

Fairhurst *et al*; Supplementary Table I

Analysis of the splenic lineage and activation 8 weeks after IFN-ADV treatment

	UT		IFN-ADV		ADV		
	B6 <i>n</i> =12	B6.Ste123 <i>n</i> =8	B6 <i>n</i> =9	B6.Ste123 <i>n</i> =8	B6 <i>n</i> =11	B6.Ste123 <i>n</i> =10	
Total B220	% # x10 ⁸	52.1±1.4 (5.8 ±0.5)	44.6±1.9 [#] (10 ±1.3 [#])	16.1±2.1* (2.8 ±0.4)	13.6±2.1* (6.5 ±1.9)	51.2±1.3 (6.7 ±0.4)	41.8±2.3 (9.4 ±1.0)
% CD69+	% # x10 ⁷	4.5±1.1 (2.4 ±0.5)	3.8±0.9 (3.2 ±0.6)	13.1±1.8* (2.1 ±0.6)	8.5±1.8 (3.4 ±0.7)	4.9±1.2 (3.3 ±0.8)	4.7±1.1 (4.3 ±0.9)
CD86 MFI		5.5±1.6	4.1±1.3	24.0±5.7* (2.1 ±0.6)	32.5±7.7* (3.4 ±0.7)	5.1±1.6	6.2±2.0
IgD MFI		202.5±177.1	197.1±18.2	75.1±9.3* (2.1 ±0.6)	61.6±16.1* (3.4 ±0.7)	203.5±11.9	169.9±16.7
IgM MFI		8.6±1.9	2.9±0.4	16.3±4.3	3.1±0.6	8.8±2.3	5.1±1.5
MZ	%B220 # x10 ⁷	8.9±0.8 (5.4 ±0.8)	6.2±0.6 (6.7 ±1.3)	30.1±4.1* (9.2 ±1.8)	9.3±4.0 (4.9 ±2.0)	8.1±0.5 (5.4 ±0.4)	5.9±1.1 (5.1 ±0.9)
T1	%B220 # x10 ⁷	5.9±0.8 (3.6 ±0.6)	8.4±1.0 (8.8 ±1.5 [#])	5.8±0.9 (1.6 ±0.3)	10.1±2.0 (6.0 ±1.6)	5.8±0.7 (3.9 ±0.4)	8.1±0.9 (7.8 ±1.3)
T2	%B220 # x10 ⁷	2.3±0.4 (1.3 ±0.3)	0.7±0.2 [#] (0.9 ±0.2)	1.5±0.2 (0.4 ±0.1)	0.2±0.1 (0.2 ±0.1)	2.4±0.5 (1.7 ±0.4)	0.8±0.3 (0.6 ±0.2)
B1a	%B220 # x10 ⁷	1.0±0.1 (0.6 ±0.1)	1.3±0.1 (1.3 ±0.2 [#])	3.4±0.9* (1.0 ±0.3)	6.9±2.9 (4.6 ±1.8)	1.1±0.1 (0.7 ±0.1)	1.7±0.2 (1.5 ±0.2)
B1b	%B220 # x10 ⁷	20.0±2.1 (11.8 ±2.1)	25.6±1.7 (25.6 ±3.7 [#])	59.6±4.9* (16.5 ±2.1)	59.5±7.7* (37.9 ±12.9)	17.8±1.6 (11.5 ±0.9)	26.1±1.9 (24.1 ±2.1)
Fo	%B220 # x10 ⁷	16.7±2.0 (9.6 ±1.6)	18.8±1.8 (18.0 ±2.8 [#])	4.3±1.1* (1.4 ±0.4 [#])	6.2±2.2* (5.1 ±2.2 [#])	16.7±1.9 (11.4 ±1.6)	22.4±4.2 (22.3 ±2.7)
GL7	%B220 # x10 ⁷	13.3±1.9 (7.3 ±1.0)	16.1±2.1 (14.2 ±1.9)	30.8±2.1* (8.4 ±1.1)	38.4±5.1* (29.0 ±10.1)	13.4±1.9 (8.5 ±0.9)	20.7±3.2 (17.7 ±2.2)
Plasma	%total # x10 ⁷	0.7±0.1 (0.9 ±0.3)	1.6±0.3 (3.4 ±0.8 [#])	1.2±0.2 (2.0 ±0.2)	3.4±0.8* (22.1 ±10.6)	0.6±0.1 (0.8 ±0.1)	2.1±0.5 (5.4 ±1.7)
Plasmablast	%total # x10 ⁷	0.9±0.2 (1.2 ±0.5)	1.2±0.2 (2.4 ±0.4 [#])	0.4±0.2 (0.5 ±0.1)	1.2±0.2 (6.6 ±2.6 [#])	0.6±0.1 (0.9 ±0.1)	1.4±0.4 (3.5 ±0.1)
CD8	%total # x10 ⁷	9.3±1.0 (10.2 ±1.6)	6.0±0.6 (12.7 ±1.7)	7.8±0.9 (14.2 ±2.3)	6.7±1.3 (23.4 ±3.9)	9.7±1.2 (14.6 ±1.8)	7.7±1.3 (15.9 ±2.1)
CD4	%total # x10 ⁷	12.3±1.1 (13.7 ±2.0)	13.1±1.8 (27.1 ±2.8 [#])	7.6±0.8 (13.7 ±2.0)	9.4±1.1 (40.2 ±8.6 [#])	12.2±1.1 (12.6 ±1.7)	13.6±1.8 (28.3 ±3.0)
CD4:CD8		1.4±0.1	2.2±0.3	1.4±0.2	2.4±1.0	1.3±0.1	2.0±0.3

