Supplementary Material

Contraction of intestinal effector T cells by retinoic acid-induced purinergic receptor P2X7

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Supplementary Table 1.

1. ChIP primers:

Site-A-F: 5’-GTG TGC CTG TTG GTG TGT AA-3’

Site-A-R: 5’- TCG TGA AGG CAG AAA GAT ACA AC-3’

Site-B-F: 5’- GAG GTG GCC TAT TCT ATA ATC CTG-3’

Site-B-R: 5’-ACC TCT TCC CAG TAG CAT CGG-3’

Site-C-F: 5’- CTG GCA CAG TCT CTA TTG CCT C-3’

Site-C-R: 5’-CTT CGG TCT TTA CGG TTA CCA TT-3’

Site-D-F: 5’-TAG AAT GCT TGG CTA GTA AGC ATG-3’

Site-D-R: 5’-CTT CTT TGA TGC AGC TGG ATG TGA-3’

Site-E-F: 5’- TGC TTT ATC CAG TCC TAC CGG-3’

Site-E-R: 5’-CTTT CTT CGT CAC CAC ATC CTT-3’

Site-F-F: 5’- GGT AGA CCA CGG AGA AGT GTA TG-3’

Site-F-R: 5’- TTC TAG CTG GAA ACA AAT AGC AGC-3’

2. P2rx7 promoter and enhancer cloning primers:

Promoter:

Forward: 5’-ATT ACT CGA GCA CTC CTT TGC ATG ACT GAG TA-3’

Reverse: 5’-ATT AAA GCT TGT CCT GGC AAC TGC CCT GAA-3’

Enhancer:

Forward: 5’-ATT AAA GCT TGA GCG ATA AGC TGT ACC A-3’

Reverse: 5’-ATT AAA GCT TCG AGA CCA GGG TGT G-3’

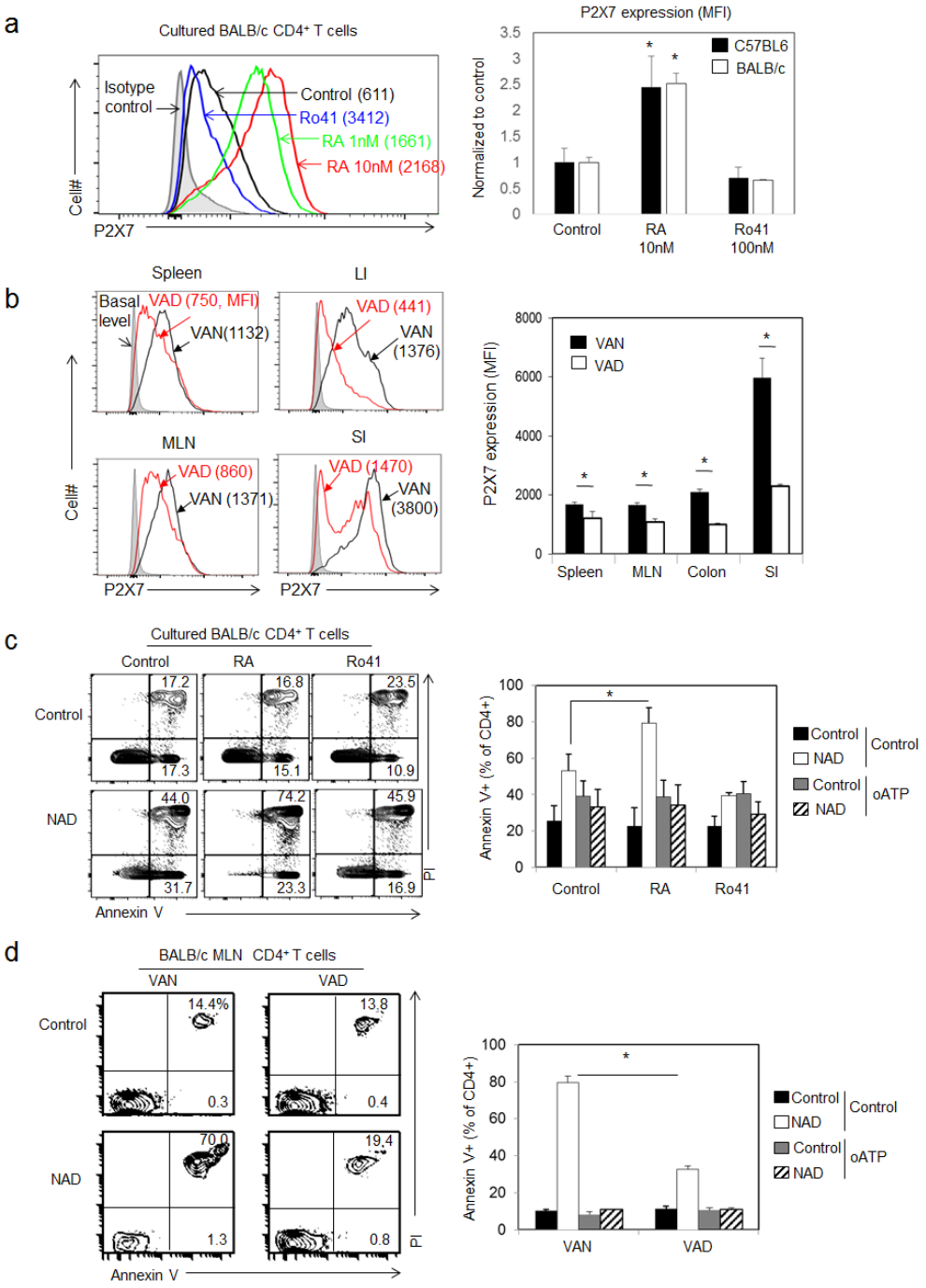
3. qRT-PCR primers:

