTCACGCTGAGC -3'

Rantes For: 5'- GGAGTATTTCTACACCAGCAGC -3'
Rev: 5'- TCTTGAACCCACTTCTTCTCTG -3'

Fasl For: 5'- GGTGCTAATGGAGGAGAAGAGG -3'
Rev: 5'- CACTGGTAAGATTGAATACTGCC -3'

Fas For: 5'- ACTTCTACTGCGATTCTCCTGG -3'
Rev: 5'- TGTATTGCTGGTTGCTGTGC -3'

Tnfr1 For: 5'- CCCCAAATGGAAATGTGCTATG -3'
Rev: 5'- GGATGCTACAGATGCGGTGG -3'

Ccl3 For: 5'-CCAATTCATCGTTGACTATTT-3'
Rev: 5'-CTTGGACCCAGGTCTCTT-3'

Ccl4 For: 5'-CCTGCTGTTTCTCTTACACC-3'
Rev: 5'-TTTGGTCAGGAATACCACAG-3'

Mcp1 For: 5'- TCTGCCCTAAGGTCTTCAGCAC -3'
Rev: 5'- GCTTGAGGTGGTTGTGGAAAAG -3'

Il17a(6) For: 5'- CAGACTACCTCAACCGTTCC -3'
Rev: 5'- AGCATCTTCTCGACCCTGAA -3'

*Purchased from Real Time Primers, LLC (Elkins Park, PA)

SUPPLEMENTARY TABLE 2

Correlation coefficient matrix for 10 genes from Figure 9A and C in a cohort of 45 patients with biliary atresia.

| | | CCR4 | IL18 | CD74 | CD86 | AREG/AREGB-1 | IL4R | BCL3 | BCL6 | IL1RL1 | IL6 |
|---------------------|--|--|--|--|--|--|--------------|-------------|-------------|---------------|------------|
| CCR4 | Pearson's r R Std Error t p-value | 1. | | | | | | | | | |
| IL18 | Pearson's r R Std Error t p-value | -0.094 0.023 -0.623 0.535 | 1. | | | | | | | | |
| CD74 | Pearson's r R Std Error t p-value | 0.165 0.022 1.100 0.277 | 0.450 0.018 3.311 0.001 | 1. | | | | | | | |
| CD86 | Pearson's r R Std Error t p-value | 0.264 0.021 1.801 0.078 | 0.341 0.020 2.380 0.021 | 0.634 0.013 5.381 <0.001 | 1. | | | | | | |
| AREG/AREGB-1 | Pearson's r R Std Error t p-value | 0.384 0.019 2.727 0.009 | 0.316 0.020 2.186 0.034 | 0.140 0.029 0.928 0.358 | 0.535 0.016 4.153 <0.001 | 1. | | | | | |
| IL4R | Pearson's r R Std Error t p-value | 0.124 0.022 0.824 0.414 | 0.117 0.022 0.778 0.440 | -0.087 0.023 -0.573 0.569 | 0.153 0.022 1.019 0.313 | 0.612 0.014 5.078 <0.001 | 1. | | | | |
| BCL3 | Pearson's r | 0.331 | 0.143 | 0.075 | 0.331 | 0.646 | 0.866 | 1. | | | |

| | | | | | | | | | | |
|---------------|-------------|-------|-------|--------|--------|--------|--------|--------|--------|--------|
| | R Std Error | 0.021 | 0.022 | 0.0231 | 0.020 | 0.013 | 0.005 | | | |
| | t | 2.307 | 0.950 | 0.494 | 2.302 | 5.556 | 11.385 | | | |
| | p-value | 0.025 | 0.347 | 0.623 | 0.026 | <0.001 | <0.001 | | | |
| BCL6 | Pearson's r | 0.127 | 0.113 | -0.025 | 0.216 | 0.549 | 0.835 | 0.815 | 1. | |
| | R Std Error | 0.022 | 0.022 | 0.023 | 0.022 | 0.016 | 0.007 | 0.007 | | |
| | t | 0.844 | 0.752 | -0.167 | 1.450 | 4.310 | 9.953 | 9.237 | | |
| | p-value | 0.402 | 0.456 | 0.868 | 0.154 | <0.001 | <0.001 | <0.001 | | |
| IL1RL1 | Pearson's r | 0.248 | 0.172 | 0.094 | 0.587 | 0.846 | 0.712 | 0.699 | 0.656 | 1. |
| | R Std Error | 0.021 | 0.022 | 0.0230 | 0.015 | 0.006 | 0.011 | 0.011 | 0.013 | |
| | t | 1.684 | 1.151 | 0.621 | 4.750 | 10.431 | 6.660 | 6.421 | 5.711 | |
| | p-value | 0.099 | 0.256 | 0.537 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | |
| IL6 | Pearson's r | 0.318 | 0.131 | -0.062 | 0.373 | 0.752 | 0.544 | 0.647 | 0.652 | 0.692 |
| | R Std Error | 0.021 | 0.022 | 0.023 | 0.020 | 0.010 | 0.016 | 0.013 | 0.013 | 0.012 |
| | t | 2.206 | 0.870 | -0.407 | 2.640 | 7.493 | 4.256 | 5.564 | 5.639 | 6.293 |
| | p-value | 0.032 | 0.388 | 0.685 | 0.011 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

