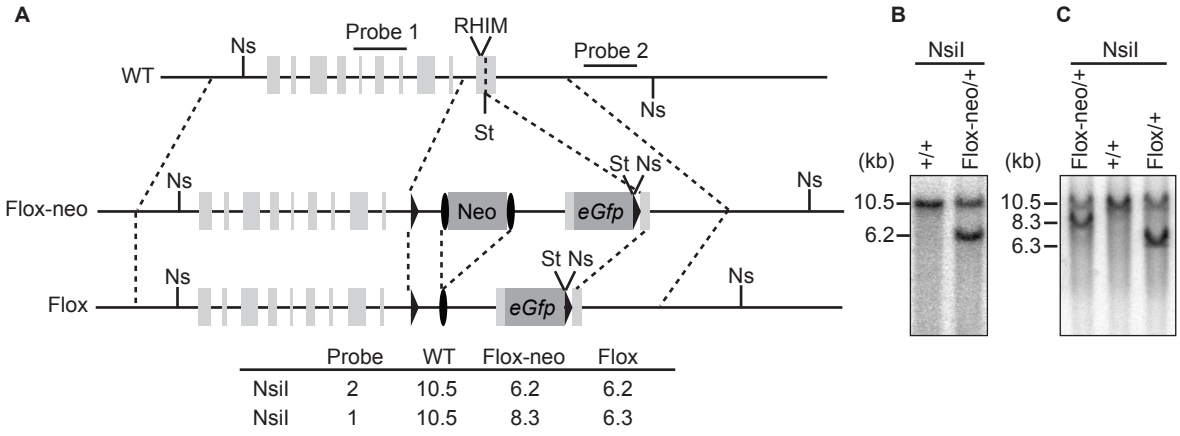
## Figure S1




| | Probe | WT | Flox-neo | Flox |
|---|---|---|---|---|
| NsiI | 2 | 10.5 | 6.2 | 6.2 |
| NsiI | 1 | 10.5 | 8.3 | 6.3 |