CD25⁺CD4⁺	%CD4 # x10 ⁷	12.7 ± 1.0 (1.7 ± 0.3)	13.6 ± 1.8 (3.4 ± 0.4) [#]	16.8 ± 1.0 (2.4 ± 0.4)	22.4 ± 2.45 [*] (9.9 ± 3.0) [#]	13.0 ± 1.3 (2.1 ± 0.3)	16.4 ± 1.4 (4.7 ± 0.7) [#]
CD4CD44^{lo}	%CD4 # x10 ⁷	49.6 ± 3.0 (7.0 ± 1.3)	27.0 ± 4.6 [#] (7.0 ± 1.2)	47.3 ± 2.8 (6.5 ± 0.9)	26.1 ± 5.5 (8.9 ± 1.3)	48.8 ± 3.5 (8.2 ± 1.3)	23.9 ± 4.4 (6.1 ± 1.8)
CD4CD44^{hi}	%CD4 # x10 ⁷	27.6 ± 2.4 (3.7 ± 0.6)	43.6 ± 4.1 [#] (11.8 ± 1.8) [#]	29.9 ± 2.9 (4.2 ± 0.8)	50.8 ± 6.0 (22.0 ± 5.9) [#]	26.2 ± 2.4 (4.2 ± 0.7)	52.6 ± 5.5 (15.5 ± 2.5) [#]
CD8CD44^{hi}	%CD8 # x10 ⁷	13.3 ± 2.8 (1.2 ± 0.2)	21.5 ± 2.7 (2.7 ± 0.4) [#]	23.8 ± 2.8 (3.5 ± 0.8)	36.5 ± 8.2 (7.1 ± 1.0)	14.0 ± 2.3 (1.7 ± 0.3)	27.9 ± 3.7 (4.7 ± 1.3)
NK1.1	%total # x10 ⁷	4.0 ± 0.1 (4.5 ± 0.2)	3.2 ± 0.3 (7.2 ± 1.1)	3.0 ± 0.4 (5.6 ± 0.8)	2.4 ± 0.4 (8.9 ± 1.6)	4.0 ± 0.2 (5.2 ± 0.4)	2.7 ± 0.2 [#] (6.0 ± 0.6)
CD11b⁺	%total # x10 ⁷	9.8 ± 0.8 (11.4 ± 1.9)	13.5 ± 2.4 (31.5 ± 7.1) [#]	10.8 ± 1.4 (20.7 ± 3.82)	7.8 ± 0.8 (39.3 ± 12.9)	9.6 ± 0.8 (12.6 ± 1.3)	12.5 ± 0.8 [#] (29.1 ± 4.2)
PMNs	%total # x10 ⁷	1.1 ± 0.1 (1.2 ± 0.2)	1.4 ± 0.4 (3.2 ± 0.8) [#]	2.1 ± 0.3 [*] (3.8 ± 0.6) [*]	1.4 ± 0.1 (6.2 ± 1.7)	1.0 ± 0.2 (1.4 ± 0.4)	1.1 ± 0.1 (2.5 ± 0.4)
PMN FcγRII/III		95 ± 9	109 ± 7	132 ± 13	125 ± 12	90 ± 8	110 ± 9
Gr1⁺7/4⁺monos	%total # x10 ⁷	0.9 ± 0.1 (1.2 ± 0.2)	1.2 ± 0.2 (2.8 ± 0.7)	1.3 ± 0.5 (2.3 ± 0.7)	0.9 ± 0.1 (5.3 ± 2.1)	1.0 ± 0.2 (1.3 ± 0.3)	1.1 ± 0.2 (2.5 ± 0.5)
FcγRII/III		89 ± 8	59 ± 5	96 ± 12	56 ± 9	78 ± 6	47 ± 4
Gr1⁺7/4⁺monos%	%total # x10 ⁷	0.8 ± 0.1 (0.9 ± 0.2)	0.9 ± 0.2 (2.2 ± 0.4) [#]	2.6 ± 0.5 [*] (4.8 ± 1.0) [*]	2.2 ± 0.5 (10.8 ± 4.2)	0.7 ± 0.1 (1.0 ± 0.2)	0.8 ± 0.1 (1.8 ± 0.2)
FcγRII/III		145 ± 6	144 ± 8	181 ± 11	91 ± 15 [#]	146 ± 12	134 ± 10
F4/80⁺ FSC^{hi}	%total # x10 ⁷	2.1 ± 0.3 (2.3 ± 0.4)	2.0 ± 0.4 (4.6 ± 1.0)	5.6 ± 0.9 [*] (10.7 ± 2.2) [*]	6.9 ± 1.6 [*] (38.4 ± 17.2) [*]	2.1 ± 0.4 (2.8 ± 0.5)	3.0 ± 0.6 (6.0 ± 0.9)
CD11b⁺CD11c⁺	%total # x10 ⁷	1.5 ± 0.1 (1.6 ± 0.2)	2.2 ± 0.5 (5.4 ± 1.6) [#]	0.8 ± 0.3 (3.1 ± 0.5)	1.4 ± 0.2 (6.7 ± 2.1)	1.4 ± 0.2 (1.7 ± 0.2)	1.5 ± 0.2 (3.5 ± 0.6)
CD8⁺CD11c⁺	%total # x10 ⁷	0.9 ± 0.2 (1.1 ± 0.2)	1.2 ± 0.1 (2.6 ± 0.4)	0.7 ± 0.2 (1.6 ± 0.4)	1.1 ± 0.3 (4.3 ± 1.2) [#]	1.0 ± 0.2 (1.3 ± 0.2)	0.9 ± 0.2 (2.4 ± 0.5)
B220⁺CD11c⁺	%total # x10 ⁷	2.2 ± 0.2 (2.4 ± 0.4)	2.0 ± 0.2 (4.2 ± 0.5)	1.1 ± 0.2 [*] (1.7 ± 0.2)	1.0 ± 0.3 (4.1 ± 1.4)	2.1 ± 0.3 (2.7 ± 0.4)	1.6 ± 0.2 (3.7 ± 0.5)

