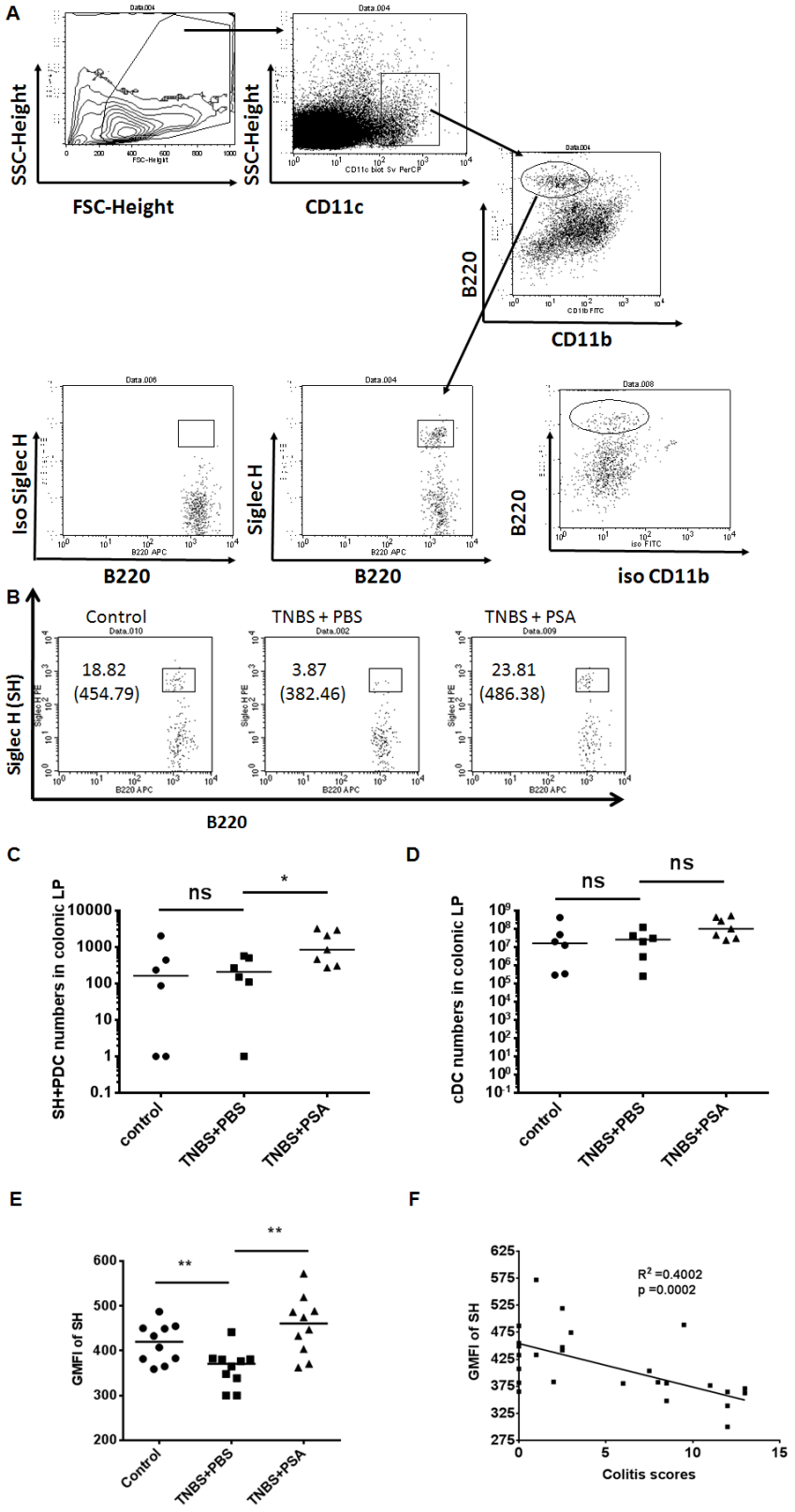
A
SSC-Height
FSC-Height
SSC-Height
CD11c
B220
CD11b
Iso Siglec H
B220
Siglec H
B220
B220
iso CD11b
B
Control
TNBS + PBS
TNBS + PSA
18.82
(454.79)
3.87
(382.46)
23.81
(486.38)
Siglec H (SH)
B220
C
SH+PDC numbers in colonic LP
ns
*
control
TNBS+PBS
TNBS+PSA
D
cDC numbers in colonic LP
ns
ns
control
TNBS+PBS
TNBS+PSA
E
GMFI of SH
**
**
Control
TNBS+PBS
TNBS+PSA
F
GMFI of SH
R² =0.4002
p =0.0002
Colitis scores

Figure S1, related to Figure 2. Tracking of SH+ PDCs. **(A)** MLN cells were selectively gated for SH+CD11b−B220+CD11c+ events. Isotype control IgG antibodies to CD11b (*right middle panel*) and SH (*right lower panel*) demonstrate the nonmyeloid SH-expressing population of PDCs. Around 5000 events were acquired in the CD11c+ gate for analysis by flow cytometry. **(B)** Representative dot plots for PDCs (boxes in the dot plots) on day 3 after intrarectal TNBS challenge of different treatment groups: controls (*left panel*, mice not receiving TNBS), TNBS+PBS (*middle panel*), and TNBS+PSA (*right panel*). The numbers outside parentheses indicate percentages of PDCs (events in the boxes) with respect to the total number of CD11b−B220+CD11c+ events (total events in the plot). Numbers in parentheses indicate geometric mean fluorescence intensities of SH. (**C** and **D**) Effect of PSA pretreatment on colonic DC subsets in the TNBS-induced model of colitis. Numbers of colonic lamina propria (LP) PDCs but not CDCs (**C** and **D**, respectively) are mildly but significantly altered in groups of mice treated with PSA and then challenged intrarectally with TNBS from the corresponding PDC and CDC numbers in groups of mice treated with PBS and TNBS. Horizontal bars in the scatter plots represent median values. Unpaired Student's *t*-test: **p*<0.05; ns, not significant. (**E** and **F**) Geometric mean fluorescence intensity (GMFI) of Siglec H in MLN PDCs in TNBS colitis. MLNs of PSA treated mice had significantly greater augmentation of GMFI of SH in PDCs (CD11b−B220+CD11c+ population) when challenged with TNBS than did PBS-treated mice challenged with TNBS (**E**). GMFI of SH in MLN PDCs is significantly but inversely correlated with colitis scores. Horizontal bars in the scatter plots represent median values (**F**). Unpaired Student's *t*-test: ***p*<0.01.

