Supplementary Fig. 1

a

empty vector   GFP   Cre recombinase

Side Scatter (y-axis) / GFP (x-axis)

B6 row: 0.13, 26.7, 0.44
iOvaFla row: 0.15, 33.7, 4.33
Nlrc4⁻/⁻; iOvaFla row: 0.14, 27, 25.8

b

Nlrc4⁻/⁻; iOvaFla; LysM-Cre$^{+/-}$
iOvaFla; LysM-Cre$^{-/-}$
iOvaFla; LysM-Cre$^{+/-}$

37 — GFP
25 — GAPDH

Immunoblot quantification

Normalized intensity (a.u.) (GFP/GAPDH)

** , *

■ *Nlrc4*⁻/⁻; iOvaFla; LysM-Cre$^{+/-}$
○ iOvaFla; LysM-Cre$^{-/-}$
● iOvaFla; LysM-Cre$^{+/-}$

c

Peritoneal Macrophages

GFP

iOvaFla; LysM-Cre$^{-/-}$
iOvaFla; LysM-Cre$^{+/-}$
Nlrc4⁻/⁻; iOvaFla; LysM-Cre$^{+/-}$

d

BM Neutrophils   Spleen Neutrophils   LN Neutrophils

GFP

e

Spleen B Cells   Spleen CD4+ T Cells   Spleen CD8+ T Cells
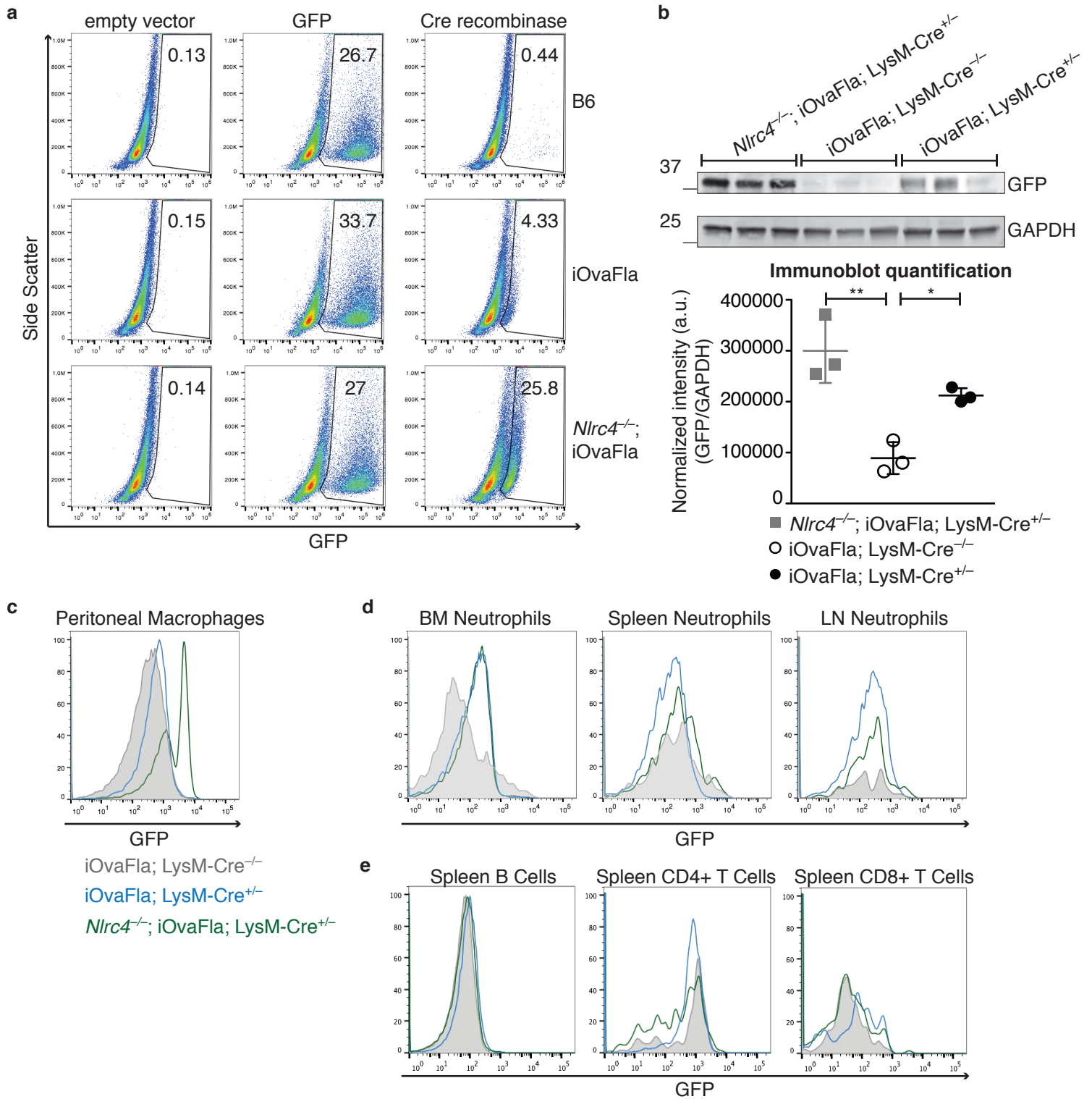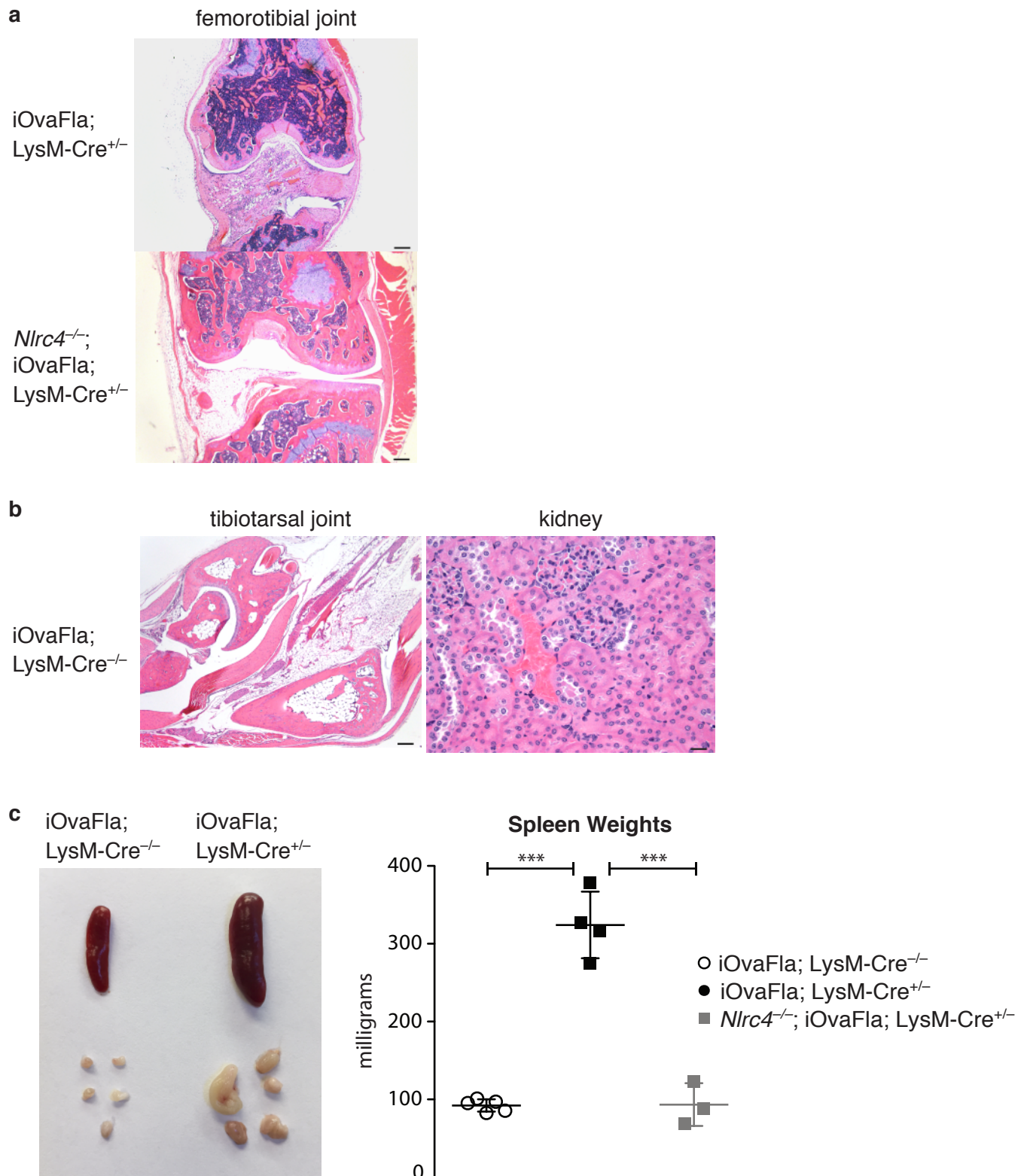
GFP