Results are expressed as mean ±SE with significant differences between treatment *p<0.05, and strain [#] p<0.05.

Fairhurst *et al*: Supplementary Table II

Analysis of the bone marrow lineage and activation 8 weeks after IFN-ADV treatment

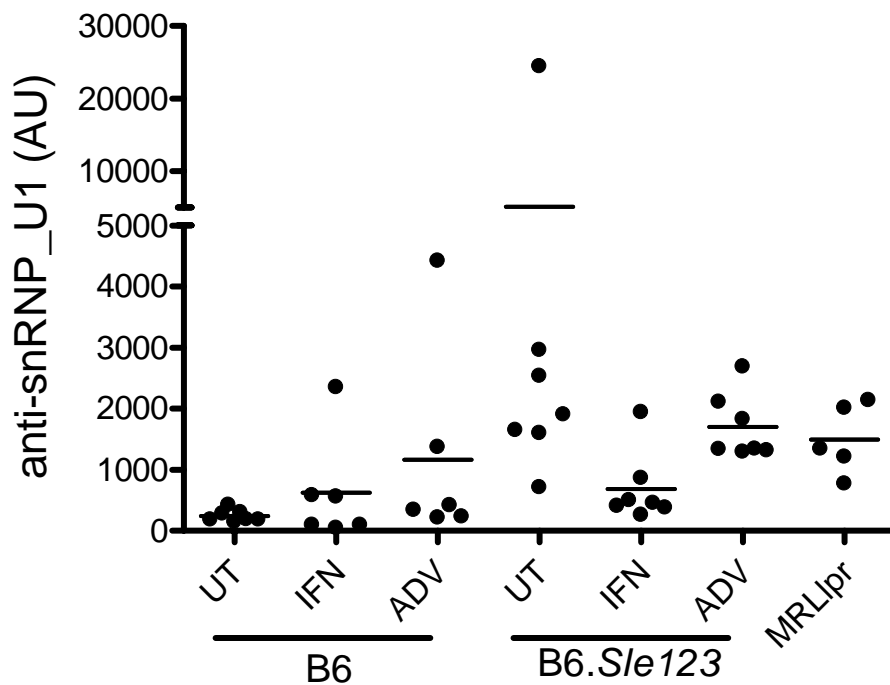
	UT		IFN-ADV		ADV	
	B6	B6.Sle123	B6	B6.Sle123	B6	B6.Sle123
B220 %total # x10 ⁶	17.3 ± 1.1 (9.6 ± 1.2)	15.1 ± 1.0 (7.8 ± 1.2)	5.6 ± 0.7* (1.7 ± 0.4 [†])	5.2 ± 1.0* (1.0 ± 0.4 [†])	17.8 ± 1.4 (9.4 ± 1.1)	12.9 ± 1.3 (6.2 ± 1.0)
Pro B %total # x10 ⁶	2.4 ± 0.3 (1.2 ± 0.2)	3.2 ± 0.3 (1.4 ± 0.1)	2.7 ± 0.4 (0.9 ± 0.2)	2.1 ± 0.4 (0.4 ± 0.1 [†])	3.2 ± 0.4 (1.6 ± 0.2)	2.9 ± 0.3 (1.4 ± 0.2)
Pre B %total # x10 ⁶	9.4 ± 1.2 (5.4 ± 1.0)	10.5 ± 0.6 (5.4 ± 1.0)	2.5 ± 0.4* (0.8 ± 0.2 [†])	2.4 ± 0.6* (0.5 ± 0.2 [†])	9.4 ± 1.1 (5.2 ± 0.9)	8.1 ± 1.1 (3.9 ± 0.7)
ImmatureB %total # x10 ⁶	6.4 ± 0.8 (3.4 ± 0.6)	3.6 ± 0.9 (1.6 ± 0.5)	1.9 ± 0.6* (0.6 ± 0.3 [†])	1.3 ± 0.5 (0.2 ± 0.1)	6.7 ± 0.9 (3.5 ± 0.4)	3.0 ± 0.5 (1.5 ± 0.3)
CD11b %total # x10 ⁷	39.0 ± 5.3 (2.0 ± 0.3)	35.4 ± 6.1 (1.7 ± 0.8)	40.6 ± 5.9 (1.2 ± 0.3)	32.5 ± 8.9 (0.5 ± 0.1)	37.3 ± 5.6 (1.7 ± 0.2)	41.2 ± 7.3 (1.9 ± 0.4)

Results are expressed as mean ±SE with significant differences between treatment *p<0.05, and strain # p<0.05.

Fairhurst *et al*; Supplemental Figure 1
SN RNP_U1 ANA assay.

Method

Immulon II plates (Dynatech Laboratories, Chantilly, VA), precoated with methylated BSA, were coated with 0.4 μ g/ml each of U1-snRNP C Protein, U1-snRNP C A Protein and U1-snRNP C 68 Protein, as recommended by the manufacturer (Diarect, Germany). After blocking with PBS/3% BSA/0.1% gelatin/3mM EDTA, 1/800 dilutions of the test sera were incubated in duplicate for 2h at room temperature (Optimization of this dilution was determined using 5 MRLlpr mice in an initial experiment). Bound IgG was detected with alkaline phosphatase (AP)-conjugated anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA or IgM-AP (Southern Biotech)) using pNPP as a substrate. Raw OD was converted to U/ml using positive control serum from an MRLlpr mouse.

