

SUPPLEMENTAL MATERIAL

Mike et al., <http://www.jem.org/cgi/content/full/jem.20121887/DC1>

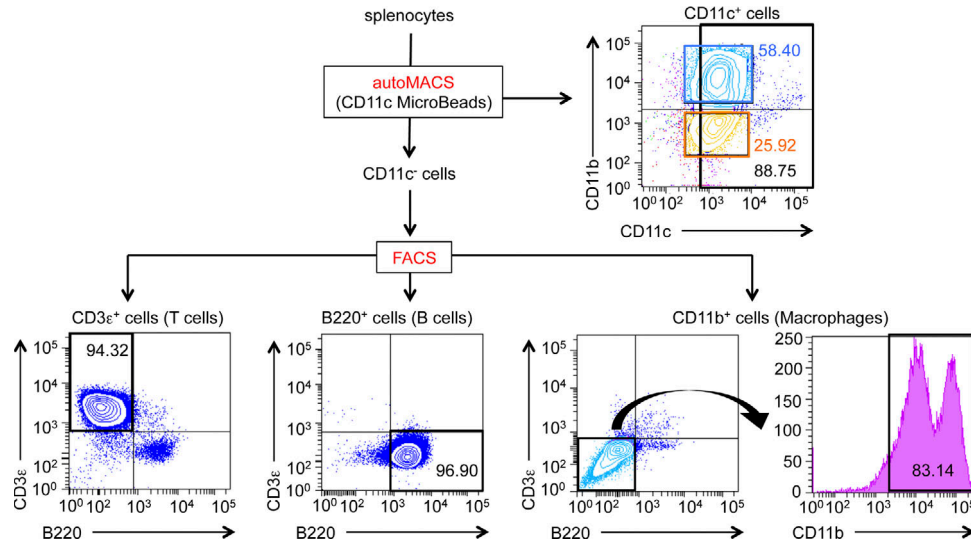


Figure S1. Flow cytometry of mouse splenocytes. Mouse splenocytes were separated into CD11c⁺ and CD11c⁻ populations by AutoMACS. CD11c⁻ cells were further sorted into CD3ε⁺ T cells, B220⁺ B cells, and CD11b⁺ macrophages. Purities of individual cell populations, which were used in Fig. 1 D, are indicated by boxes.

Table S1. Serum biochemical parameters in *Pla2g2d^{+/+}* and *Pla2g2d^{-/-}* mice under normal housing conditions

Parameters	Genotypes		Units
	<i>Pla2g2d^{+/+}</i>	<i>Pla2g2d^{-/-}</i>	
Alkaline phosphatase	135.3 ± 70.5	105.8 ± 41.2	U/liter
Alanine aminotransferase	30.9 ± 11.1	24.5 ± 3.1	U/liter
Bilirubin	0.3 ± 0.1	0.3 ± 0.1	mg/dl
Albumin	3.7 ± 0.5	3.6 ± 0.4	mg/dl
Nitrogen urea	28.5 ± 4.9	29.5 ± 11.5	mg/dl
Total cholesterol	77.4 ± 28.1	83.0 ± 11.5	mg/dl

Male 15-wk-old *Pla2g2d^{+/+}* and *Pla2g2d^{-/-}* mice were anesthetized and blood samples were collected by cardiac puncture. Sera were applied to a clinical chemistry analyzer VetScan with V-DPP rotors (*n* = 8). Data are pooled results from two experiments. None of these parameters differed significantly between the genotypes.

Table S2. Circulating blood cells in *Pla2g2d^{+/+}* and *Pla2g2d^{-/-}* mice under normal housing conditions

Cell populations	Genotypes		Units
	<i>Pla2g2d^{+/+}</i>	<i>Pla2g2d^{-/-}</i>	
Leukocytes	8.18 ± 1.11	7.89 ± 1.40	x 10 ⁹ cells/liter
Lymphocytes	7.02 ± 1.04	6.47 ± 1.07	x 10 ⁹ cells/liter
Granulocytes	0.76 ± 0.11	0.96 ± 0.29	x 10 ⁹ cells/liter
Monocytes	0.39 ± 0.15	0.46 ± 0.18	x 10 ⁹ cells/liter
Erythrocytes	9.97 ± 0.36	9.98 ± 0.13	x 10 ¹² cells/liter
Platelets	0.59 ± 0.04	0.55 ± 0.04	x 10 ¹² cells/liter

Blood samples of 15-wk-old male *Pla2g2d^{+/+}* and *Pla2g2d^{-/-}* mice were collected from tail biopsy with 10 mM EDTA as an anti-coagulant. The samples were applied to the clinical blood cell analyzer VetScan HM to determine the numbers of circulating blood cells ($n = 9$). Data are pooled results from two experiments. None of these parameters differed significantly between the genotypes.