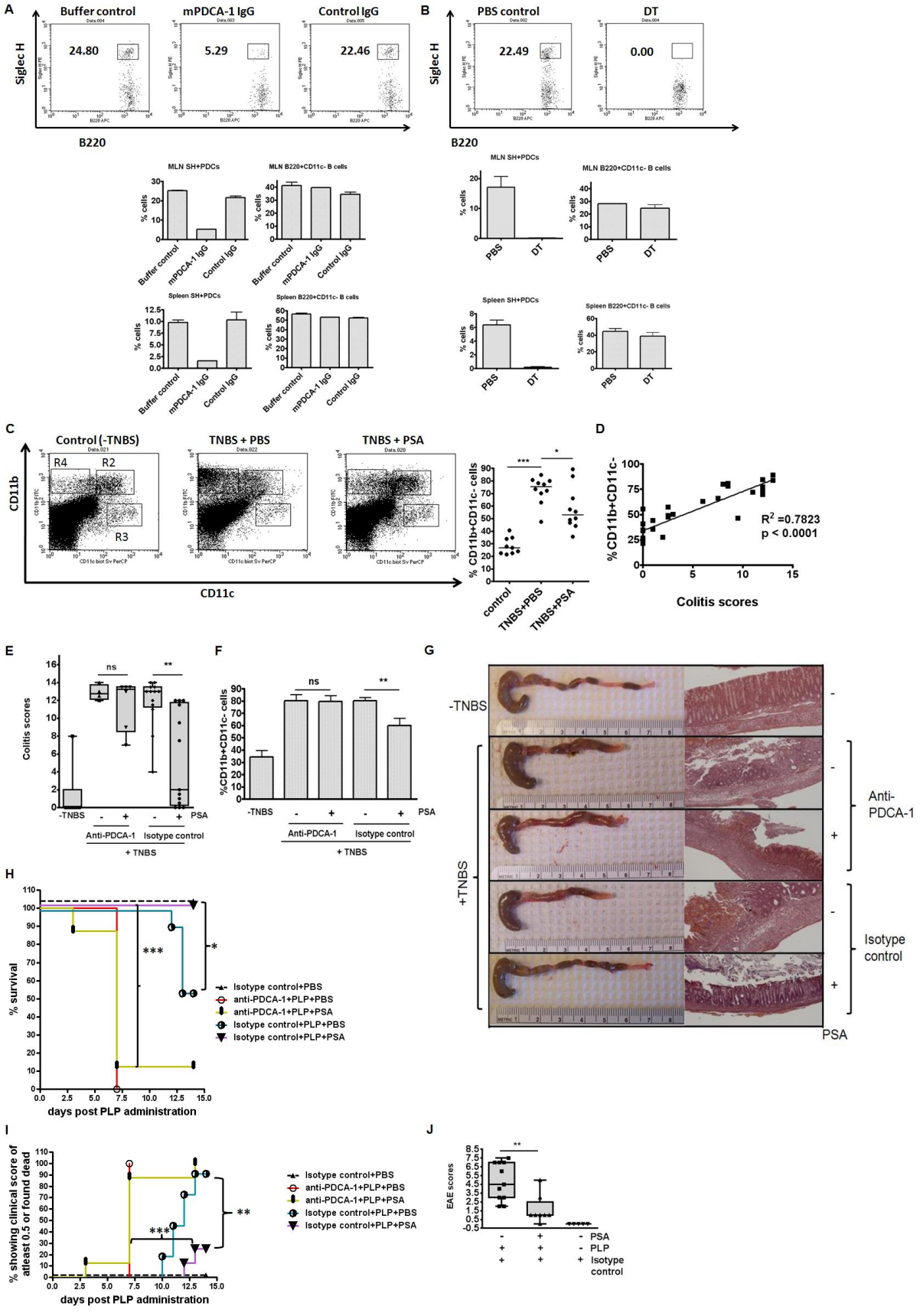
A
Buffer control
mPDCA-1 IgG
Control IgG
24.80
5.29
22.46
Siglec H
B220
MLN SH+PDCs
MLN B220+CD11c- B cells
Spleen SH+PDCs
Spleen B220+CD11c- B cells
% cells
Buffer control
mPDCA-1 IgG
Control IgG
B
PBS control
DT
22.49
0.00
PBS
DT
C
Control (-TNBS)
TNBS + PBS
TNBS + PSA
R4
R2
R3
CD11b
CD11c
% CD11b+CD11c- cells
control
TNBS+PBS
TNBS+PSA
D
% CD11b+CD11c-
Colitis scores
R2 =0.7823
p < 0.0001
E
Colitis scores
-TNBS
PSA
Anti-PDCA-1
Isotype control
+ TNBS
F
%CD11b+CD11c- cells
G
-TNBS
+TNBS
Anti-PDCA-1
Isotype control
PSA
H
% survival
days post PLP administration
Isotype control+PBS
anti-PDCA-1+PLP+PBS
anti-PDCA-1+PLP+PSA
Isotype control+PLP+PBS
Isotype control+PLP+PSA
I
% showing clinical score of atleast 0.5 or found dead
days post PLP administration
J
EAE scores
PSA
PLP
Isotype control

Figure S2, related to Figure 3. PDC depletion: strategies and consequences. **(A)** PDC depletion after two intraperitoneal doses of buffer control, 250 μg of isotype control antibody, or 250 μg of mAb to PDCA-1 (mPDCA-1 IgG). Flow cytometry plots show SH+CD11b−B220+CD11c+ cells in the boxes, with respective percentages indicated outside the boxes, from MLNs in the various treated groups. For this analysis, 5000 cells were acquired in the CD11c+ gate. Bar graphs on the left indicate depletion (~80%) of SH+ PDCs in MLNs and spleen with mPDCA-1 IgG vs. buffer control and isotype control IgG. The frequency of B220+CD11c− B cells remained unchanged in all treatment groups (*bar graphs, right*). **(B)** Similar analysis in BDCA2-DTR mice treated with PBS or DT (200 ng/dose, 2 doses on successive days; analysis on the third day). PDC depletion was almost complete with DT. (**C** and **D**) Enhanced frequency of CD11b+CD11c− cells in colonic lamina propria serves as a strong disease phenotype. Flow cytometry plots representing colonic lamina propria cells from differently treated (PSA or PBS) WT animals in the TNBS colitis model. R4, R2, and R3 represent CD11b+CD11c−, CD11b+CD11c+, and