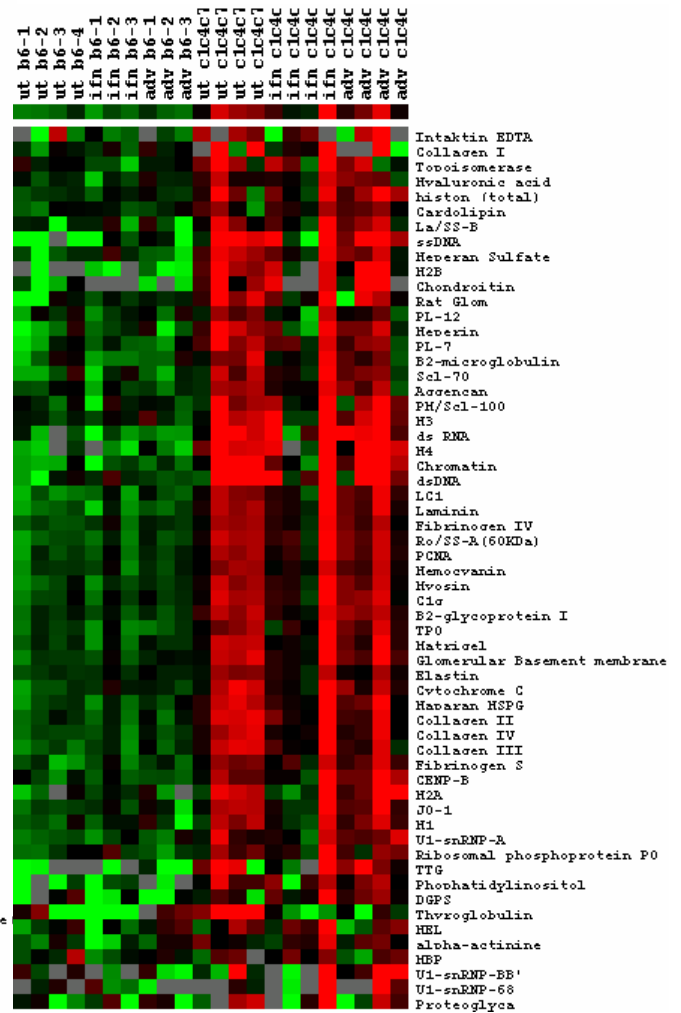
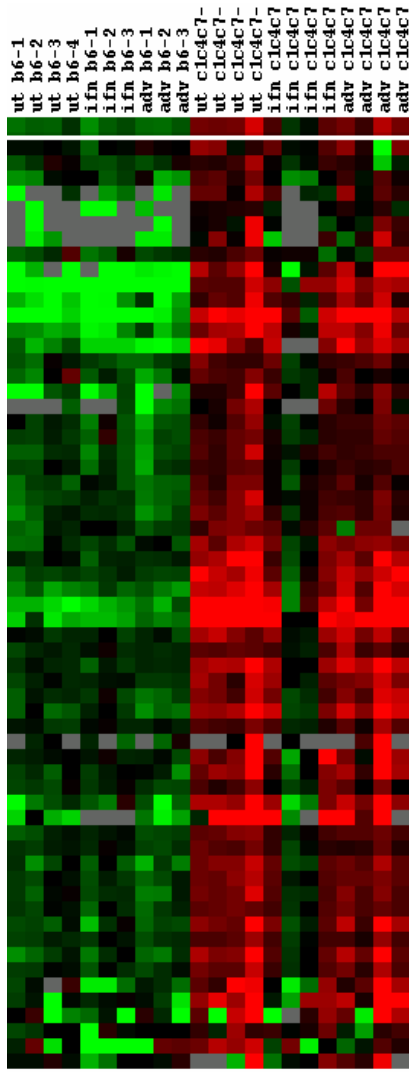


Fairhurst *et al*; Supplemental Figure 2
Autoantigen array of treated B6 and B6.*Slc123* mice.

Autoantigen arrays specific to IgG (A) and IgM (B) on serum samples were completed at the UT Southwestern Microarray Core facility (Li *et al. J. Clin. Inv.* 115:3428, 2005).

