

Saxena et al. Science Translation Medicine Sept 2011

Supplementary Table S1

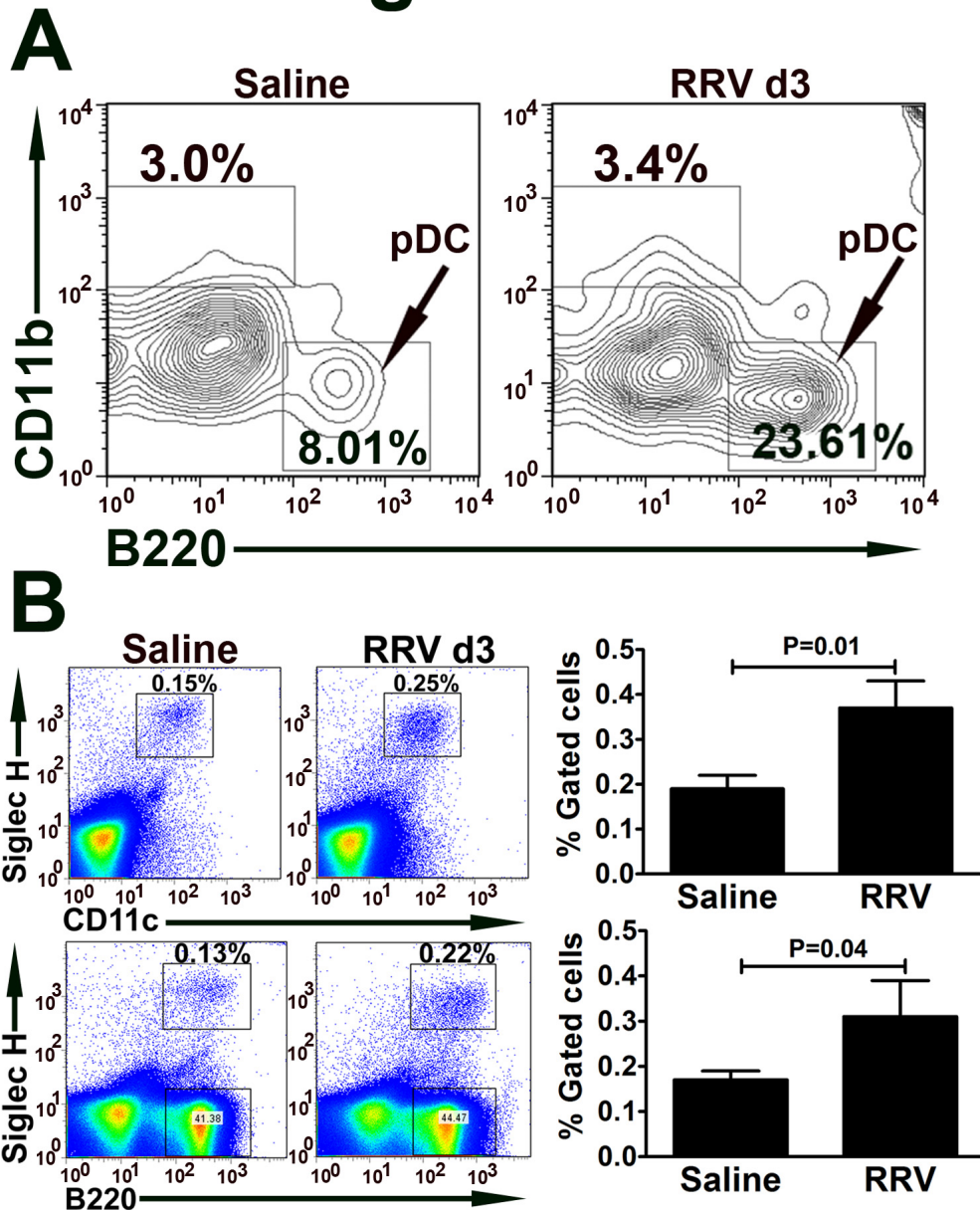
Oligonucleotide primer sequences and PCR product sizes used in real-time PCRs to quantify the expression of murine cytokine, RRV gene and Human DC related gene