SUPPLEMENTARY TABLE 3

Correlation coefficient matrix for 10 genes from Figure 9A and C in a cohort of 7 normal controls.

| | | CCR4 | IL18 | CD74 | CD86 | AREG/AREGB-1 | IL4R | BCL3 | BCL6 | IL1RL1 | IL6 |
|---------------------|--------------------|-------------|-------------|--------------|-------------|---------------------|-------------|-------------|-------------|---------------|------------|
| CCR4 | Pearson's r | 1. | | | | | | | | | |
| | <i>R Std Error</i> | | | | | | | | | | |
| | <i>t</i> | | | | | | | | | | |
| | <i>p-value</i> | | | | | | | | | | |
| IL18 | Pearson's r | -0.537 | 1. | | | | | | | | |
| | <i>R Std Error</i> | 0.142 | | | | | | | | | |
| | <i>t</i> | -1.425 | | | | | | | | | |
| | <i>p-value</i> | 0.214 | | | | | | | | | |
| CD74 | Pearson's r | 0.748 | -0.310 | 1. | | | | | | | |
| | <i>R Std Error</i> | 0.088 | 0.181 | | | | | | | | |
| | <i>t</i> | 2.521 | -0.729 | | | | | | | | |
| | <i>p-value</i> | 0.053 | 0.499 | | | | | | | | |
| CD86 | Pearson's r | 0.503 | 0.271 | 0.275 | 1. | | | | | | |
| | <i>R Std Error</i> | 0.149 | 0.185 | 0.185 | | | | | | | |
| | <i>t</i> | 1.301 | 0.631 | 0.641 | | | | | | | |
| | <i>p-value</i> | 0.250 | 0.556 | 0.550 | | | | | | | |
| AREG/AREGB-1 | Pearson's r | 0.032 | 0.050 | 0.258 | -0.447 | 1. | | | | | |
| | <i>R Std Error</i> | 0.200 | 0.199 | 0.187 | 0.160 | | | | | | |
| | <i>t</i> | 0.072 | 0.113 | 0.596 | -1.118 | | | | | | |
| | <i>p-value</i> | 0.945 | 0.915 | 0.577 | 0.315 | | | | | | |
| IL4R | Pearson's r | 0.431 | -0.272 | 0.770 | -0.059 | 0.421 | 1. | | | | |
| | <i>R Std Error</i> | 0.163 | 0.185 | 0.081 | 0.199 | 0.165 | | | | | |