P2rx7-F: 5’-GTG TGG AAA TTG CCT TCC GTC-3’

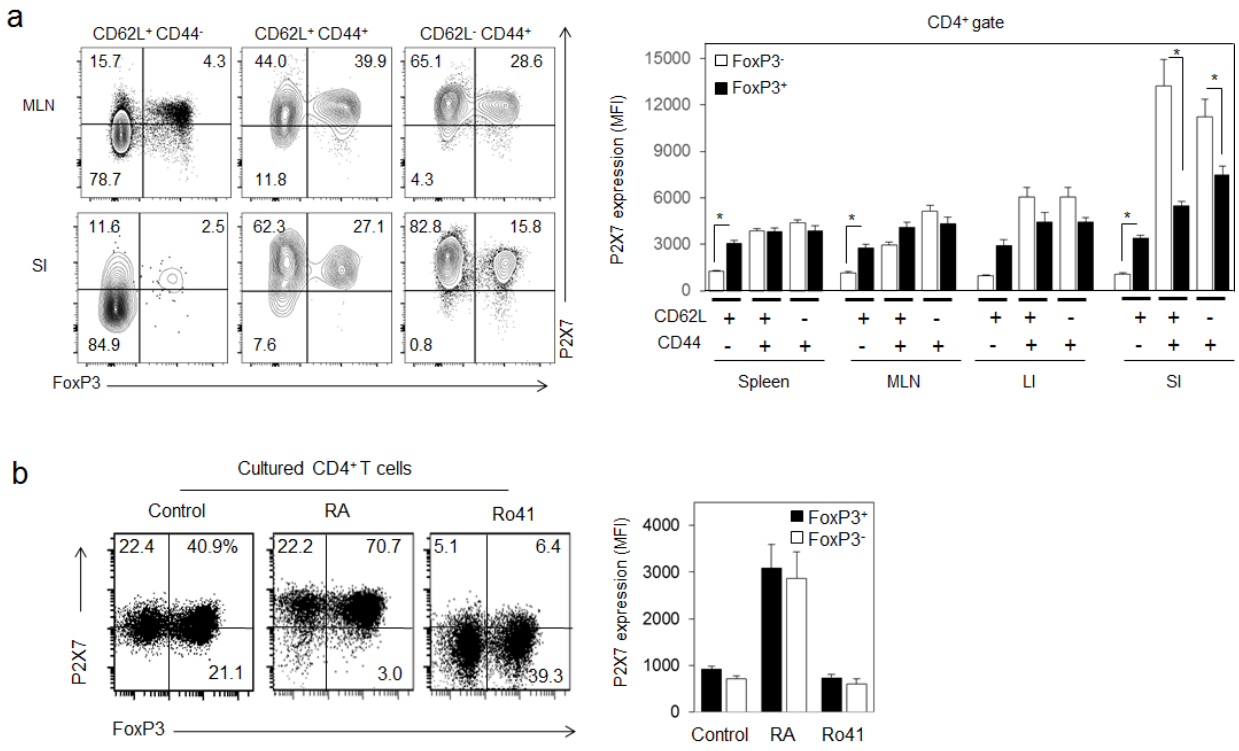
P2rx7-R: 5’-GTA GTC ATC CCT ATG AAC TTC GG-3

