Figure S1

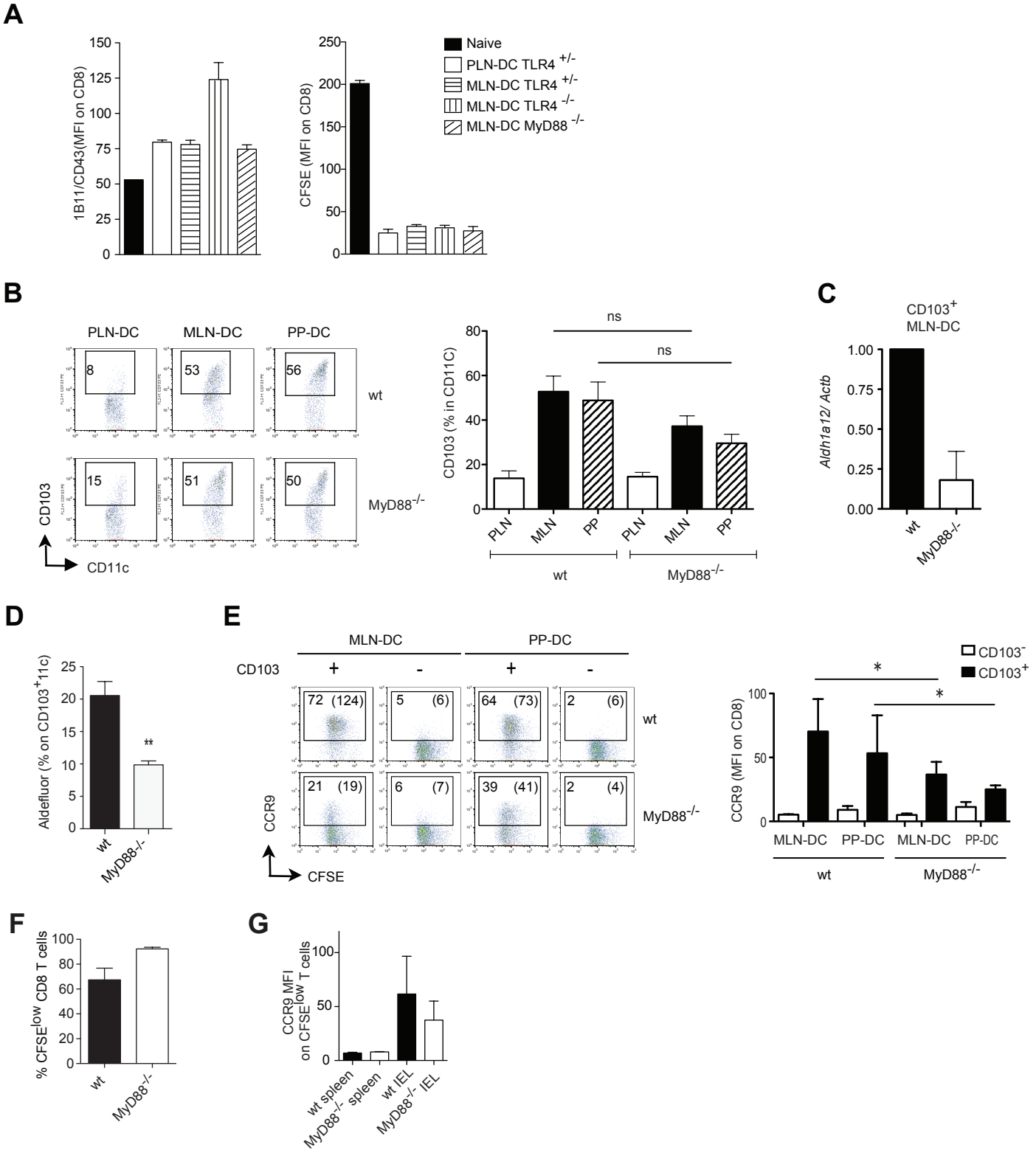

Figure S1, related to Figure 1. (**A**) Expression of 1B11/CD43 (marker of effector CD8 T cells) and CFSE dilution on CD8 T cells activated with TLR4$^{+/-}$, TLR4$^{-/-}$ or MyD88$^{-/-}$ DC (triplicate). Naïve T cells are shown for comparison. (**B**) DC were isolated from wild type or MyD88$^{-/-}$ mice and analyzed for the percentages of CD103^{+} cells among CD11c^{+} DC (n= 7). (**C**) *Aldh1a2* mRNA (encoding Raldh2) was determined by TaqMan in sorted CD11c^{+}CD103^{+} MLN-DC from wild type or MyD88$^{-/-}$ mice (n=2). (**D**) Raldh activity (aldefluor staining) in CD11c^{+}CD103^{+} MLN-DC from untreated (non-Flt3L DC expanded) wild type or MyD88$^{-/-}$ mice (n=8-10). (**E**) Naïve CD8 T cells were activated with sorted CD11c^{+}CD103^{+} or CD11c^{+}CD103^{-} MLN-DC from wild type or MyD88$^{-/-}$ mice and then analyzed for their expression of CCR9 (n=4). (**F, G**) Wild type or MyD88$^{-/-}$ mice (Thy1.2^{+}) were adoptively transferred with wild type CFSE-labeled OT-I CD8 T cells (Thy1.1^{+}) and then immunized i.p. with ovalbumin plus Alum. Four days later the thansfered Thy1.1^{+} CD8 T cells were analyzed for their proliferation (CFSE dilution) in MLN (**F**) and their CCR9 expression in the small bowel intraepithelial lymphocyte compartment (IEL) and the spleen (**G**) (n=3). Graphs show mean ± SEM. *p<0.05, **p<0.01