Gene	Primer Sequences	Temp (°C)	Size (bp)
<i>Il15</i>	For: 5'-AGGAATACATCCATCTCGTGCTAC-3' Rev: 5'-ACTGACACAGCCCCAAAATGAAG-3'	50.1	96
<i>Gapdh</i>	For: 5'-TGGTTTGACAATGAATACGGCTAC-3' Rev: 5'-GGTGGGTGGTCCAAGGTTTC-3'	53.4	92
<i>Nsp3</i>	For: 5'-TGTCAAGAGAATACCTGGGAAATC-3' Rev: 5'-GGAATCATCAACTTCAACTTCACC-3'	48.2	97
<i>LILRA4</i>	For: 5'-TACAGGTGCTACGGCTCACG-3' Rev: 5'-CTGACTTCTTTTGTGCTGGATTG -3'	55.8	118
<i>TLR7</i>	For: 5'- CAGAAGTCCAAGTTCCTCCAGC-3' Rev: 5'- GACACTGCCAGAAGTATGGGTG -3'	54.2	100
<i>NRP1</i>	For: 5'- AAGGGGACAGAACTGGATGC -3' Rev: 5'- GGTCTATTTGGAGCCACTCATTG -3'	54.6	111
<i>GAPDH</i>	For: 5'- TGACATCAAGAAGGTGGTGAAGC -3' Rev: 5'- TCAAAGGTGGAGGAGTGGGTG -3'	56.8	120