Table S3. A list of primers used in quantitative RT-PCR

Gene	Accession number	Forward primer	Reverse primer	Probe number (Roche)
<i>Ccl2</i>	NM_011333.3	5'-CATCCACGTGTTGGCTCA-3'	5'-GATCATCTGCTGGTGAATGAGT-3'	62
<i>Ccr2</i>	NM_009915.2	5'-ACCTGTAATGCCATGCAAGT-3'	5'-TGTCTTCCATTTCCTTTGATTG-3'	27
<i>Cd11b</i>	NM_001082960.1	5'-CAATAGCCAGCCTCAGTGC-3'	5'-GAGCCCAGGGGAGAAGT-3'	76
<i>Cd11c</i>	NM_021334.2	5'-ATGGAGCCTCAAGACAGGAC-3'	5'-GGATCTGGGATGCTGAAATC-3'	20
<i>Cd68</i>	NM_009853.1	5'-GACCTACATCAGAGCCCGAGT-3'	5'-CGCCATGAATGTCCACTG-3'	96
<i>Foxp3</i>	NM_054039.1	5'-TCAGGAGCCACCAGTACA-3'	5'-TCTGAAGGCAGAGTCAGGAGA-3'	78
<i>Gapdhs</i>	NM_008085.1	5'-CCTTGAGATCAACACGTACCAG-3'	5'-CGCCTGTACACTCCACCAC-3'	1
<i>Irfng</i>	NM_008337.1	5'-ATCTGGAGGAACTGGCAAAA-3'	5'-TTCAAGACTCAAAGAGTCTGAGGTA-3'	21
<i>Il1b</i>	NM_008361.3	5'-TGTAATGAAAGACGGCACACC-3'	5'-TCTTCTTTGGGTATTGCTGG-3'	78
<i>Il6</i>	NM_031168.1	5'-GCTACCAAACCTGGATATAATCAGGA-3'	5'-CCAGGTAGCTATGGTACTCCAGAA-3'	6
<i>Il10</i>	NM_010548.1	5'-CAGAGCCACATGCTCCTAGA-3'	5'-TGCCAGCTGGTCTTTGTT-3'	41
<i>Il12a</i>	NM_008351.1	5'-ATCACAACCATCAGCAGATCA-3'	5'-CGCCATTATGATTAGAGACTG-3'	49
<i>Pla2g2d</i>	NM_011109.1	5'-GCTCTGGGCTGGAACATGA-3'	5'-CCTGGGTTGCAGTTATACCG-3'	66
<i>Pla2g5</i>	NM_011110.2	5'-CTCACACTGGCTTGGTTCCT-3'	5'-CATGGACTTGAGTTCTAGCAAGC-3'	95
<i>S100a9</i>	NM_009114.2	5'-CACCTGAGCAAGAAGGAAT-3'	5'-TGTCATTTATGAGGGCTTCATT-3'	31
<i>Tgfb1</i>	NM_011577.1	5'-TGGAGCAACATGTGGAATC-3'	5'-CAGCAGCCGGTTACCAAG-3'	72
<i>Tnf</i>	NM_013693.1	5'-TCTTCTATTCTGCTTGTGG-3'	5'-GGTCTGGCCATAGAACTGA-3'	49
<i>Rn18s</i>	-	5'-TCGAGGCCCTGTAATTGGAA-3'	5'-CCCTCAATGGATCCTCGTT-3'	-
<i>Pla2g1b</i>		Taq man probe (Applied Biosystems) No. 00478249		
<i>Pla2g2d</i>		Taq man probe (Applied Biosystems) No. 00478250		
<i>Pla2g2e</i>		Taq man probe (Applied Biosystems) No. 00478870		
<i>Pla2g2f</i>		Taq man probe (Applied Biosystems) No. 00478872		
<i>Pla2g5</i>		Taq man probe (Applied Biosystems) No. 00448162		
<i>Pla2g10</i>		Taq man probe (Applied Biosystems) No. 00449532		

Table S4. A list of antibodies used in flow cytometry

Molecule	Clone	Ig subclass	Fluorescent labeling	Source
CD3 ϵ	145-2C11	Hamster IgG	Alexa Fluor 488 APC FITC	BioLegend eBioscience BioLegend
CD4	GK1.5	Rat IgG2b	phycoerythrin	BioLegend
CD8 α	53-6.7	Rat IgG2a	Alexa Fluor 647	BioLegend
CD11b	M1/70	Rat IgG2b	FITC phycoerythrin	BioLegend BioLegend
CD11c	N418	Hamster IgG	Alexa Fluor 700 Biotin Pacific blue phycoerythrin	BioLegend BioLegend BioLegend eBioscience
CD16/32	2.4G2 93	Rat IgG2b Rat IgG2a	- -	BD BioLegend
CD25	PC61	Rat IgG1	Alexa Fluor 488	BioLegend
CD44	IM7	Rat IgG2b	phycoerythrin	BD
CD45R/B220	RA3-6B2	Rat IgG2a	Alexa Fluor 647	BD
CD62L	MEL-14	Rat IgG2a	APC	BioLegend
CD80	16-10A1	Hamster IgG	APC	BioLegend
CD86	GL-1	Rat IgG2a	Biotin	BioLegend
FOXP3	FJK-16s	Rat IgG2a	Biotin	eBioscience
MHC class II	M5/114.15.2	Rat IgG2b	FITC	BioLegend
Streptavidin ^a	-	-	FITC PE/Cy7	eBioscience BioLegend

^aUsed in combination with biotin-labeled antibodies.