Figure S2

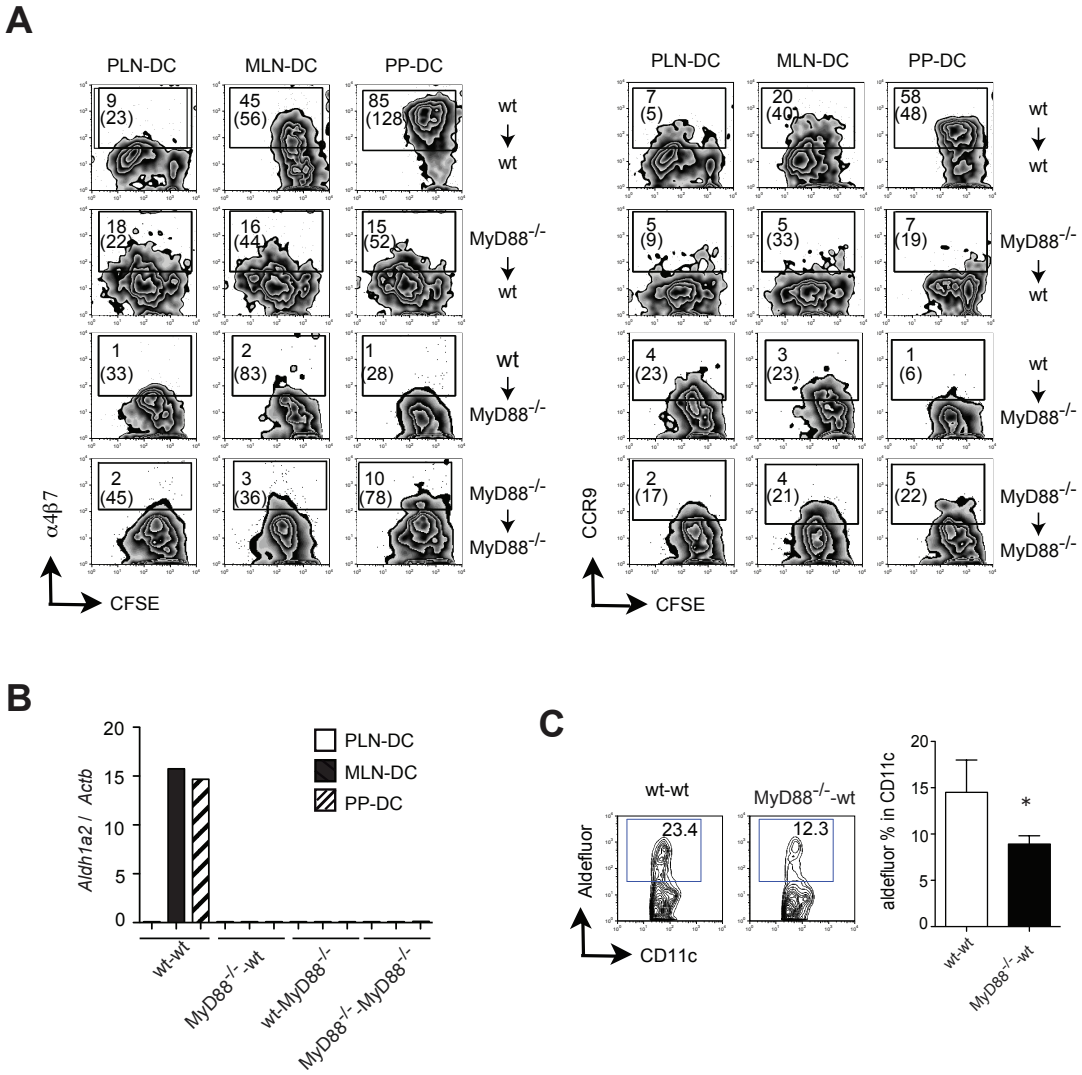

Figure S2, related to Figure 2. **(A)** Flow cytometry plots show α4β7 and CCR9 staining on CD8 T cells activated by DC from bone marrow (BM) chimeras in which BM from either wild type or MyD88$^{-/-}$ donors was transplanted into irradiated wild type or MyD88$^{-/-}$ recipient mice. (**B)** *Aldh1a2* mRNA expression in DC from BM chimeras. Graph representative of two experiments with similar results.(**C**) MLN-DC from BM chimeras were analyzed for their Raldh activity (aldefluor assay) (n=5). Graphs show mean ± SEM. *p<0.05