Temp: annealing temperature

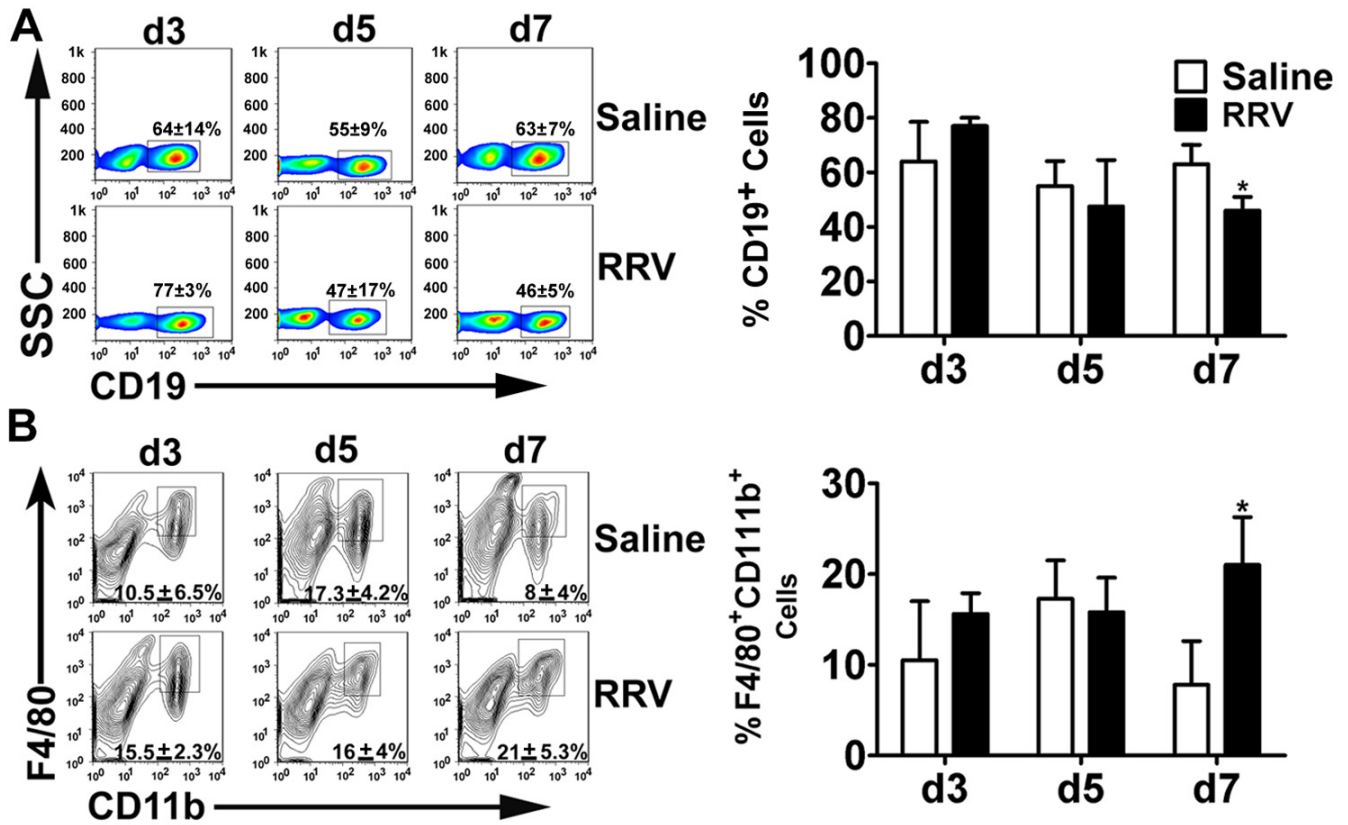
Size (bp): product size in base pairs

Figure S1



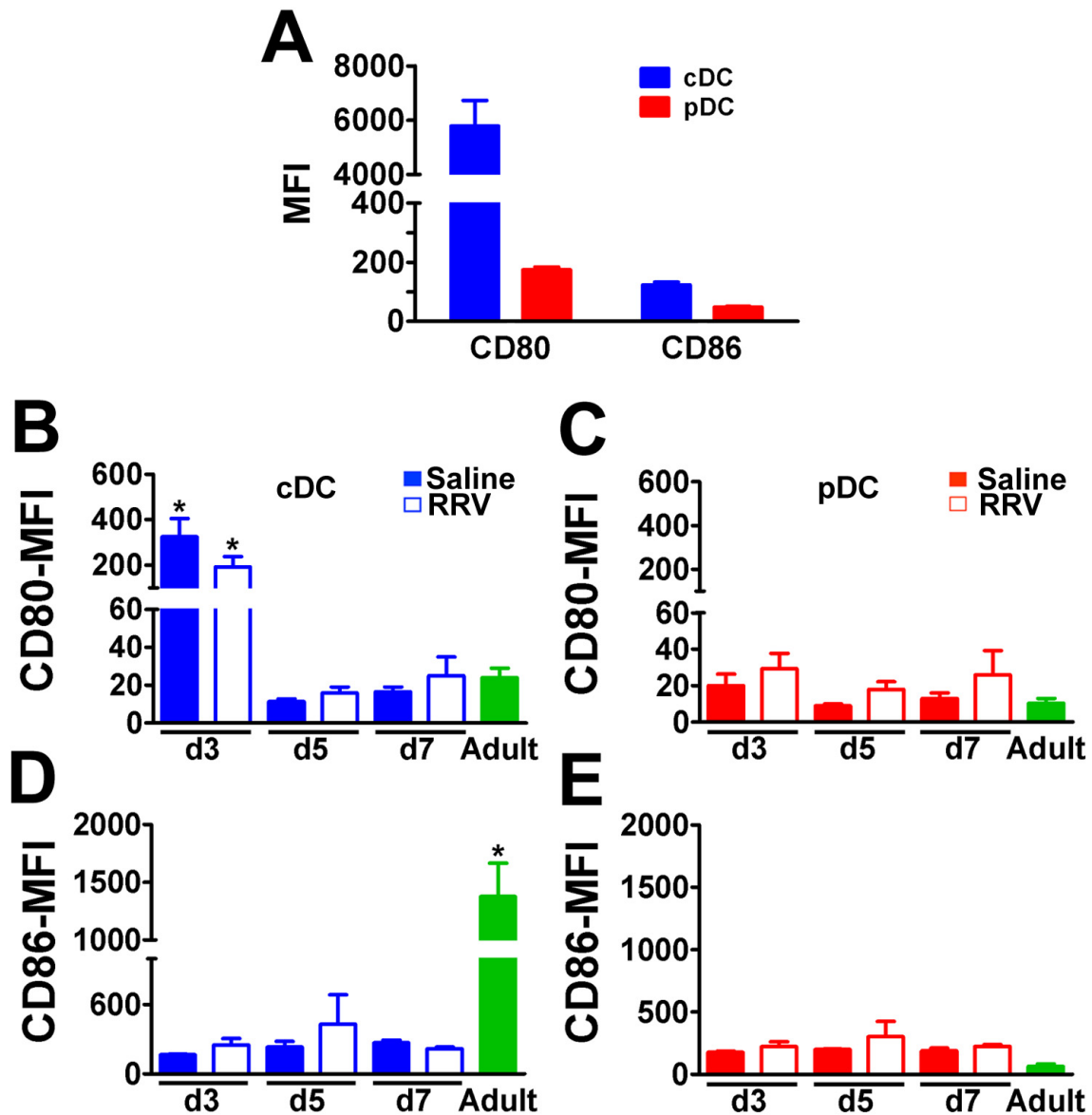
Quantification of hepatic pDCs after RRV challenge. Flow cytometric analyses of hepatic mononuclear cells included an initial size gating, exclusion of 7AAD⁺ cells, gating on CD11c⁺ cells, then quantification of CD11b⁻B220⁺ cells (panel **A**) or Siglec H (panel **B**) 3 days after saline or RRV challenge. Numbers in contour or dot plots represent % of gated cells. Graphs in panel **B** show the mean \pm S.D. of % gated cells from 3 different experiments; N=4-7 livers per group in each experiment).