CD11b−CD11c+ cells, respectively **(C)**. The frequency of CD11b+CD11c− cells is calculated as [R4/(R4+R2+R3)] × 100 for each sample, and frequencies are grouped on the basis of treatment, as represented by the scatter plot on the right. Horizontal bars in the scatter plot represent median values. Data were analyzed by unpaired Student's *t*-test. **p*<0.05; ****p*<0.001. Frequency of CD11b+CD11c− colonic lamina propria cells in individual mice correlates significantly and directly with cumulative clinical scores **(D)**. **(E)** IgG antibodies (250 μg/dose) were administered on each of the 2 days before intrarectal administration of TNBS. Boxplots show median (*horizontal bar inside box*) and quartile distributions in the TNBS model. PSA is protective in the isotype control IgG–treated groups (*fourth and fifth boxes*) but not in the anti-PDCA-1-treated groups (*second and third boxes*); protection was assessed as cumulative clinical scores. The latter scores, calculated as the sum of percentage of weight lost, stool softness, apparent colon thickness, and histopathology, were assessed for statistical significance by two-tailed nonparametric Mann-Whitney test. ***p*<0.01; ns, not significant. **(F)** Frequency of CD11b+CD11c− cells in colonic lamina propria of mice in different treatment groups, as shown in **A**. CD11b+CD11c− frequency was calculated as a percentage of total CD11b+CD11c−, CD11b+CD11c+, and CD11b−CD11c+ gated cell populations. Error bars in the CD11b+CD11c− frequency plot represent SEM values for untreated controls (n = 6), TNBS +

PBS + anti-PDCA-1 recipients (n = 4), TNBS + PSA + anti-PDCA-1 recipients (n = 6), TNBS + PBS + isotype controls (n = 13), and TNBS + PSA (100 μg/dose) + isotype controls (n = 13). Data were analyzed by unpaired Student's t-test.**p<0.01; ns, not significant. **(G)** Gross colonic appearance (*left panels*) and histopathologic appearance (*right panels*, stained with hematoxylin and eosin, 10× magnification) demonstrate lack of colon thickening, formed stool pellets, and reduced epithelial hyperplasia and inflammation in the isotype control IgG + PSA + TNBS–treated group (*bottom panel*)—findings similar to those