Figure S3

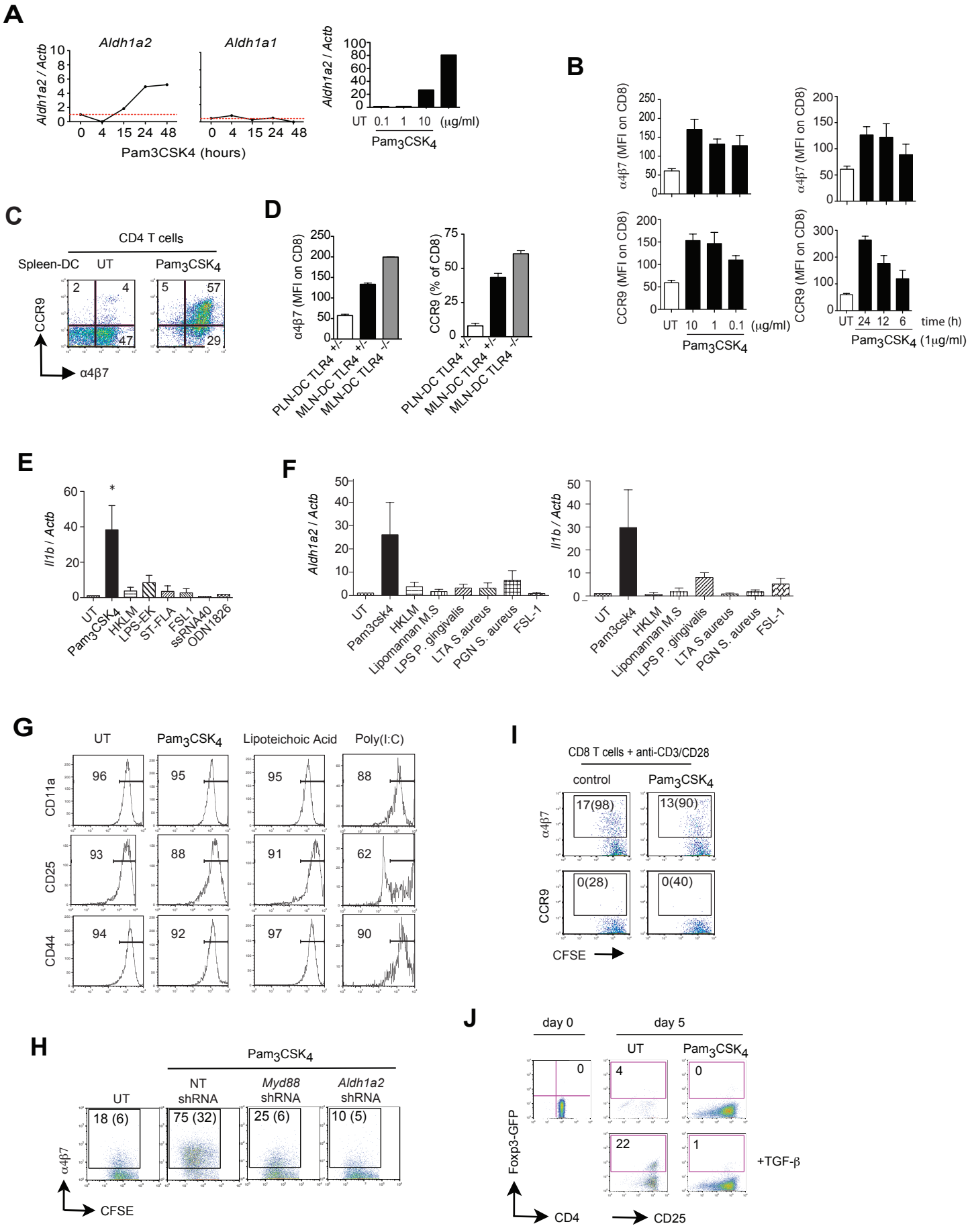
A
Aldh1a2
Aldh1a1
Aldh1a2 / Actb
Pam3CSK4 (hours)
UT 0.1 1 10 (μg/ml)
Pam3CSK4
B
α4β7 (MFI on CD8)
CCR9 (MFI on CD8)
UT 10 1 0.1 (μg/ml)
Pam3CSK4
UT 24 12 6 time (h)
Pam3CSK4 (1μg/ml)
C
CD4 T cells
Spleen-DC UT Pam3CSK4
CCR9
α4β7
D
α4β7 (MFI on CD8)
CCR9 (% of CD8)
PLN-DC TLR4 +/-
MLN-DC TLR4 +/-
MLN-DC TLR4 -/-
E
Il1b / Actb
UT
Pam3CSK4
HKLM
LPS-EK
ST-FLA
FSL1
ssRNA40
ODN1826
F
Aldh1a2 / Actb
Il1b / Actb
UT
Pam3csk4
HKLM
Lipomannan M.S
LPS P. gingivalis
LTA S.aureus
PGN S. aureus
FSL-1
G
UT
Pam3CSK4
Lipoteichoic Acid
Poly(I:C)
CD11a
CD25
CD44
96
95
95
88
93
88
91
62
94
92
97
90
H
Pam3CSK4
UT
NT shRNA
Myd88 shRNA
Aldh1a2 shRNA
18 (6)
75 (32)
25 (6)
10 (5)
α4β7
CFSE
I
CD8 T cells + anti-CD3/CD28
control
Pam3CSK4
17(98)
13(90)
0(28)
0(40)
α4β7
CCR9
CFSE
J
day 0
day 5
UT
Pam3CSK4
0
4
0
22
1
Foxp3-GFP
CD4
CD25
+TGF-β