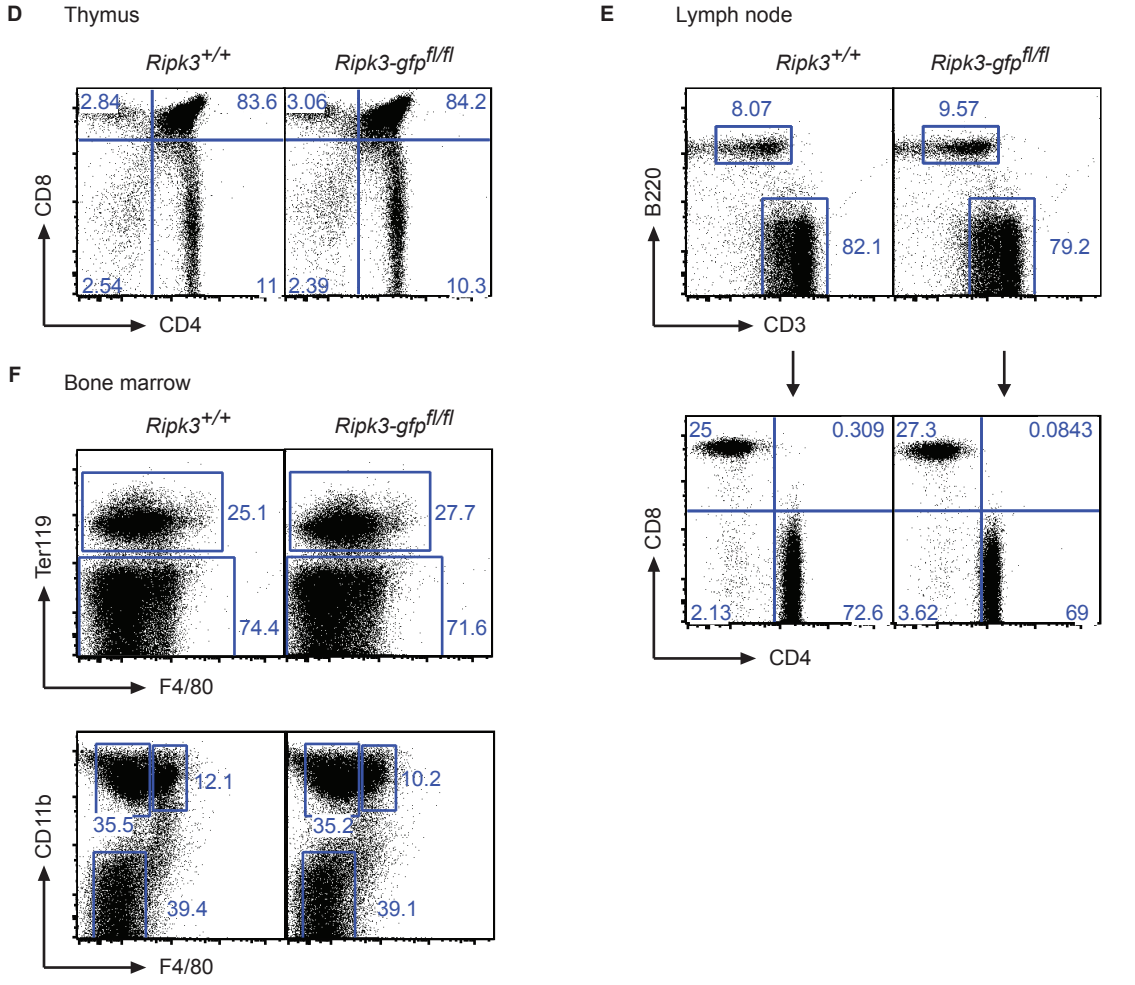


**Figure S1. Generation of RIPK3-GFP reporter mice, Related to Figure 1.**

**(A)** Schematic representation of the wild-type mouse *Ripk3* allele, the *Ripk3-gfp* allele with the *neo* cassette (Flox-neo), and the floxed *Ripk3-gfp* allele (Flox). Exons are shown as light gray boxes. LoxP and FRT sequences are indicated as triangle and oval, respectively. St indicates stop codon. Ns = NsiI site. Southern blot probes used for genotyping were shown as solid lines. Table below the genomic organization of the *Ripk3* alleles shows the expected size of bands in Southern blotting using the indicated probes. **(B)** Genomic DNA from mice of the indicated genotypes was digested by NsiI and subjected to Southern blotting using **(B)** probe 2 or **(C)** probe 1. Representative FACS plots of **(D)** developing thymocytes, **(E)** lymphocytes from inguinal lymph nodes, and **(F)** bone marrow cells of 6-week old mice of the indicated genotypes.