Supplementary Fig. 1. Genetic system for cell type-specific inflammasome activation in vivo. (a) Retroviral transduction of B6, iOvaFla$^{+/+}$, and *Nlrc4*⁻/⁻; iOvaFla$^{+/+}$ BMMs with either an empty MSCV vector, MSCV vector containing only GFP, or MSCV vector expressing Cre Recombinase. **(b)** GFP immunoblot and quantification performed on *ex vivo* peritoneal macrophages. Quantification was performed using Licor image studio light. GFP histograms of **(c)** peritoneal macrophages, **(d)** neutrophils from the bone marrow, spleen, or lymph nodes, and **(e)** splenic B cells (CD19⁺ TCRb⁻), CD4⁺ (TCRb⁺ C19⁻ CD4⁺), and CD8⁺ T cells (TCRb⁺ C19⁻ CD8⁺). Data in (c - e) are representative from n=3 biological replicates and (a - d) two or (e) three independent experiments. Error bars are s.d. Results were analyzed with an one-way ANOVA and Bonferroni post-tests; * = p < 0.05, ** = p < 0.01.
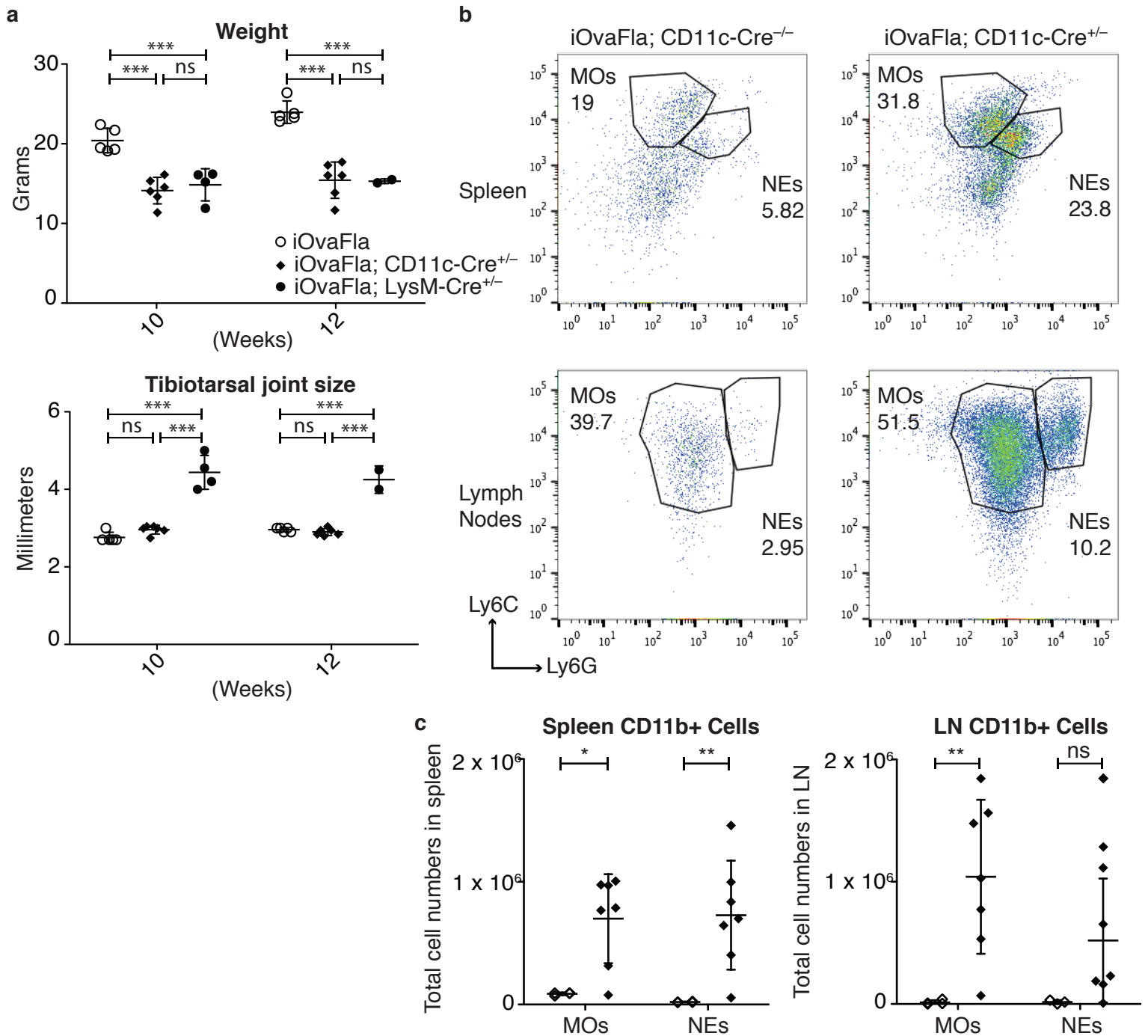
Supplementary Fig. 2