| | | | | | | | | | | | |
|---------------|--------------------|--------|--------|--------|--------|--------|--------|-------|--------|-------|----|
| | <i>t</i> | 1.068 | -0.631 | 2.701 | -0.131 | 1.038 | | | | | |
| | <i>p-value</i> | 0.334 | 0.556 | 0.043 | 0.901 | 0.347 | | | | | |
| BCL3 | Pearson's r | 0.001 | 0.114 | 0.585 | -0.047 | 0.062 | 0.741 | 1. | | | |
| | <i>R Std Error</i> | 0.200 | 0.197 | 0.132 | 0.200 | 0.199 | 0.090 | | | | |
| | <i>t</i> | 0.003 | 0.256 | 1.612 | -0.104 | 0.139 | 2.470 | | | | |
| | <i>p-value</i> | 0.998 | 0.808 | 0.168 | 0.921 | 0.895 | 0.057 | | | | |
| BCL6 | Pearson's r | -0.025 | 0.145 | 0.472 | -0.337 | 0.456 | 0.323 | 0.554 | 1. | | |
| | <i>R Std Error</i> | 0.200 | 0.196 | 0.155 | 0.177 | 0.158 | 0.179 | 0.139 | | | |
| | <i>t</i> | -0.056 | 0.327 | 1.198 | -0.800 | 1.145 | 0.762 | 1.489 | | | |
| | <i>p-value</i> | 0.958 | 0.757 | 0.285 | 0.460 | 0.304 | 0.480 | 0.197 | | | |
| IL1RL1 | Pearson's r | -0.457 | 0.745 | -0.468 | 0.260 | -0.216 | -0.171 | 0.096 | -0.205 | 1. | |
| | <i>R Std Error</i> | 0.158 | 0.089 | 0.156 | 0.186 | 0.191 | 0.194 | 0.198 | 0.192 | | |
| | <i>t</i> | -1.149 | 2.499 | -1.183 | 0.603 | -0.495 | -0.388 | 0.215 | -0.467 | | |
| | <i>p-value</i> | 0.303 | 0.055 | 0.290 | 0.573 | 0.642 | 0.714 | 0.838 | 0.660 | | |
| IL6 | Pearson's r | 0.411 | -0.164 | 0.316 | 0.439 | -0.573 | 0.302 | 0.419 | -0.044 | 0.300 | 1. |
| | <i>R Std Error</i> | 0.166 | 0.195 | 0.180 | 0.161 | 0.134 | 0.182 | 0.165 | 0.200 | 0.182 | |
| | <i>t</i> | 1.007 | -0.371 | 0.746 | 1.094 | -1.564 | 0.709 | 1.032 | -0.098 | 0.703 | |
| | <i>p-value</i> | 0.360 | 0.726 | 0.489 | 0.324 | 0.179 | 0.510 | 0.349 | 0.926 | 0.514 | |

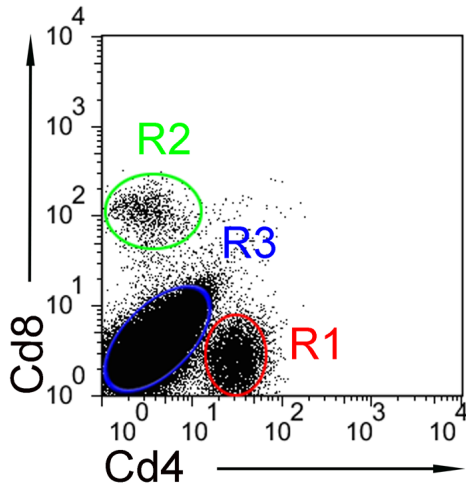
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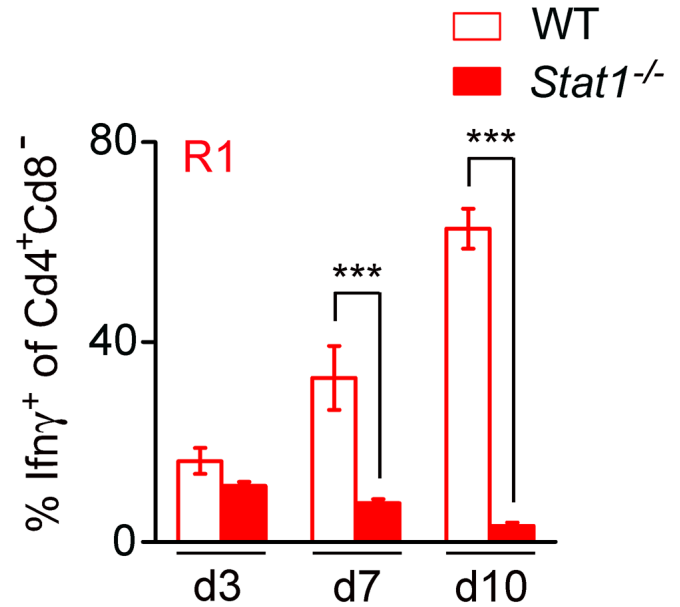
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Supplementary Figure 1