Art2b-F: 5’-GTG GCT AAC CCA GCA GGT GAC T-3’

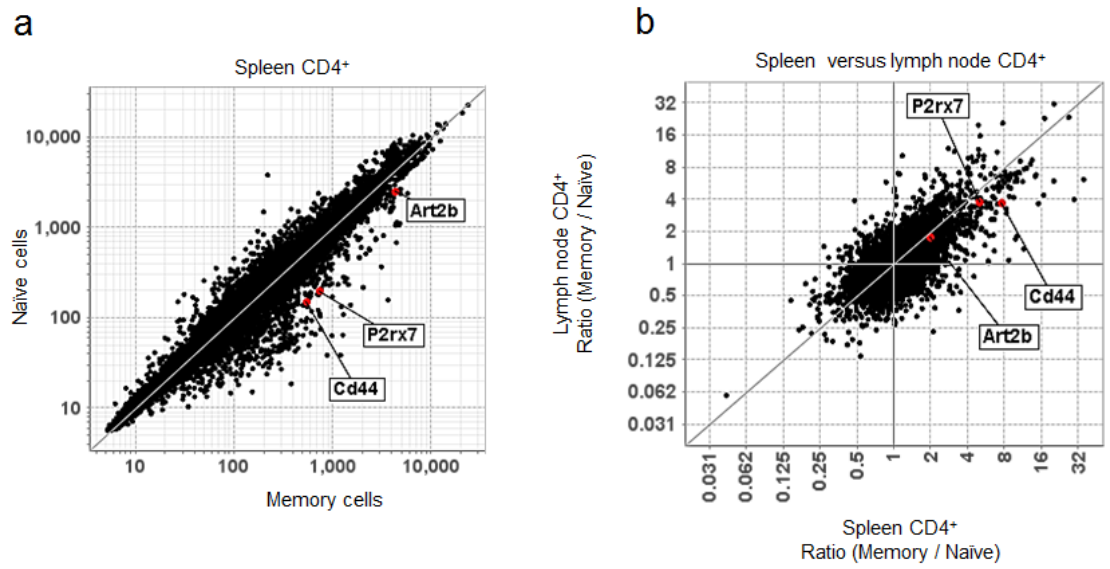
Art2b-R: 5’-CTT GAA GTC TCT AAC AGC TC-3’