Figure S2



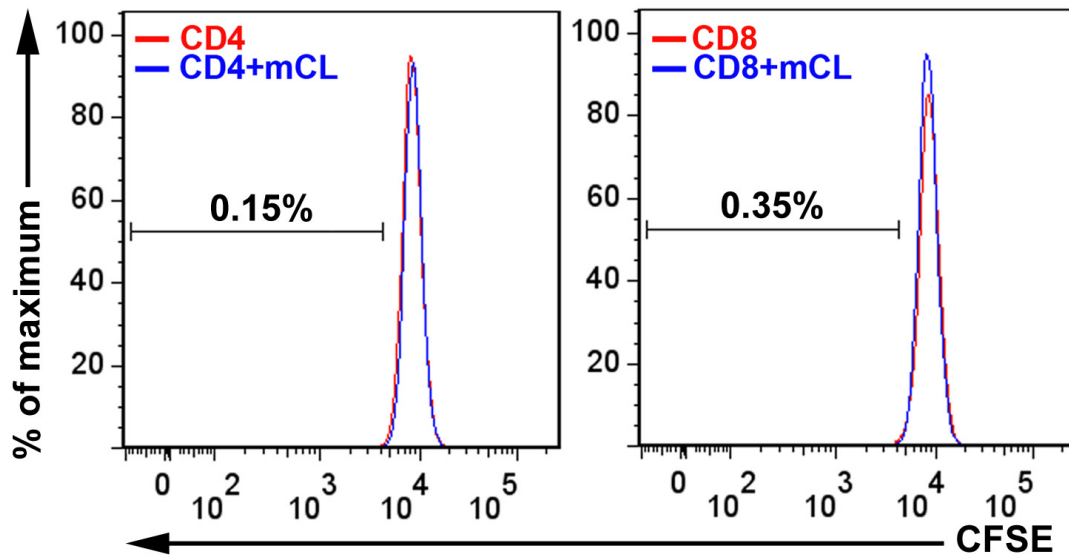
Quantification of hepatic B cells and macrophages after RRV challenge. Panel **A** shows dot plots from flow cytometric analyses of hepatic mononuclear cells stained with anti-CD19 antibodies at days 3, 5, and 7 after saline or RRV injection of newborn mice. In panel **B**, contour plots of cells stained with anti-F4/80 and CD11b antibodies in the same experimental setting. Results depict mean \pm S.D. N=3-4 mice per group and time point; *P<0.05 (all other groups have P>0.05).