for the TNBS-untreated control group (*top panel*) and contrasting with all other TNBS-treated groups (*middle three panels*).The images shown are representative sections from animals in 2 independent experiments. (**H, I** and **J**) PSA treatment prevents SJL/J mice with PLP-induced EAE from developing disease when PDCs are not depleted; however, when PDCs are depleted, nearly all mice die early on. Of 16 mice treated with anti-PDCA-1 and induced with PLP, 15 were found dead on the day after the last IgG treatment (7 days after PLP administration). PSA treatment did not lower this early mortality rate (**H)**. (Red and green lines represent treatment with PLP+PBS and PLP+PSA, respectively.) In contrast, all isotype control IgG–treated mice survived this early period. (Black dashed, blue, and purple lines represent controls not treated with PLP, PLP+PBS-treated mice, and PLP+PSA-treated mice, respectively.) However, later mortality rates (with mice either found dead or sacrificed due to severe illness) were significantly higher among PLP-induced mice receiving PBS + isotype control IgG than among their counterparts given PSA + isotype control IgG. The PLP-induced PBS + isotype control IgG group (*blue line*) developed observable clinical symptoms earlier than did their counterparts given PSA + isotype control IgG (*purple line*) **(I)**. For the PBS + isotype control IgG group not receiving PLP, n = 5. All of the other groups received PLP for induction of EAE; n = 8, 8, 11, and 8 for the PBS + anti-PDCA-1 mAb group, the PSA + anti-PDCA-1 mAb group, the PBS + isotype control IgG group, and the PSA + isotype control IgG group, respectively. Data shown in **H** and **I** were analyzed by log-rank test. *, **, and *** denote p<0.05, p<0.01, and p<0.001, respectively. Boxplots show median (*horizontal bar inside box*) and quartile distributions in EAE cumulative clinical scores at the time of death or sacrifice **(J)**. PBS + isotype control IgG mice had significantly more severe disease than did PSA + isotype control IgG mice. Cumulative clinical scores were assessed for statistical significance by two-tailed nonparametric Mann-Whitney test. **p<0.01.