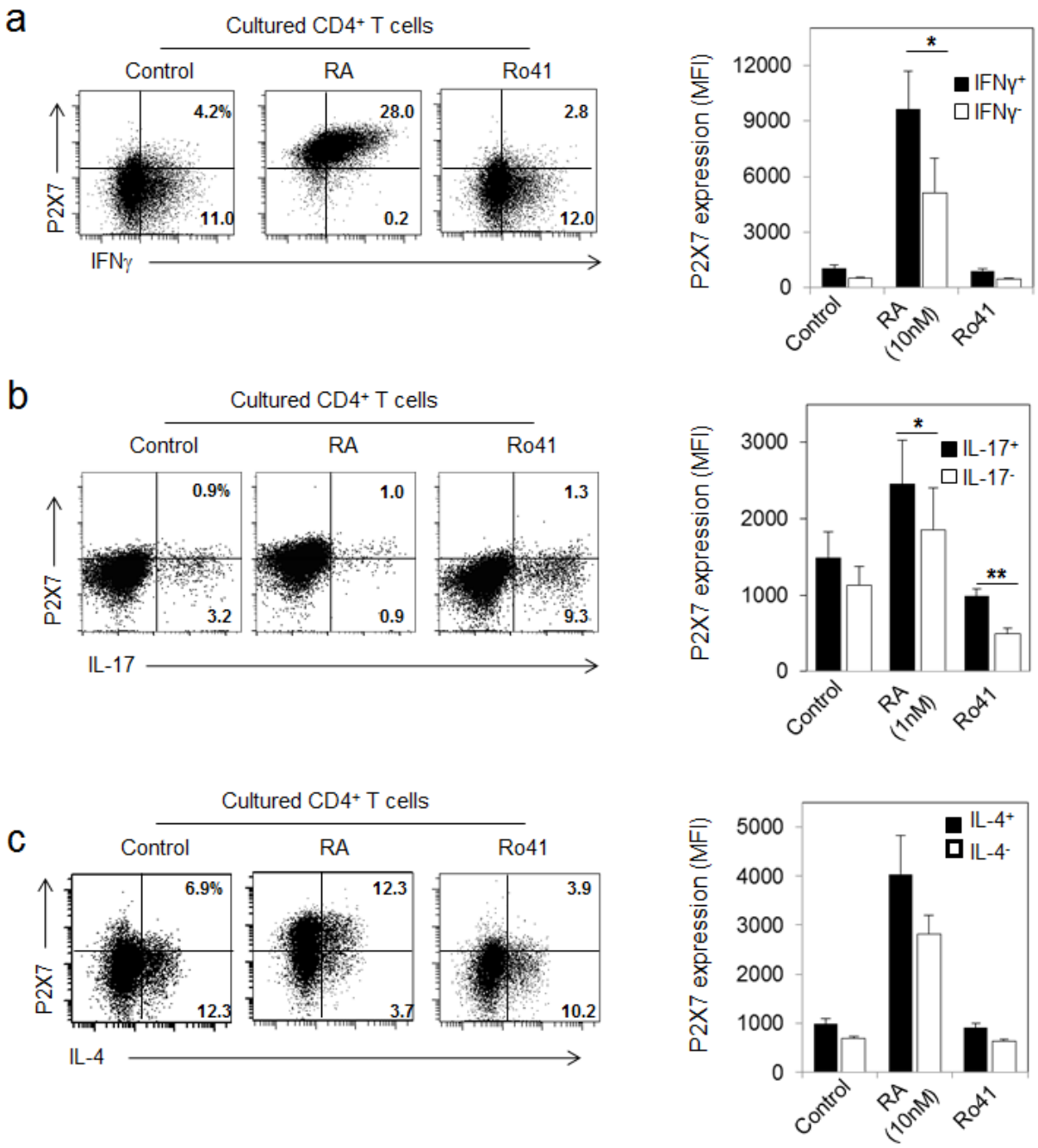
Supplementary Figure 1. Comparison of P2X7 expression and NAD-induced apoptosis of C57BL/6 versus BALB/c CD4$^+$ T cells. (a) Comparison of P2X7 expression by cultured CD4$^+$ T cells, isolated from the spleen and lymph nodes of C57BL/6 and BALB/c mice. (b) P2X7 expression by CD4$^+$ T cells in various organs of BALB/c mice. (c) Sensitivity of cultured BALB/c CD4$^+$ T cells to NAD-induced apoptosis. Naïve CD4$^+$ T cells were cultured with concanavalin A (a, c) in the presence of IL-2 and RA (or Ro41-5253) for 5 days. (d) Sensitivity of BALB/c SI CD4$^+$ T cells to NAD-induced apoptosis *ex vivo*. Representative and combined data (n=3 for a, c, d. n=5 for b) are shown. Error bars indicate SEM.