A IgG ANA

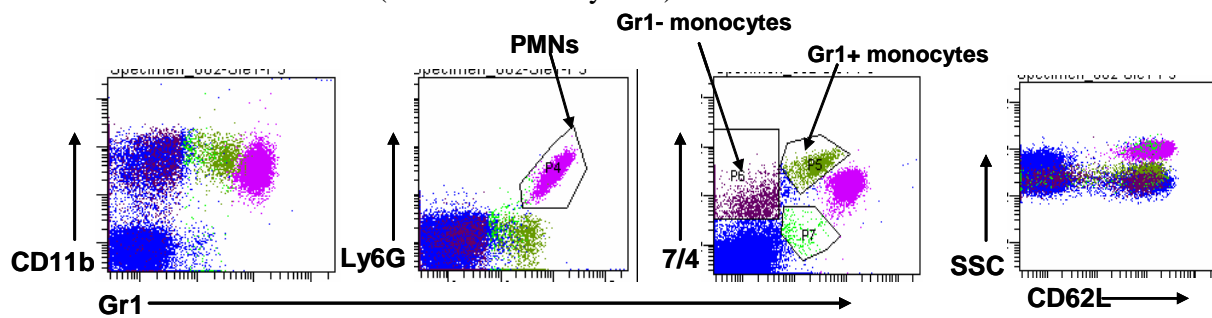
B IgM ANA



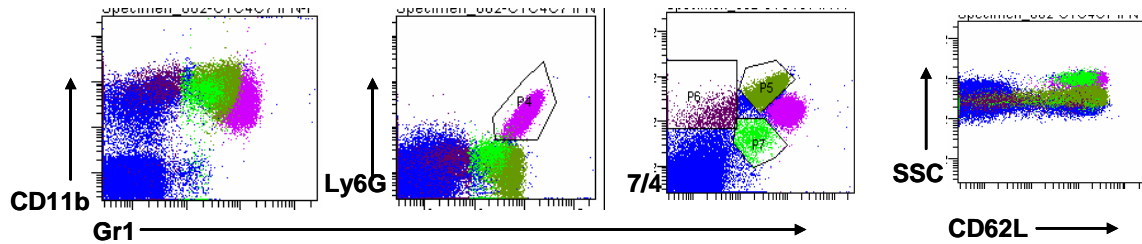
Fairhurst *et al*: Supplemental Figure 3
Defining monocytes and neutrophils

When myeloid expansion occurs, as demonstrated when comparing a non-diseased mouse (A) and an IFN-ADV injected B6.Sle123 mouse (B), resolution of the myeloid populations is lost. Using the markers Neu7/4, CD11b and Gr1 as shown below, the different populations can be resolved. The neutrophils are stained with the specific marker Ly6G (clone IA8, BD) to identify this population through other gating systems. Neutrophils demonstrate a higher side scatter (SSC) compared with monocytes due to their granularity, they express higher levels of Gr1 (Ly6C & Ly6G; clone RB6-8C6), and a lower level of Neu7/4 than Gr1+ monocytes.

A: Non-diseased B6.*Sle1* (minimal monocytosis)