Figure S3



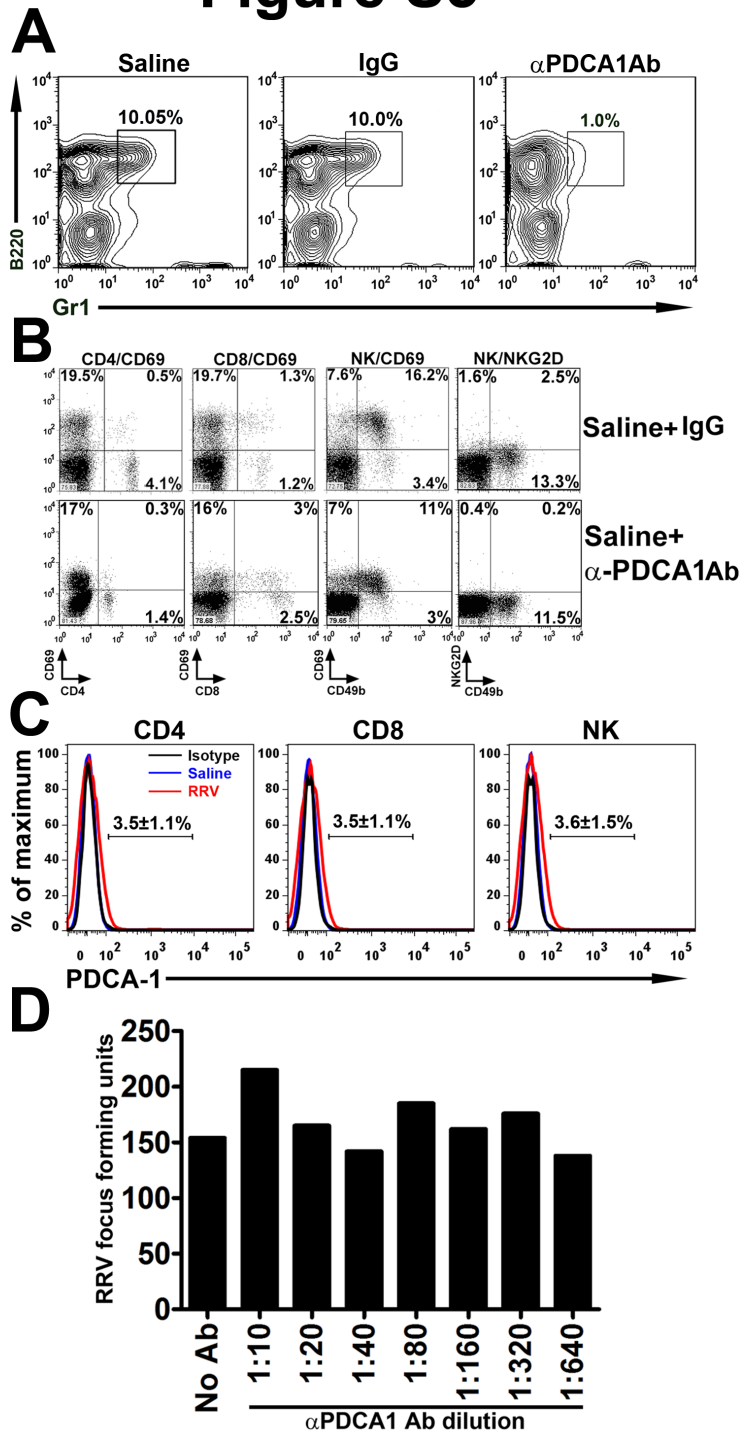
Expression of co-stimulatory markers in hepatic DCs. Panel A shows the mean fluorescence intensity (MFI) of co-stimulatory markers CD80 and CD86 expression in live (7AAD-) hepatic mononuclear cell gated for pDC (CD11c⁺CD11b⁻PDCA1⁺B220⁺) and cDCs (CD11c⁺PDCA1⁻) at birth. Panel B shows the MFI at day 3 (d3), day 5 (d5) and day 7 (d7) after injection of saline or RRV soon after birth and in adult Balb/c mice. The data for saline and adult groups are the same as Figure 3. Data are shown as mean \pm SD of 3 separate experiments; N=4-7 livers per group per time point. *P<0.01 (all other groups have P>0.05).