## Figure S2

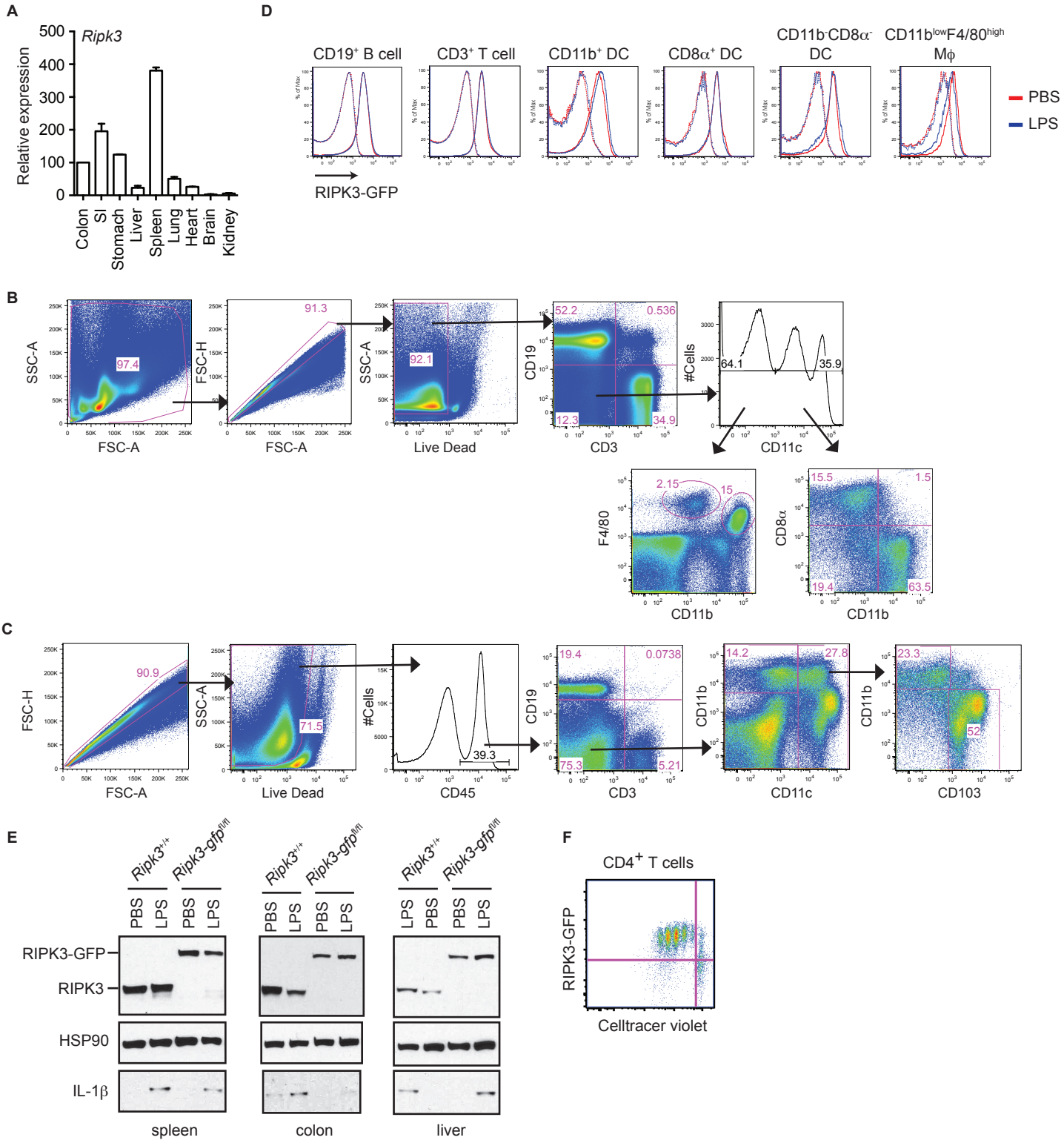

A
Ripk3
Relative expression
500
400
300
200
100
0
Colon
SI
Stomach
Liver
Spleen
Lung
Heart
Brain
Kidney
D
CD19+ B cell
CD3+ T cell
CD11b+ DC
CD8α+ DC
CD11b-CD8α- DC
CD11blowF4/80high Mϕ
PBS
LPS
RIPK3-GFP
B
SSC-A
FSC-A
97.4
FSC-H
91.3
92.1
Live Dead
52.2
0.536
CD19
CD3
12.3
34.9
#Cells
64.1
35.9
CD11c
F4/80
CD11b
2.15
15
CD8α
15.5
1.5
19.4
63.5
C
FSC-H
90.9
71.5
CD45
39.3
19.4
0.0738
75.3
5.21
14.2
27.8
23.3
52
CD103
E
Ripk3+/+
Ripk3-gfpfl/fl
PBS
LPS
RIPK3-GFP
RIPK3
HSP90
IL-1β
spleen
colon
liver
F
CD4+ T cells
RIPK3-GFP
Celltracer violet

**Figure S2. Characterization of *Ripk3-gfp*$^{fl/fl}$ reporter mice, Related to Figure 1.**

**(A)** Relative expression of *Ripk3* mRNA in various mouse tissues was determined by Q-PCR. **(B-C)** Gating strategy for FACS analysis in (**B**) Fig. 1B and (**C**) Fig. 1C are shown. **(D)** Representative histograms of RIPK3-GFP fluorescence intensity in various splenic immune subsets from LPS- or PBS-injected *Ripk3*$^{+/+}$ (dashed lines) and *Ripk3-gfp*$^{fl/fl}$ (solid lines). **(E)** Expression of RIPK3 and RIPK3-GFP in tissues of LPS-treated mice was determined by Western blotting. (**F)** CD4$^{+}$ T cells were purified from *Ripk3-gfp*$^{fl/fl}$ mice and subsequently stained with cell tracer violet. The cells were stimulated with plate-bound anti-CD3 and anti-CD28 antibodies. Results shown are mean ± SEM.