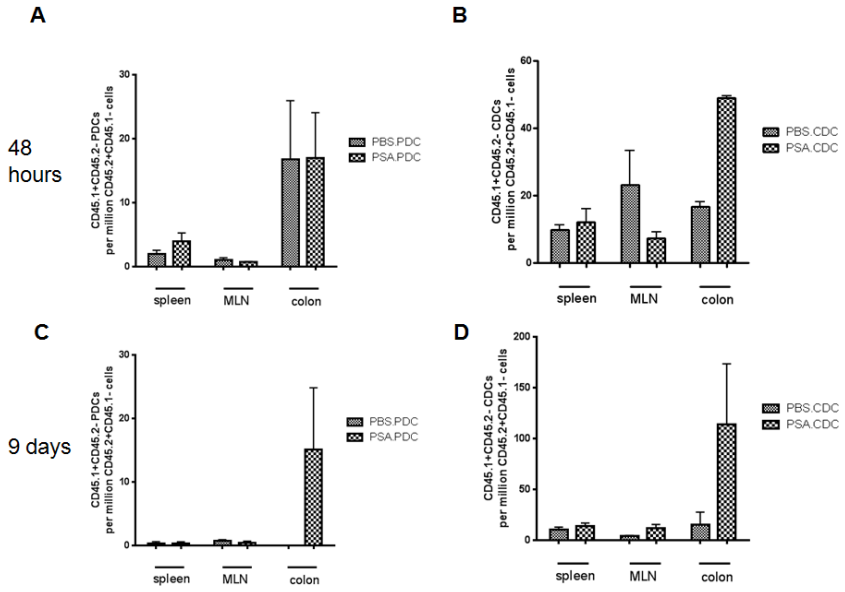

Figure S3, related to Figure 4. Distribution of CD45.1+CD45.2– donor DCs after adoptive transfer to CD45.2+ B6 mice. After *in vitro* treatment with PBS or PSA (300 μg/ml), unsorted CD45.1+ (SJL/J.B6) murine BMDCs containing ~0.2 × 10^5 PDCs and ~9 × 10^5 CDCs were injected intraperitoneally into CD45.2+ B6 mice. Recipient mice were sacrificed 48 hours (**A**, **B**) or 9 days (**C, D**) after cell transfer, and donor cells were analyzed for PDCs (SH+ or PDCA-1+ and CD11c+; **A**, **C**) and CDCs (SH– or PDCA-1– and CD11c+; **B**, **D**) by flow cytometry. Donor PDCs and CDCs were observed in spleen, MLNs, and colon of recipient mice 48 hours and even 9 days after transfer. For each treatment group, *n* = 3 or 4. Error bars indicate SEM values.