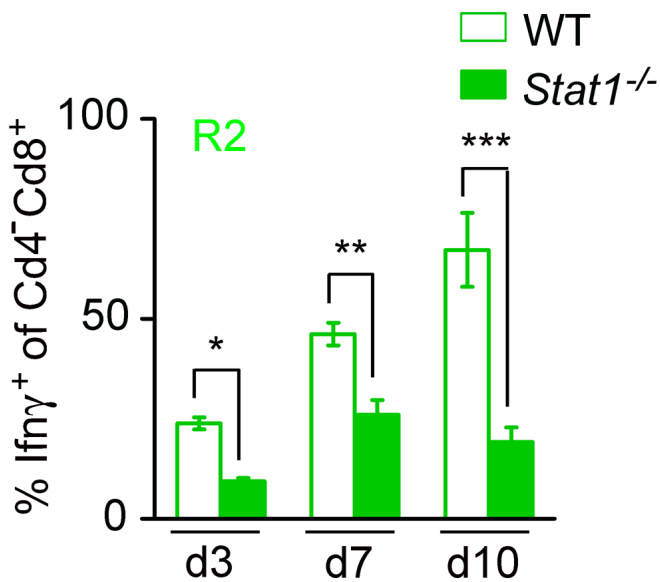
A



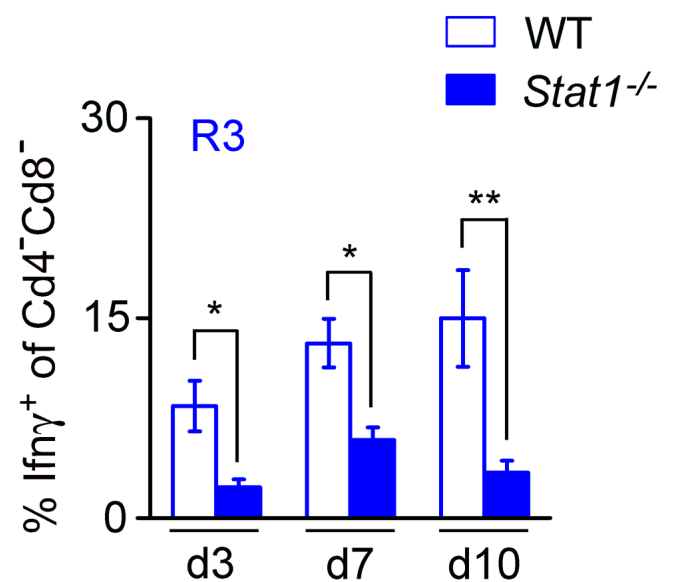
B



C

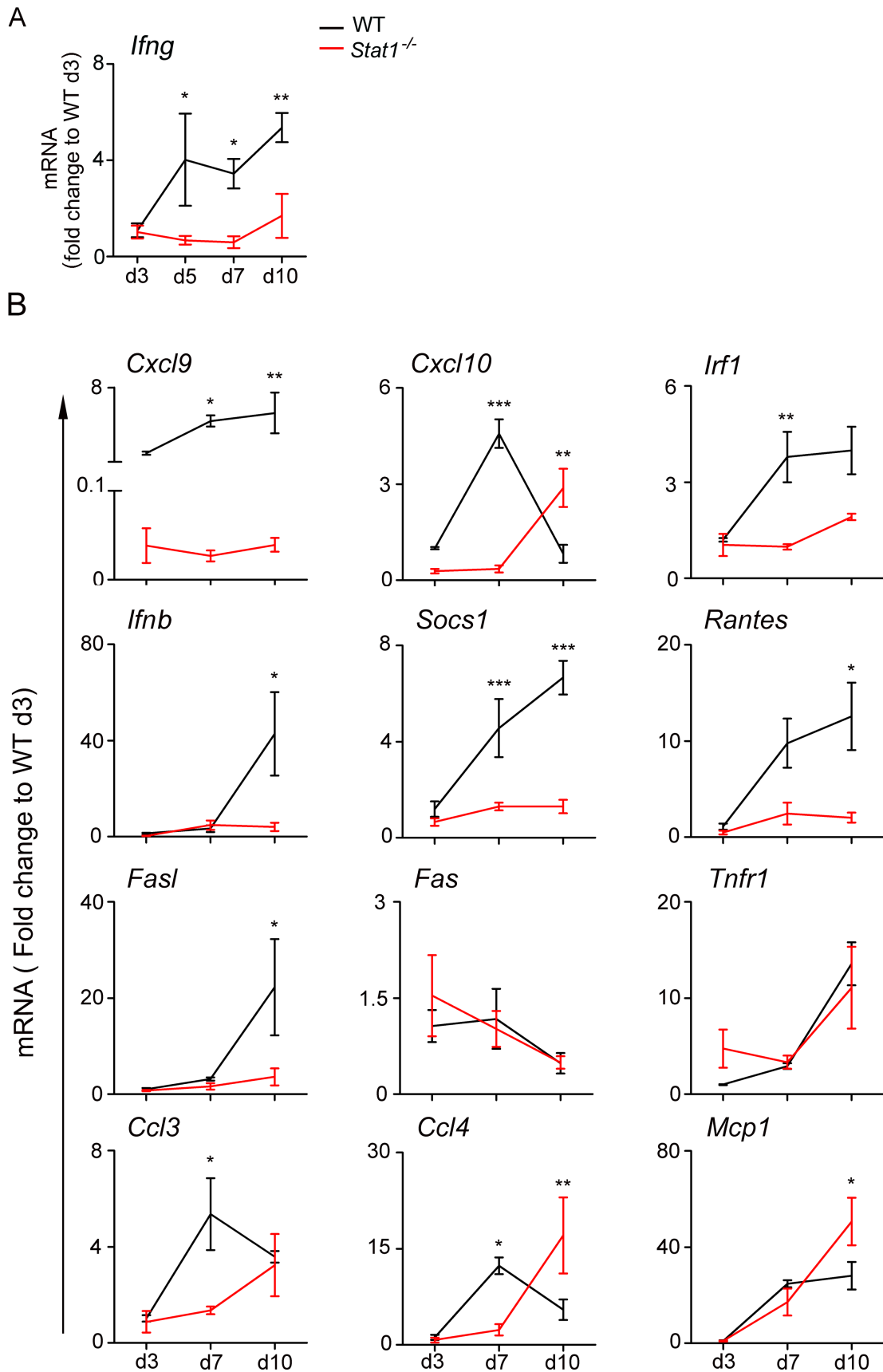


D



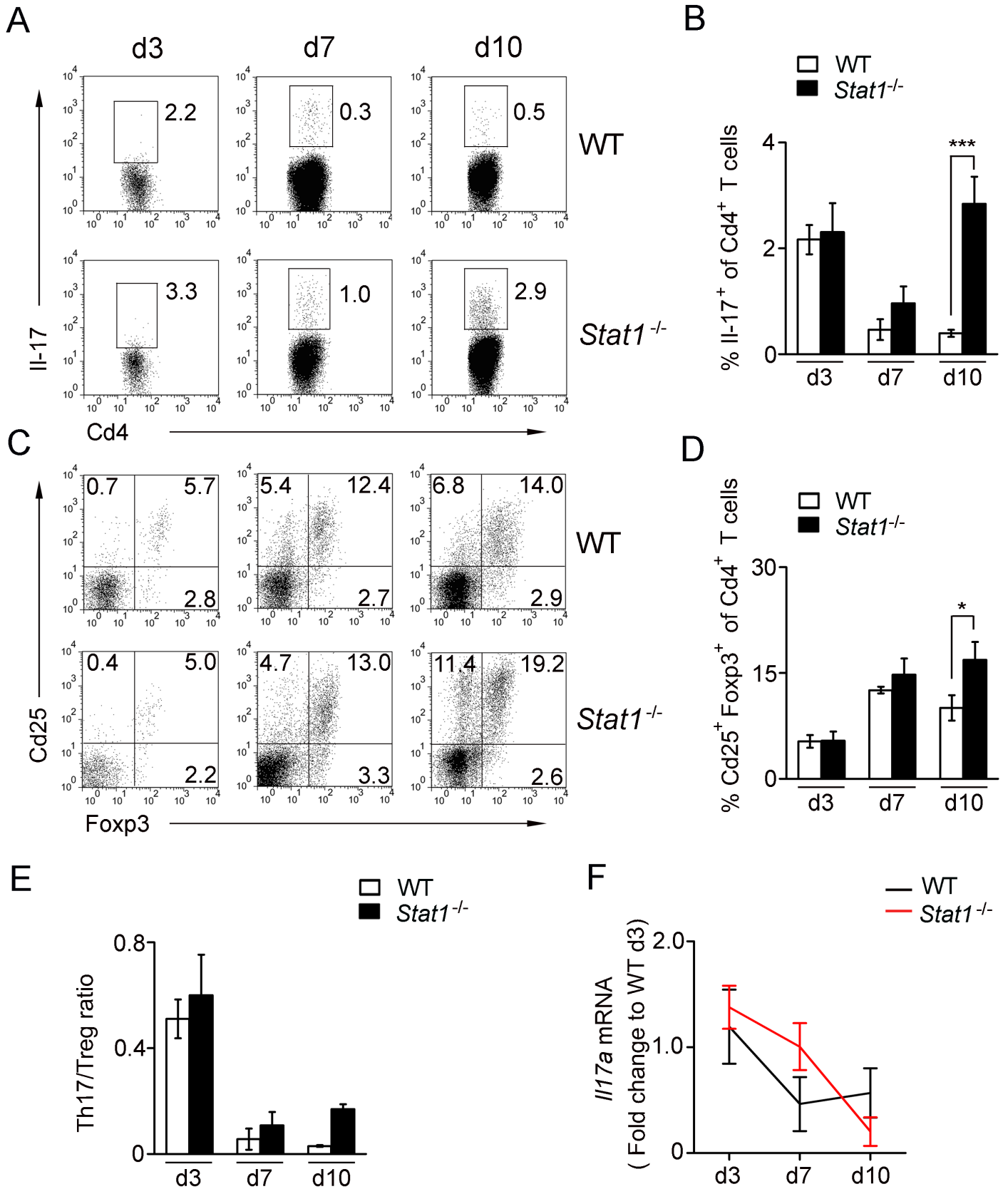
Decrease in Ifnγ production in *Stat1*^{-/-} mice after RRV. Gating strategy for Cd4⁺ (R1), Cd8⁺ (R2) and Cd4⁻Cd8⁻ (R3) hepatic mononuclear cells (A). The quantification of the percent of these cells expressing Ifnγ at days 3, 7 and 10 after RRV infection is higher in WT than *Stat1*^{-/-} mice. *P<0.05, **P<0.01, ***P<0.001. Note that panel B is also in Figure 3B, but it is shown here to demonstrate the gating strategy to quantify these cells.