## Figure S3

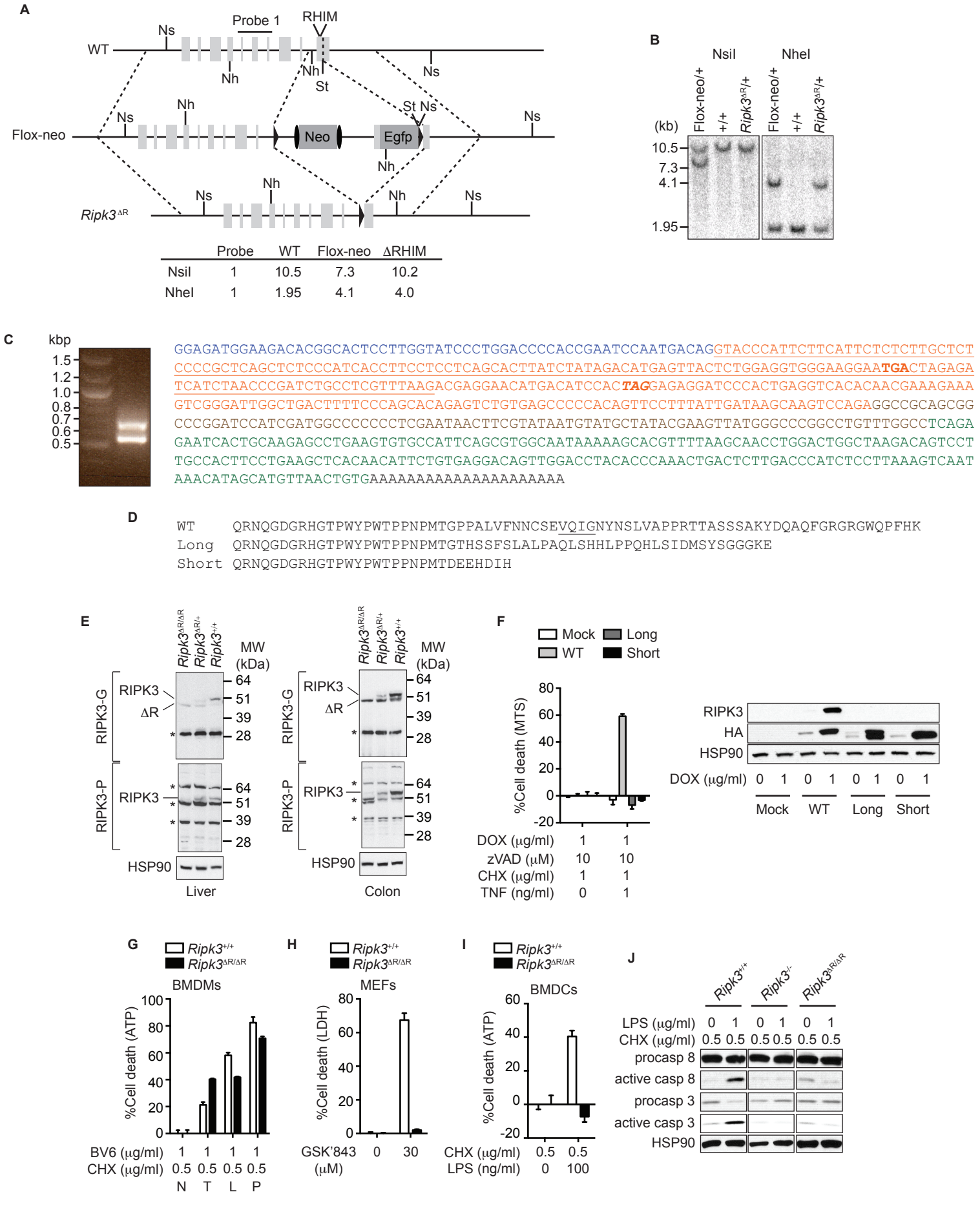

A
Probe 1
RHIM
WT
Ns
Nh
St
Flox-neo
Neo
Egfp
Ripk3ΔR
B
NsiI
NheI
Flox-neo/+
+/+
Ripk3ΔR/+
(kb)
10.5
7.3
4.1
1.95
Probe WT Flox-neo ΔRHIM
NsiI 1 10.5 7.3 10.2
NheI 1 1.95 4.1 4.0
C
kbp
1.5
1.2
1.0
0.8
0.7
0.6
0.5
GGAGATGGAAGACACGGCACTCCTTGGTATCCCTGGACCCCACCGAATCCAATGACAGGTACCCATTCTTCATTCTCTCTTGCTCT
CCCCGCTCAGCTCTCCCATCACCTTCCTCCTCAGCACTTATCTATAGACATGAGTTACTCTGGAGGTGGGAAGGAATGACTAGAGA
TCATCTAACCCGATCTGCCTCGTTTAAGACGAGGAACATGACATCCACTAGGAGAGGATCCCACTGAGGTCACACAACGAAAGAAA
GTCGGGATTGGCTGACTTTTCCCAGCACAGAGTCTGTGAGCCCCCACAGTTCCTTTATTGATAAGCAAGTCCAGAGGCCGCAGCGG
CCCGGATCCATCGATGGCCCCCCCTCGAATAACTTCGTATAATGTATGCTATACGAAGTTATGGGCCCGGCCTGTTTGGCCTCAGA
GAATCACTGCAAGAGCCTGAAGTGTGCCATTCAGCGTGGCAATAAAAAGCACGTTTTAAGCAACCTGGACTGGCTAAGACAGTCCT