Figure S4



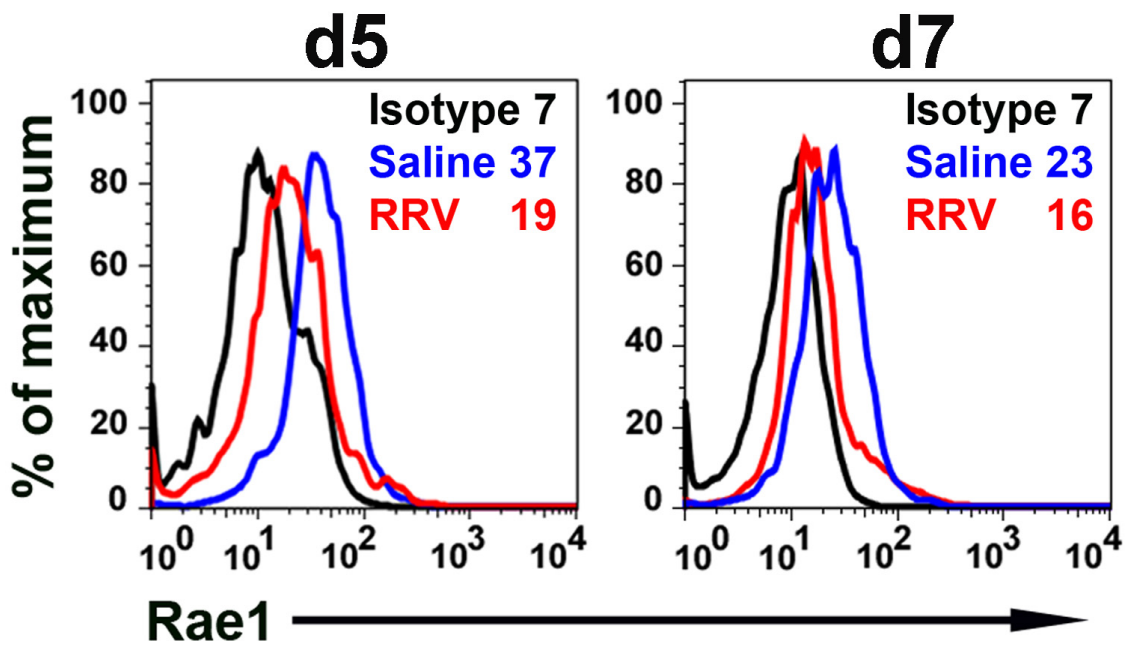
Effect of cholangiocytes on proliferation of CD4⁺ and CD8⁺ cells. Co-culture of a murine cholangiocytes (mCL) cell line infected with RRV does not induce proliferation of hepatic CD4⁺ and CD8⁺ cells (isolated from livers of 3 day old mice injected with saline) after 48 hours of culture.

Figure S5



Depletion of pDCs by anti-PDCA1 antibodies. In panel **A**, flow cytometric contour plots show a decrease in B220⁺Gr1⁺ cells from livers of mice 24 hours after injection of 50 μ g of anti-PDCA1 antibodies or IgG control isotype (also shown is a saline control group without administration of anti-PDCA1 or IgG isotype). N=3 mice per group. In **B**, flow cytometric analyses showing the impact of administration of anti-PDCA antibodies to control mice (i.e. injected with saline + IgG isotype, not with RRV). Values in each quadrant represent % cells positive for respective cell surface markers; representative results of 3 experiments. Panel **C** shows minimal changes in the expression of PDCA1 by CD4⁺, CD8⁺, and NK cells 3 days after injection of RRV when compared to saline and IgG isotype (values within the histogram represent mean \pm S.D. of % gated cells). In **D**, viral titer is depicted after MAE cells are cultured in the absence or with different dilutions of anti-PDCA1 antibody for 48 hours.

Figure S6



Rae1 expression in hepatic pDCs. Flow cytometric histograms show downregulation of Rae1 levels in hepatic pDCs 5 and 7 days after RRV challenge comparable to levels seen in saline (representative data from two independent experiments).