Supplementary Figure 2



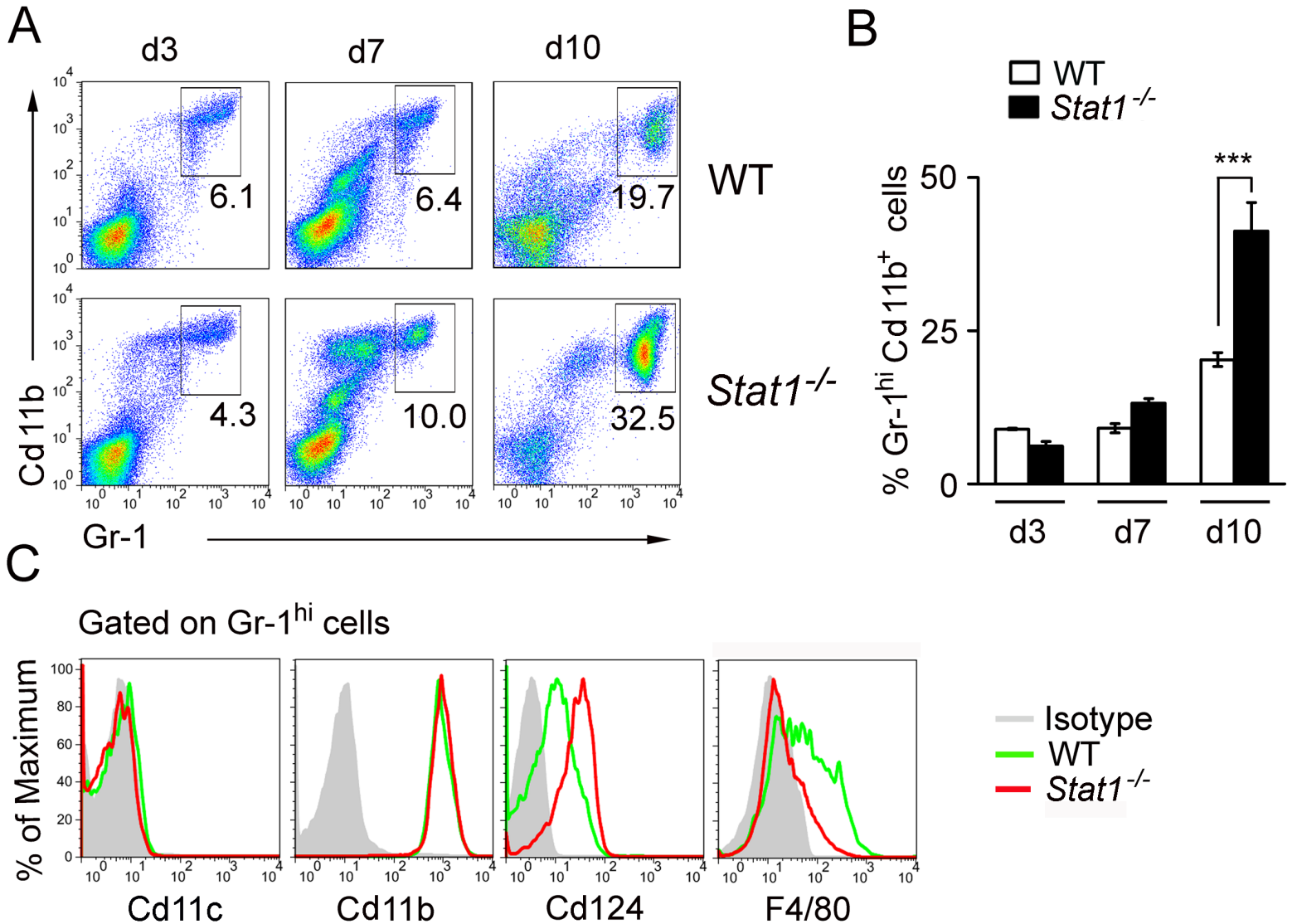
mRNA expression for *Ifng* and target genes. Individual graphs display the hepatic mRNA expression for *Ifng* (A) and *Ifng*-target genes (B) in WT and *Stat1*^{-/-} mice at different time points after RRV challenge. mRNA expression for most genes is decreased in *Stat1*^{-/-} livers when compared to WT. mRNA expression was first normalized to internal *Hprt* control and then normalized to the value from WT mice at day 3. N=3-4 livers per group and time point; *P<0.05, **P<0.01, ***P<0.001.