a femorotibial joint

iOvaFla; LysM-Cre$^{+/-}$

Nlrc4$^{-/-}$; iOvaFla; LysM-Cre$^{+/-}$

b tibiotarsal joint   kidney

iOvaFla; LysM-Cre$^{-/-}$

c iOvaFla; LysM-Cre$^{-/-}$ iOvaFla; LysM-Cre$^{+/-}$

Spleen Weights

milligrams

○ iOvaFla; LysM-Cre$^{-/-}$
● iOvaFla; LysM-Cre$^{+/-}$
■ *Nlrc4*$^{-/-}$; iOvaFla; LysM-Cre$^{+/-}$

Supplementary Fig. 2. Systemic inflammation in iOvaFla$^{+/-}$; LysM-Cre$^{+/-}$ mice. Histology images showing (a) femorotibial joint in 10 week iOvaFla; LysM-Cre$^{+/-}$ mice compared to *Nlrc4*$^{-/-}$; iOvaFla; LysM-Cre$^{+/-}$ mice (representative from 3 biological replicates) and **(b)** tibiotarsal joint and kidney of iOvaFla; LysM-Cre$^{-/-}$ mice (representative from 2 biological replicates; femorotibial and tibiotarsal joints at magnification X40 and scale bar is 500 microns; kidney at magnification X400 and scale bar is 20 microns). **(c)** Spleen and lymph nodes of iOvaFla; LysM-Cre$^{-/-}$ and iOvaFla; LysM-Cre$^{+/-}$ mice and spleen weights from iOvaFla; LysM-Cre$^{-/-}$, iOvaFla; LysM-Cre$^{+/-}$, *Nlrc4*$^{-/-}$; iOvaFla; LysM-Cre$^{+/-}$ mice (representative from two separate experiments). Error bars are s.d. Results were analyzed with a one-way ANOVA and Bonferroni post-tests; *** = $p < 0.001$.