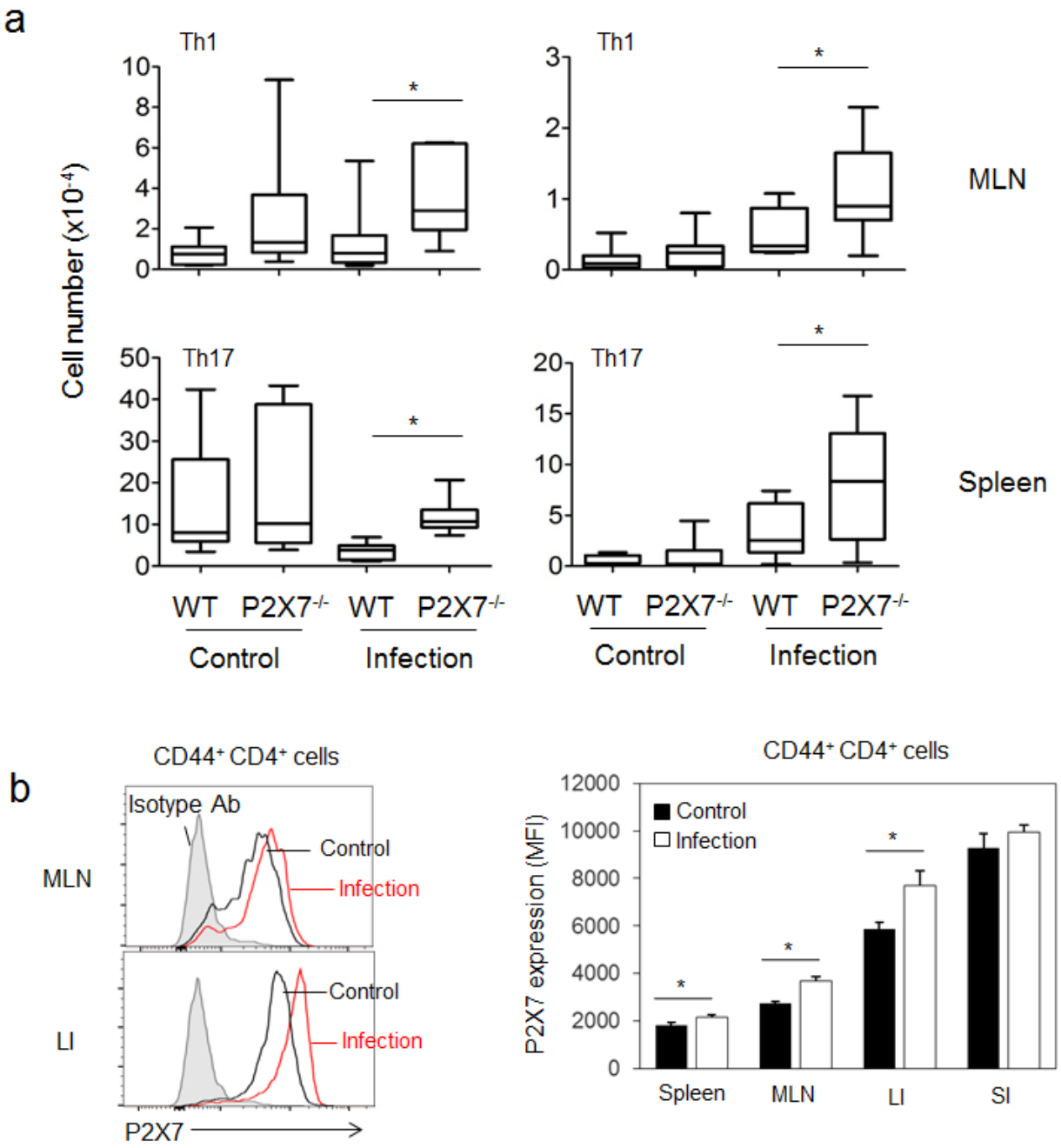
Supplementary Figure 2. Comparison of P2X7 expression between FoxP3$^+$ and FoxP3$^-$ CD4$^+$ memory/effector T cells. (a) P2X7 expression by memory versus naïve FoxP3$^+$ CD4$^+$ T cells in the spleen, MLN, and intestinal LP. (b) P2X7 expression by cultured FoxP3$^+$ versus FoxP3$^-$ CD4$^+$ T cells. Naïve CD4$^+$ T cells were cultured with TGFβ1 and and concanavalin A in the presence or absence of retinoids for 5 days. Representative and combined data (n=5) are shown.