TGCCACTTCCTGAAGCTCACAACATTCTGTGAGGACAGTTGGACCTACACCCAAACTGACTCTTGACCCATCTCCTTAAAGTCAAT
AAACATAGCATGTTAACTGTGAAAAAAAAAAAAAAAAAAAAA
D
WT QRNQGDGRHGTPWYPWTPPNPMTGPPALVFNNCSEVQIGNYNSLVAPPRTTASSSAKYDQAQFGRGRGWQPFHK
Long QRNQGDGRHGTPWYPWTPPNPMTGTHSSFSLALPAQLSHHLPPQHLSIDMSYSGGGKE
Short QRNQGDGRHGTPWYPWTPPNPMTDEEHDIH
E
Ripk3ΔR/ΔR
Ripk3ΔR/+
Ripk3+/+
MW (kDa)
RIPK3-G
RIPK3
ΔR
RIPK3-P
HSP90
64
51
39
28
Liver
Colon
F
Mock
Long
WT
Short
%Cell death (MTS)
DOX (μg/ml)
zVAD (μM)
CHX (μg/ml)
TNF (ng/ml)
HA
HSP90
Mock
WT
Long
Short
G
Ripk3+/+
Ripk3ΔR/ΔR
BMDMs
%Cell death (ATP)
BV6 (μg/ml)
CHX (μg/ml)
N T L P
H
MEFs
%Cell death (LDH)
GSK'843 (μM)
I
BMDCs
%Cell death (ATP)
CHX (μg/ml)
LPS (ng/ml)
J
Ripk3+/+
Ripk3-/-
Ripk3ΔR/ΔR
LPS (μg/ml)
CHX (μg/ml)
procasp 8
active casp 8
procasp 3
active casp 3
HSP90