Supplementary Figure 3



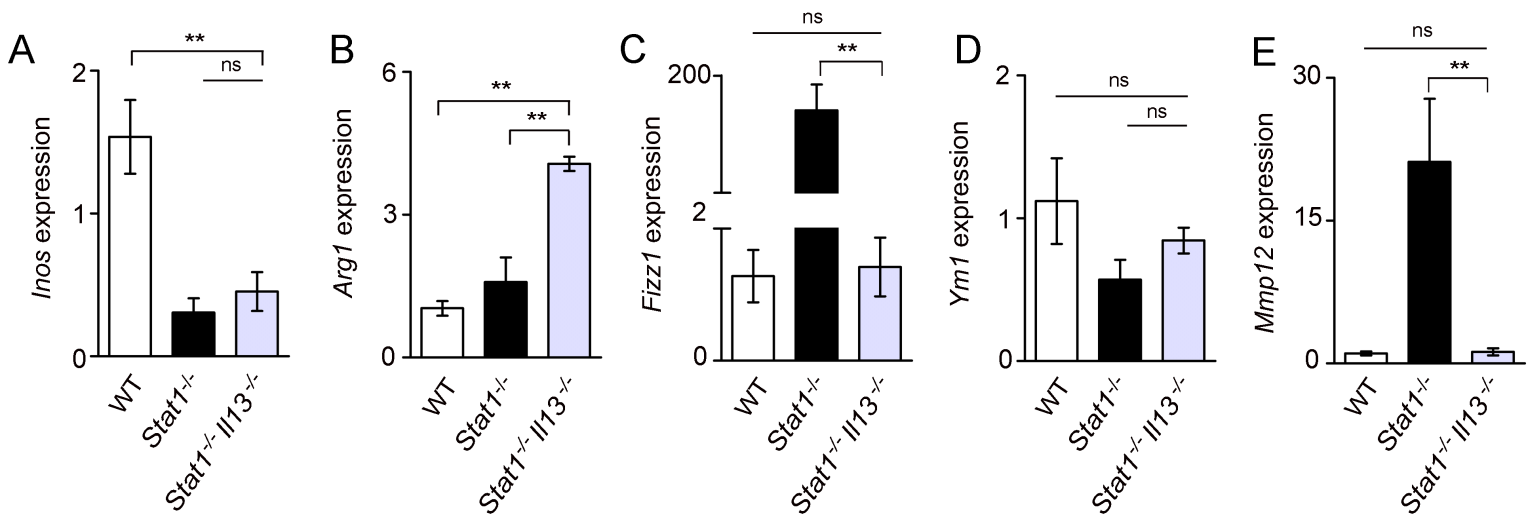
Th17 expression in *Stat1*^{-/-} mice after RRV. Flow cytometric analysis showing the expression of IL-17 (A,B) or Cd25⁺Foxp3⁺ (C,D) by hepatic Cd4⁺ cells in WT and *Stat1*^{-/-} mice at different time points after RRV. Panel E shows a Th17:Treg ratio below 1, and panel F shows a similar pattern of hepatic *Il17a* mRNA expression in both groups after RRV. Data are shown as representative dot plots (A,C) and mean±SEM (B,D); N=3 livers per group per time point; *P<0.05, ***P<0.001.

Supplementary Figure 4



Expression of IIR α and F4/80 in Gr-1^{hi} cells in *Stat1*^{-/-} livers after RRV. Flow cytometry analyses show similar levels of Gr-1^{hi} cells in WT and *Stat1*^{-/-} livers 3 and 7 days after RRV, which increase in *Stat1*^{-/-} at day 10 (A,B). Expression of markers linked to the myeloid-derived suppressor cell (MDSC) phenotype shows an increased number of Gr-1^{hi} cells co-expressing Cd124 (IIR α) in *Stat1*^{-/-} livers but only few express F4/80⁺ 10 days after RRV (C). N=6-10 livers per group and time point; experiments repeated twice; ***P<0.001.