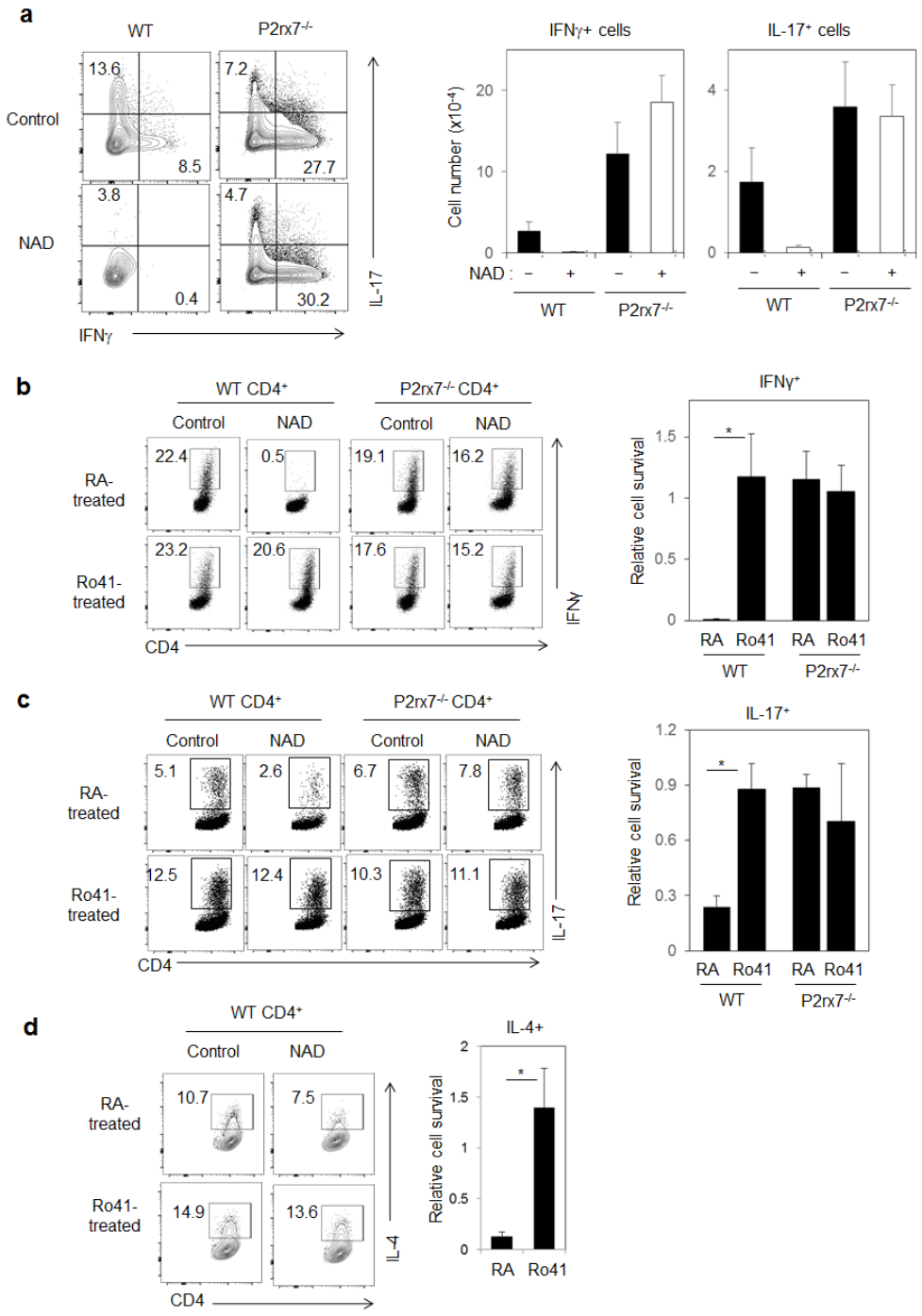
Supplementary Figure 3. Comparison of *P2rx7* and *Art2b* expression between naïve and memory CD4$^+$ T cells. Publicly available microarray data (GSE15907) was analyzed using the Multiplot module of GenePattern. Mouse naïve T cells were defined as CD3$^+$ CD19$^-$ CD4$^+$ CD62L^{high}, memory T cells were difined as βTCR$^+$ CD4$^+$ CD44^{high} CD122^{high}. (a) Absolute expression levels of naïve versus memory CD4$^+$ T cells in the spleen. (b) Fold differences between memory and naïve CD4$^+$ T cells in the spleen and lymph nodes.