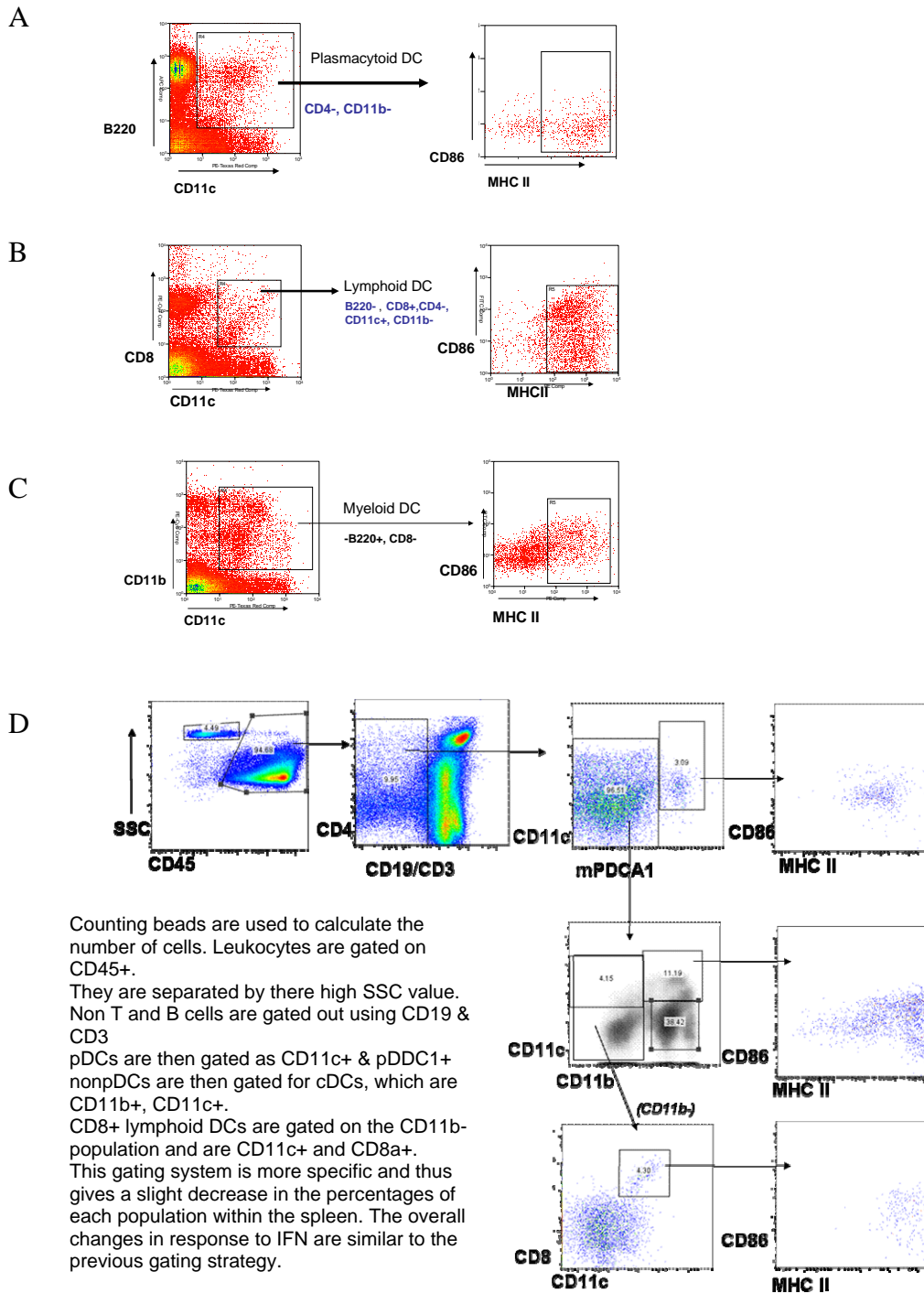
B. B6.*Sle123*+IFN-ADV (monocytosis)



Fairhurst *et al*; Supplementary Figure 4

Dendritic Cell population gating

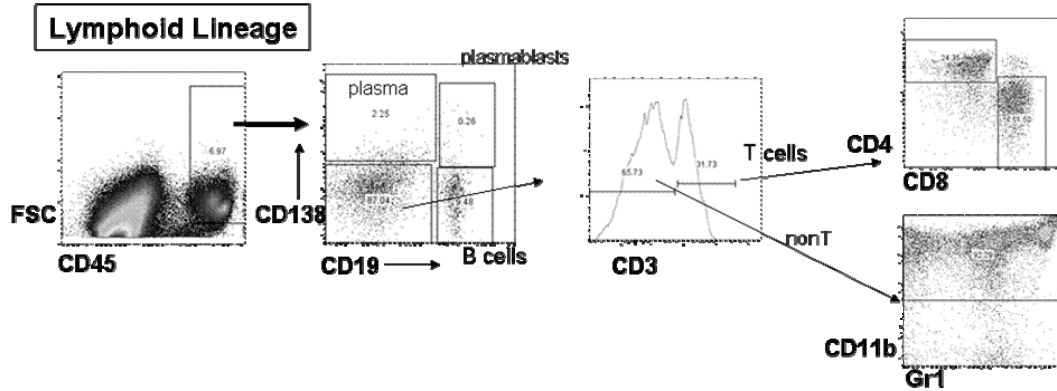
In examples A-C, dendritic cell analysis was completed using a 7 color staining procedure with acquisition and analysis on a DAKO[®] Cyan flow cytometer. Subsets are shown as: pDC populations (A) Lymphoid DCs (B) and Myeloid (conventional) (C). The experiment was repeated with a new cohort of mice, using a 10 color system on a BD LSR II[®] cytometer, with FlowJo[®] analysis (D). Both experiments demonstrated an increase in DC subset number in response to IFN in both strains.



Fairhurst *et al*; Supplementary Figure 5
Flow Cytometry analysis within the Kidney

Kidneys were prepared as described previously Wang A *et al* 2008 (57) and as outlined in the methods. Using a 2 panels of 10-color flow cytometry, the composition of the lymphoid lineage (A) and myeloid subsets (B) were determined within the kidney.

A



B