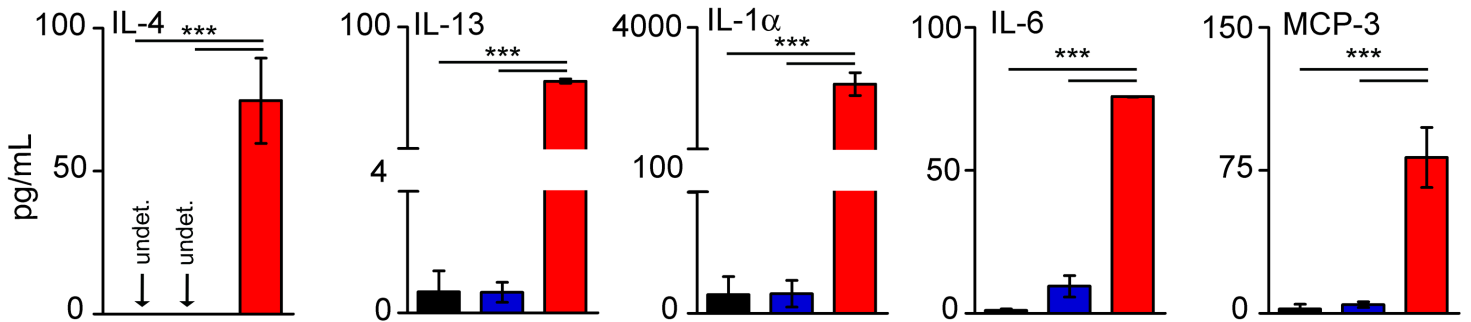
Supplementary Figure 5



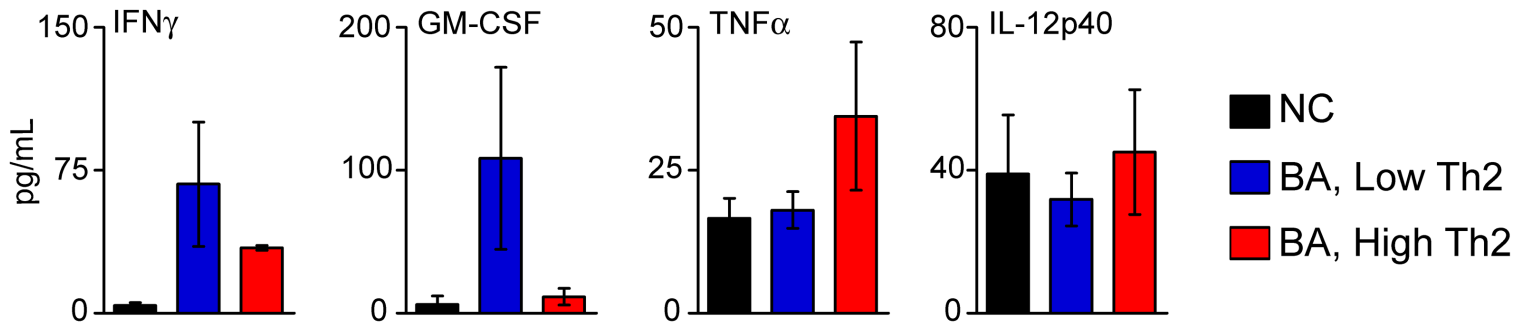
Decreased M2 activation after depletion of Il-13 in *Stat1*^{-/-} mice. Hepatic mRNA expression for M1 and M2 genes in WT, *Stat1*^{-/-} and *Stat1*^{-/-}/*Il13*^{-/-} mice 7 days after RRV. Values are expressed as a ratio to *Hprt* and then normalized to WT; N=3-5 livers per genotype; **P<0.01; ns=no significant difference.

Supplementary Figure 6

A



B



Serum levels of Th1 and Th2 cytokines/chemokines at diagnosis of biliary atresia. Mean (\pm S.E.) serum concentration of Th1 and Th2 cytokines/chemokines for infants at the time of diagnosis of biliary atresia (BA; age < 4 months) and age-matched normal infants (normal controls=NC). Data in panel A are shown in three groups: NC (N=3), BA Low Th2 group (N=9) and BA High Th2 group (N=2). ***P<0.001 (one-way ANOVA).