**Figure S3. Generation of *Ripk3*$^{\Delta R/\Delta R}$ mice, Related to Figure 2.**

**(A)** Schematic diagram of the wild-type mouse *Ripk3* allele, the *Ripk3-gfp* allele with loxP sites and *neo* cassette (Flox-neo), and the *Ripk3*$^{\Delta R}$ allele. Ns = NsiI site; Nh = NheI site. **(B)** Genomic DNA from mice with indicated genotypes was digested by NsiI or NheI and subjected to Southern blotting using probe 1. **(C)** Second PCR product in 3' RACE experiment was run on a 1% agarose gel. DNA fragments were extracted from two distinct bands shown on the left side, cloned into pUC19 vector, and subjected to DNA sequence analysis. The mRNA sequence is shown on the right. Blue, orange, brown, and green colored sequences are derived from exon 9, intron 9, targeting vector, untranslated region on exon 10, respectively. The underlined sequence was found in the *Ripk3*$^{\Delta R}$ long isoform, but spliced out in the *Ripk3*$^{\Delta R}$ short isoform. The stop codons for the long and short isoforms were highlighted in bold and bold italic, respectively. **(D)** Predicted C-terminal amino acid sequences of wild type, *Ripk3*$^{\Delta R}$ long isoform, and *Ripk3*$^{\Delta R}$ short isoform. The tetra-peptide core amino acid sequence of the RHIM (VQIG) is underlined. **(E)** Western blotting of tissue extracts from mice of the indicated genotypes. * = non-specific signals. **(F)** *Ripk3*$^{\Delta R}$ long and short isoforms were stably expressed in *Ripk3*$^{-/-}$ 3T3 fibroblasts. After induction of RIPK3 proteins by DOX for 9 hours, the cells were treated with zVAD, CHX, and TNF for 14 hours. The right panel shows DOX-inducible expression of RIPK3 WT and ΔRHIM proteins. **(G-J)** *Ripk3*$^{+/+}$ and *Ripk3*$^{\Delta R/\Delta R}$ BMDMs (**G**), MEFs **(H)** and BMDCs **(I)** were stimulated with as indicated for 14 hours. N, no stimulation; T, 100 ng/ml TNF; L, 100 ng/ml LPS; P, 20 μg/ml poly(I:C). **(J)** BMDCs pretreated with CHX for 1 hour were stimulated with LPS for 1 hour. Whole cell extracts were subjected to Western blotting. The lanes were run on the same gel but were noncontiguous. Results shown are mean ± SEM.