Figure S3 (Legend)

Figure S3, related to Fig. 3. (A) Kinetics for *Aldh1a2* and *Aldh1a1* mRNA expression (TaqMan) and dose-response curve for *Aldh1a2* mRNA induction in Pam_3CSK_4-treated spleen-DC. Representative of two experiments with similar results. **(B)** Spleen-DC were treated for 24 h with the indicated concentrations of Pam_3CSK_4 (left panels) or with 1 μg/ml Pam_3CSK_4 for the indicated times (right panels) and then used to activate naive CD8 T cells. Four days later the activated T cells were analyzed for their expression of α4β7 and CCR9. **(C)** Flow cytometry plots showing α4β7 and CCR9 expression on OT2 CD4 T cells activated with Spleen-DC pre-treated or not with Pam_3CSK_4. **(D)** Expression of α4β7 and CCR9 on CD8 T cells activated with DC from $TLR4^{-/-}$ or $TLR4^{+/-}$ mice (as controls). Results show one experiment in triplicate. **(E)** *Il1b* mRNA (encoding IL-1β) expression in Spleen-DC untreated (UT) or pre-incubated for 24 h in the presence of the indicated TLR agonists (n=2-6). **(F)** Spleen-DC were untreated (UT) or pre-incubated for 24 h in the presence of the indicated TLR1/2 or TLR2/6 agonists and then analyzed for their expression of *Aldh1a2* and *Il1b* mRNA (n=2-6). **(G)** Spleen-DC were untreated (UT) or pre-incubated for 24 h with the indicated TLR agonists and then used to activate naïve CD8 T cells. Four days later the activated T cells were analyzed for their expression of CD11a/LFA-1, CD25 and CD44. Numbers in FACS plots indicate % of positive cells. **(H)** Bone marrow-derived DC were transduced with lentiviruses codifying for short hairpin RNA (shRNA) targeting *Myd88*, *Aldh1a2* or a non-targeting (NT) sequence and then were pretreated with Pam_3CSK_4 for 24h. After that, DC were used to activate CFSE-labeled naïve CD8 T cells. 4 days later CD8 T cells were analyzed for α4β7 expression. Numbers indicate percentage of positive cells and in parenthesis MFI of total cells. **(I)** Flow cytometry plots show α4β7 and CCR9 expression on CD8 T cells activated with plate-bound anti-CD3 plus anti-CD28 antibodies (without DC) and supplemented with Pam_3CSK_4 . **(J)** Flow cytometry plots showing Foxp3/GFP or Foxp3 staining in OT-II/Foxp3-GFP or OT-II/$RAG2^{-/-}$ splenocytes, respectively, before or after 5 days of culture with peptide-pulsed Spleen-DC pre-treated or not with 1 μg/ml Pam_3CSK_4 (right). Graphs show mean ± SEM. *$p<0.05$.