Supplementary Figure 4. Expression of P2X7 by Th1, Th17 and Th2 cells that were treated with RA or Ro41-5253 *in vitro*. (a) Naïve $CD4^+$ T cells were cultured in a Th1 condition (IL-2, IL-12, anti-IL4) with indicated retinoids. (b) Naïve $CD4^+$ T cells were cultured in a Th17 condition (TGFβ1, IL-6, IL-23, IL-1β, TNFα, anti-IFNγ, and anti-IL4). (c) Naïve $CD4^+$ T cells were cultured in a Th2 condition (IL-4, IL-2, and anti-IFNγ). Concanavalin A was used to activate T cells. Shown are the frequencies of cytokine-expressing and non-expressing $CD4^+$ cells. Representative and combined data (n=4) are shown.



Supplementary Figure 5. Numbers of Th1 and Th17 cells in the MLN and spleen of WT and *P2rx7*$^{-/-}$ mice during *C. rodentium* infection. (a) Frequencies and absolute numbers of WT and *P2rx7*$^{-/-}$ Th1 and Th17 cells following infection by *C. rodentium*. (b) P2X7 expression by memory/effector CD4^{+} T cells before and after infection. The mice were infected i.g. with *C. rodentium* and sacrificed 14 days later. Representative and combined data (n=14 for a, n=8 for b) are shown. The boxes and bars in panel a represent the 25-75th and 0-100th percentile ranges.



Supplementary Figure 6. Sensitivity of WT and *P2rx7*$^{-/-}$ Th1 and Th17 cells to NAD-induced apoptosis. (a) Sensitivity of SI Th1 and Th17 cells to NAD-induced apoptosis *ex vivo*. Sensitivity of in vitro generated Th1 (b), Th17(c), and Th2 (d) cells to NAD-induced cell death. For panel B through D, naïve CD4^{+} T cells were cultured with RA or Ro41-5253 for 4-5 days in respective T helper-cell polarization conditions. The cells were incubated with NAD for 2 h. Relative cell survival rates are the ratios of NAD-untreated and treated live Th1 or Th17 cells. Representative and combined data (n=3 or 4) are shown.