## Figure S4

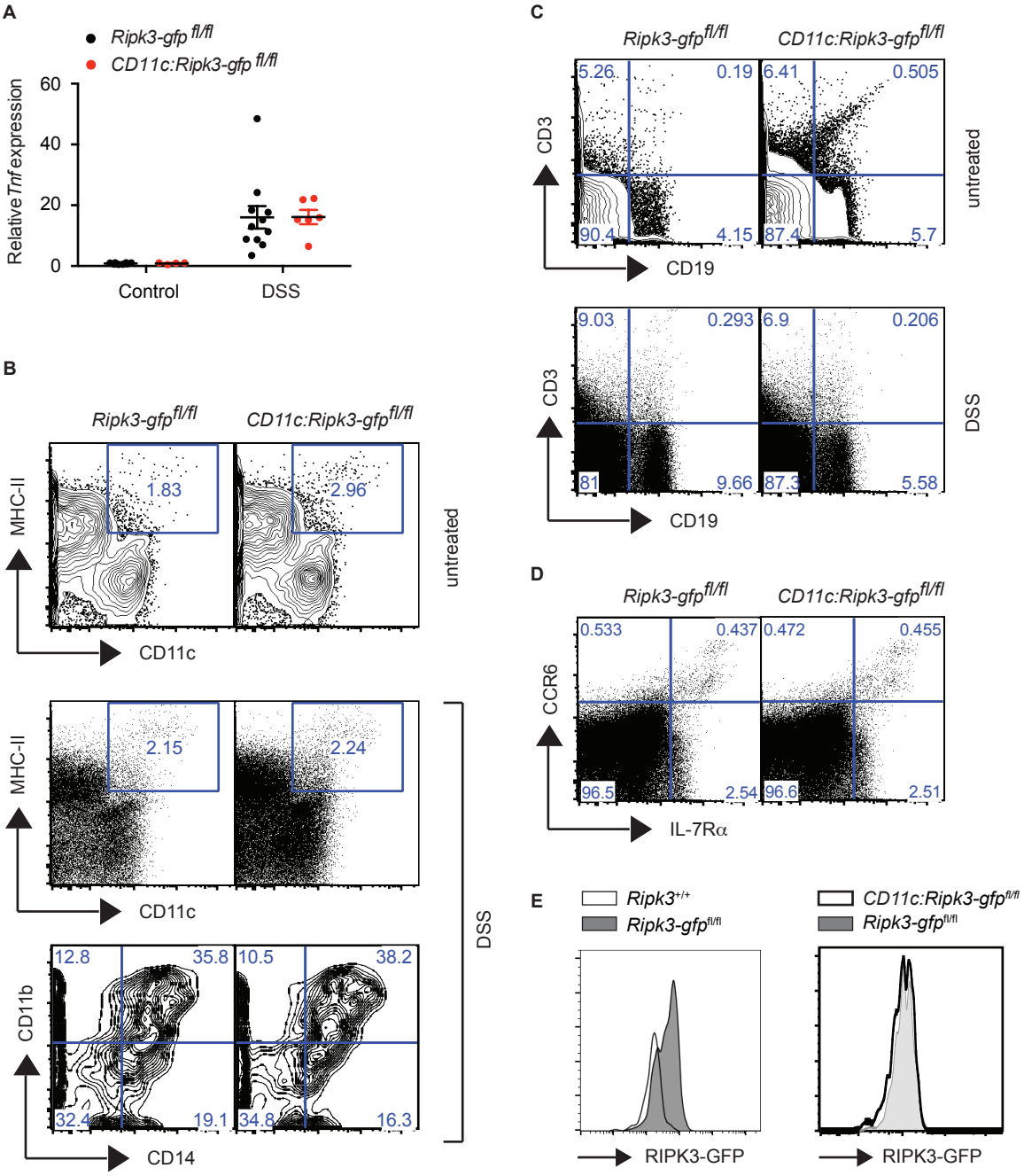

A
Ripk3-gfp fl/fl
CD11c:Ripk3-gfp fl/fl
Relative Tnf expression
60
40
20
0
Control
DSS
B
Ripk3-gfpfl/fl
CD11c:Ripk3-gfpfl/fl
MHC-II
1.83
2.96
CD11c
untreated
MHC-II
2.15
2.24
CD11c
12.8
35.8
10.5
38.2
32.4
19.1
34.8
16.3
CD11b
CD14
DSS
C
Ripk3-gfpfl/fl
CD11c:Ripk3-gfpfl/fl
5.26
0.19
6.41
0.505
90.4
4.15
87.4
5.7
CD3
CD19
untreated
9.03
0.293
6.9
0.206
81
9.66
87.3
5.58
CD3
CD19
DSS
D
Ripk3-gfpfl/fl
CD11c:Ripk3-gfpfl/fl
0.533
0.437
0.472
0.455
96.5
2.54
96.6
2.51
CCR6
IL-7Rα
E
Ripk3+/+
Ripk3-gfpfl/fl
CD11c:Ripk3-gfpfl/fl
Ripk3-gfpfl/fl
RIPK3-GFP
RIPK3-GFP

**Figure S4. Characterization of *CD11c:Ripk3-gfp*$^{fl/fl}$ mice treated with DSS, Related to Figure 5.**

**(A)** Mice treated for 7 days with DSS were analyzed for *Tnf* expression in the colon. **(B-D)** Representative FACS analysis of CD11c$^{+}$ or CD11b$^{+}$ APCs **(B)**, T and B cells **(C)**, and type 3 innate lymphoid cells **(D)** from DSS-treated mice. **(E)** RIPK3-GFP expression in CCR6$^{+}$ ILC3 from untreated (left panel) and DSS-treated mice (right panel).