Figure S4

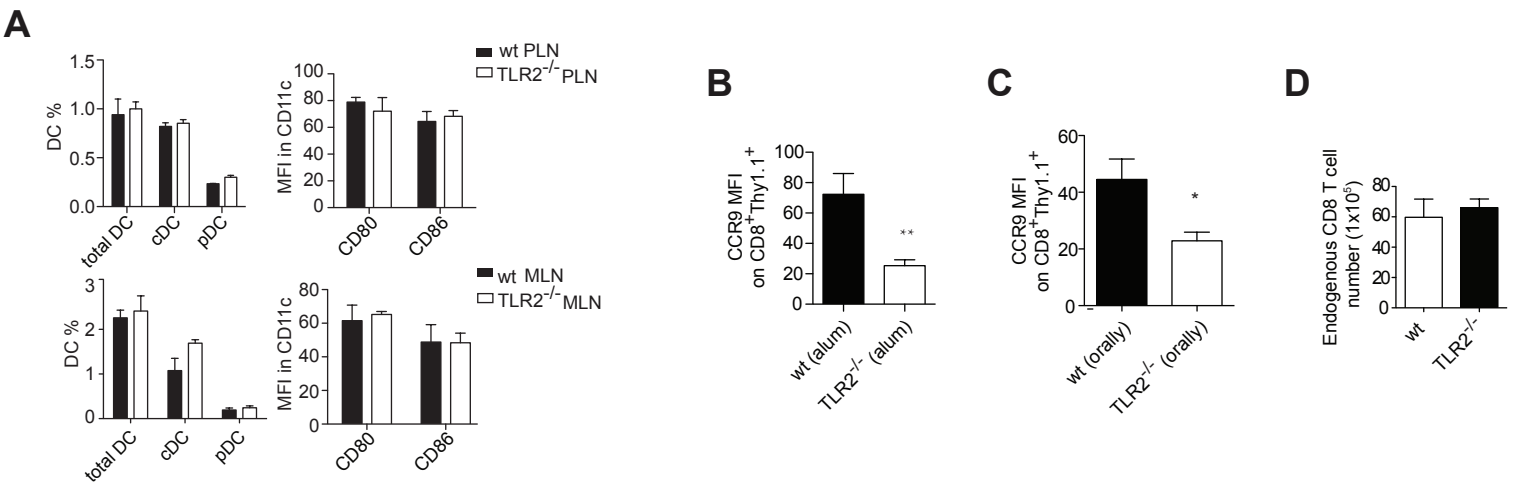

Figure S4, related to Fig. 4. (**A**) Comparison between TLR2$^{-/-}$ and wild type DC from PLN and MLN. (**B, C**) Wild type or TLR2$^{-/-}$ mice (Thy1.2^{+}) were adoptively transferred with wild type CFSE-labeled OT-I CD8 T cells (Thy1.1^{+}) and then immunized i.p. with OVA plus Alum (**B**) or with OVA orally (**C**). Four days later the mice were analyzed for the expression of CCR9 on activated Thy1.1^{+} CD8 T cells in MLN (n=4). (**D**). Endogenous CD8 T cell number in the small bowel LP of wild type or TLR2$^{-/-}$ mice (n=2). Graphs show mean ± SEM. *p<0.05, ***p<0.001