Supplementary Fig. 3

a

b

c

● iOvaFla; LysM-Cre$^{+/-}$
■ *Nlrc4*$^{-/-}$; iOvaFla; LysM-Cre$^{+/-}$

d

e
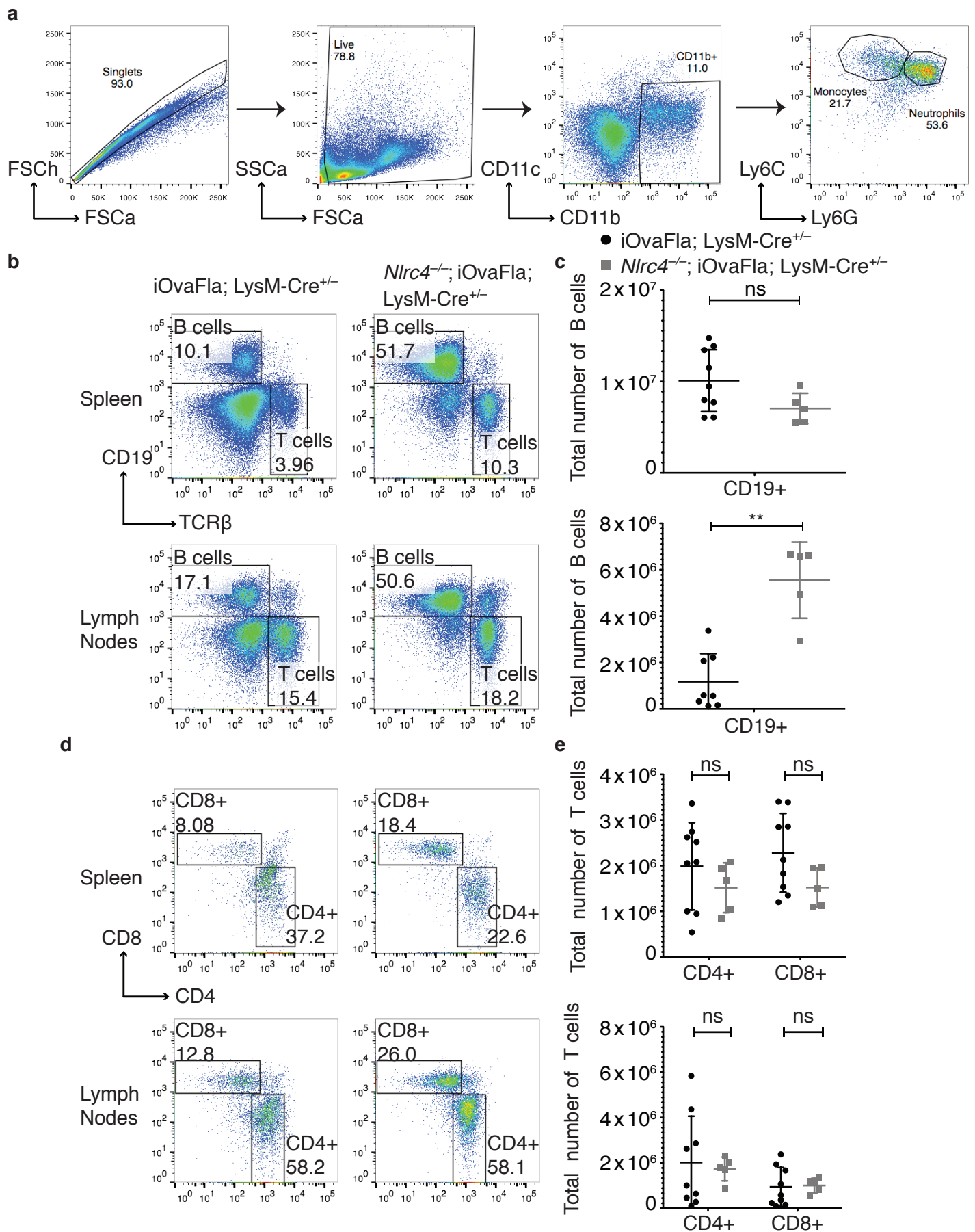
Supplementary Fig. 3. Lymphocyte populations mostly unchanged in iOvaFla; LysM-Cre$^{+/-}$ mice. (a) Gating strategy to determine monocytes and neutrophils. Example is an iOvaFla; LysM-Cre$^{+/-}$ mouse. **(b)** Flow cytometry and **(c)** quantification of B cells (CD19$^+$ TCRb$^-$). **(d)** Flow cytometry and **(e)** quantification of CD4$^+$ (TCRb$^+$ C19$^-$ CD4$^+$) and CD8$^+$ T cells (TCRb$^+$ C19$^-$ CD8$^+$). b – e are representative of 3 independent experiments. Error bars are s.d. Results were analyzed with either a Mann-Whitney t-test or two-way ANOVA and Bonferroni post-tests; * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Supplementary Fig. 4

a

b

c

Supplementary Fig. 4. iOvaFla; CD11c-Cre$^{+/-}$ mice have a less severe inflammatory phenotype. (a) Weight and tibiotarsal joint swelling as iOvaFla; Cre$^{-/-}$, iOvaFla; CD11c-Cre$^{+/-}$, and iOvaFla; LysM-Cre$^{+/-}$ mice age. **(b** and **c).** Flow cytometry plots and cell number graphs are a compilation of two separate experiments of mice aged 12-20 weeks old. For the flow cytometry plot, the top row is from the spleen and the second row from peripheral and mesenteric lymph nodes. Each column represents a different genotype. **(b)** Flow cytometry and **(c)** quantification of monocytes (MO; CD11b$^+$ Ly6C^{Hi} Ly6G^{Lo}) and neutrophils (NE; CD11b$^+$ Ly6C^{Lo} Ly6G^{Hi}). Data in (b, c) are representative of two independent experiments. Error bars are s.d. Results were analyzed with a two-way ANOVA and Bonferroni post-tests; * = p < 0.05, ** = p < 0.01, *** = p < 0.001.

Supplemental Table 1

Antibody	Clone	Used at	Supplier
Anti-CD11b PB	M1/70	1:250	eBiosciences 48-0112
Anti-CD11c APC	N418	1:250	eBiosciences 17-0114
Anti-Ly6G PE Cy7	1A8	1:250	Biolegend 127618
Anti-Ly6C PerCP Cy5.5	HK1.4	1:250	eBiosciences 45-5932
Anti-MHCII PE	M5/114.15.2	1:500	eBiosciences 12-5321
Anti-CD19 PE	eBio1D3	1:250	eBiosciences 12-0193
Anti-CD3 APC	145-2C11	1:250	eBiosciences 17-0031
Anti-CD4 PerCP e710	GK1.5	1:250	eBiosciences 25-0041
Anti-CD8 APC Cy7	53-6.7	1:250	eBiosciences 47-0081

Table listing all the flow cytometry antibodies used, the antibody clone, concentration used, and the supplier and catalogue number.