## Figure S5

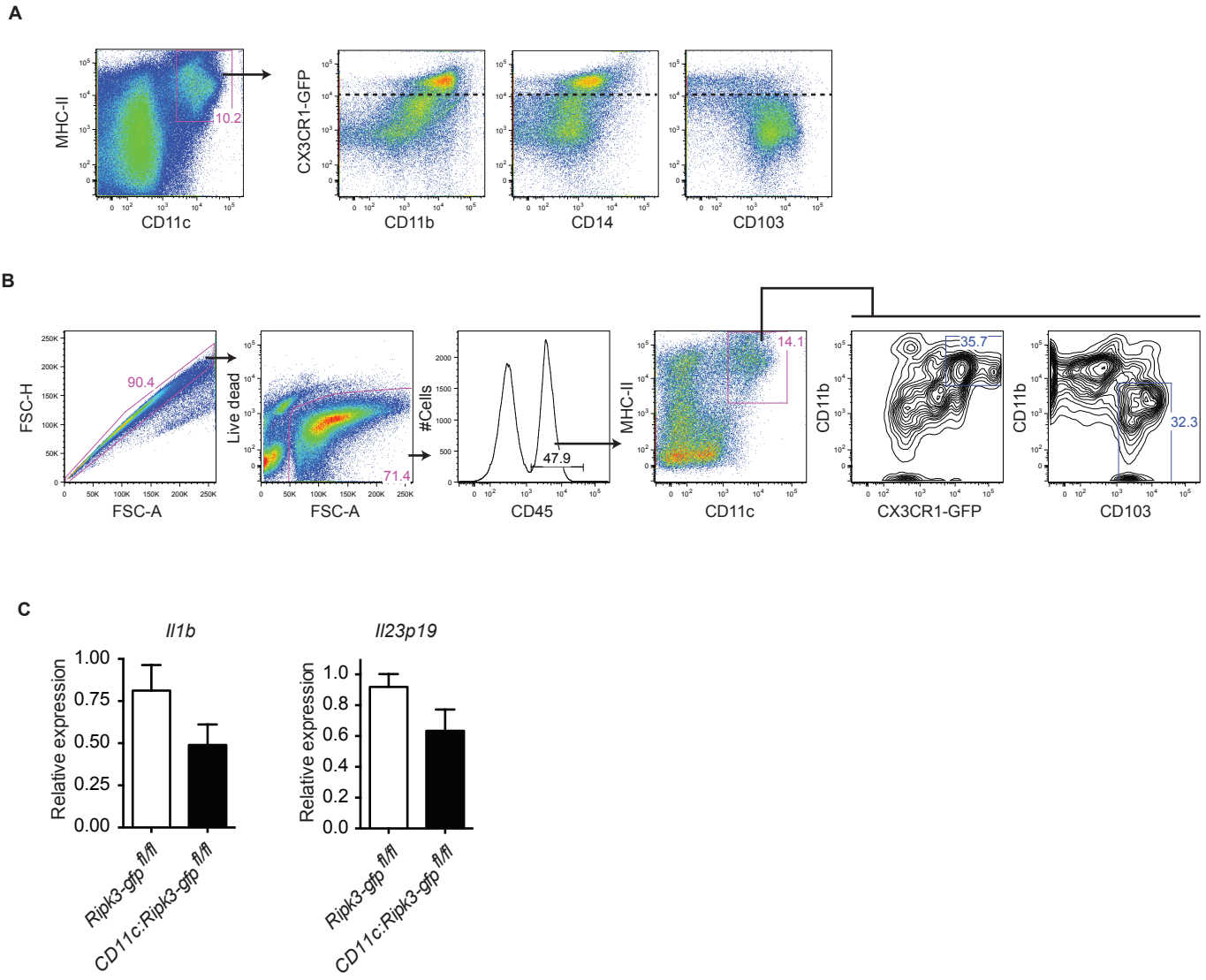

A
MHC-II
10.2
CD11c
CX3CR1-GFP
CD11b
CD14
CD103
B
FSC-H
90.4
FSC-A
Live dead
71.4
FSC-A
#Cells
47.9
CD45
MHC-II
14.1
CD11c
CD11b
35.7
CX3CR1-GFP
CD11b
32.3
CD103
C
Il1b
Relative expression
1.00
0.75
0.50
0.25
0.00
Ripk3-gfp fl/fl
CD11c:Ripk3-gfp fl/fl
Il23p19
Relative expression
1.0
0.8
0.6
0.4
0.2
0.0
Ripk3-gfp fl/fl
CD11c:Ripk3-gfp fl/fl

**Figure S5. $CX_3CR1^+$ MNPs express CD11b and CD14, but not CD103, Related to Figure 6.**

**(A)** Representative FACS plots show that $CD11c^+MHC\text{-}II^+CX_3CR1^+$ MNPs express CD11b and CD14, but not CD103. **(B)** Gating strategy for FACS analysis in Fig. 6B is shown. **(C)** $CD11c^+CD11b^+CD14^+CD103^-$ MNPs were sorted from DSS-treated *Ripk3-gfp*$^{fl/fl}$ (n=4) and *CD11c:Ripk3-gfp*$^{fl/fl}$ mice (n=4) and analyzed for *Il1b* and *Il23p19* expression by Q-PCR.