Figure S5

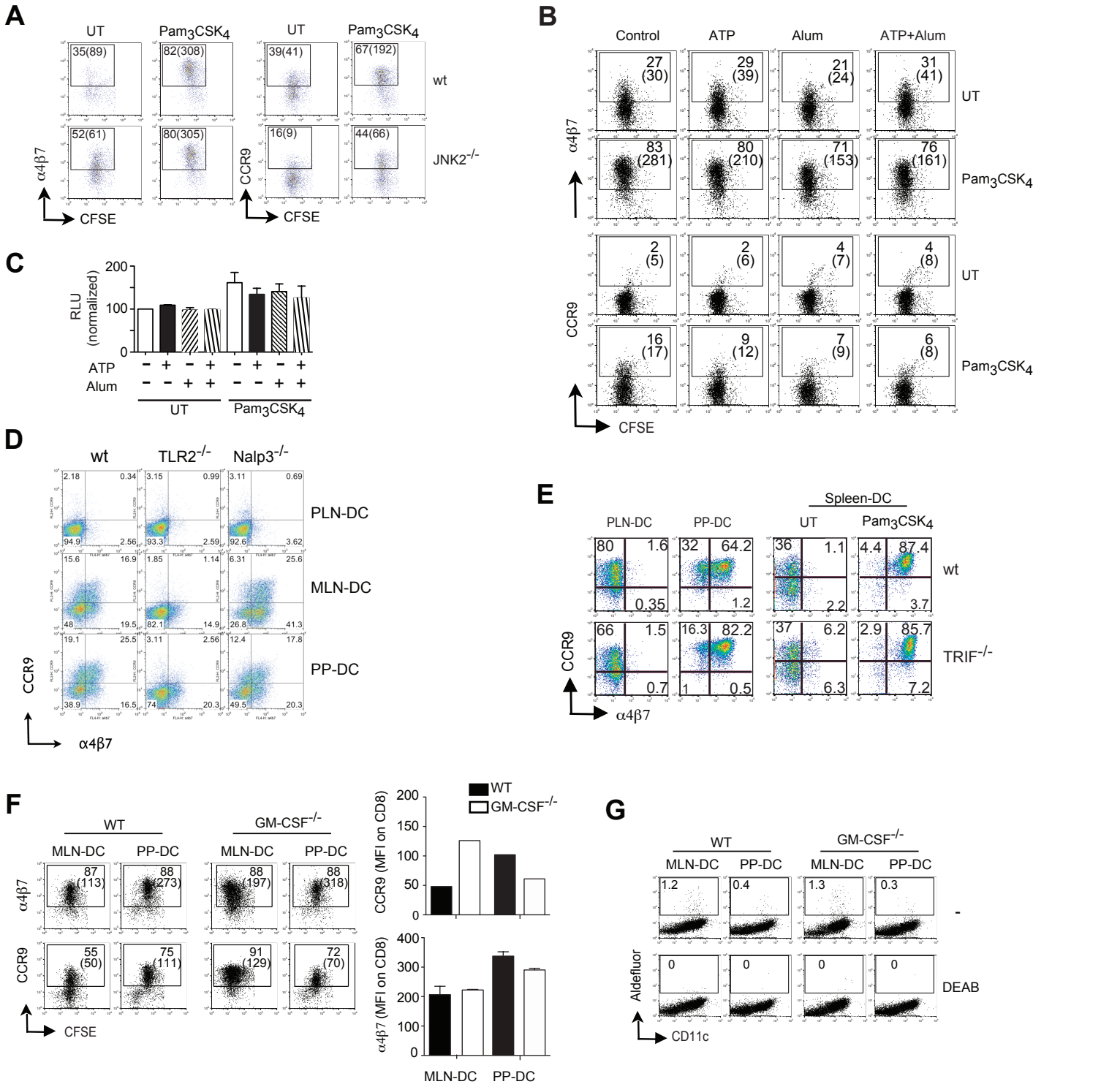

Figure S5, related to Figure 5. (**A**) Flow cytometry plots show α4β7 and CCR9 expression on CD8 T cells activated with Spleen-DC from wild type or JNK2$^{-/-}$ mice untreated (UT) or pre-treated with Pam_3CSK_4. (**B**) α4β7 and CCR9 expression on CD8 T cells activated with Spleen-DC untreated (UT) or pre-treated with Pam_3CSK_4 and either in the presence or the absence of ATP and/or Alum. (**C**) Relative Luciferase Units (RLU) in Spleen-DC from DR5-luciferase mice UT or pre-treated Pam_3CSK_4 and either in the presence or the absence of ATP and/or Alum (n=2). (**D**) Naïve CD8 T cells were activated with DC from wild type, TLR2$^{-/-}$ or Nalp3$^{-/-}$ mice and then analyzed for their expression of α4β7 and CCR9. Representative of two experiments with similar results. (**E**) Naïve CD8 T cells were activated with DC from wild type or TRIF$^{-/-}$ mice and then analyzed for their expression of α4β7 and CCR9. Spleen-DC were UT o pre-treated with Pam_3CSK_4. Representative of two experiments with similar results. (**F**) α4β7 and CCR9 expression on CD8 T cells activated with MLN-DC or PP-DC from wild type or GM-CSF$^{-/-}$ mice (n=3). Results were normalized respect to wild type MLN-DC. (**G**) Raldh activity in DC from wild type or GM-CSF$^{-/-}$ mice. Representative of three experiments with similar results. Graphs show mean ± SEM.