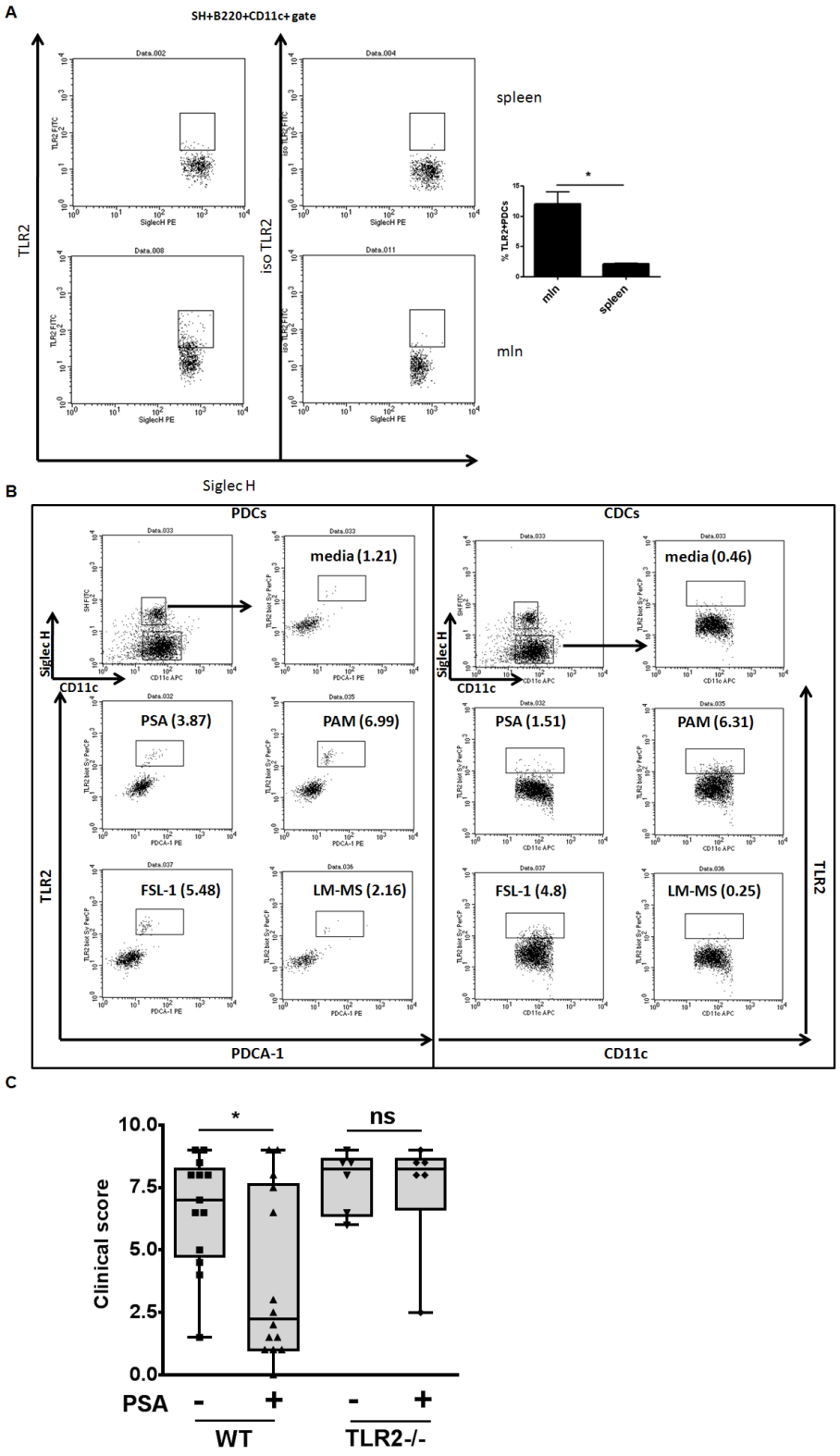

Figure S4, related to Figure 6. Association of TLR2 with PDCs. (**A**) Higher-level TLR2 expression in PDCs from MLNs than in splenic PDCs in the steady state. *Left:* Representative

dot plots show TLR2+SH+ cells in the SH+B220+CD11c+ gate in MLNs (n = 5) and spleens (n = 3) from WT C57BL/6 mice. *Right:* Average values for each tissue after subtraction of the respective values for the isotype control. Data were analyzed by unpaired Student's *t*-test. *p<0.05. Error bars indicate SEM values. (**B**)TLR2 expression in bone marrow–derived PDCs and CDCs after incubation with various TLR2 ligands [PSA, 60 μg/ml; Pam3CSK4 (PAM), 0.1 μg/ml; FSL-1, 0.1 μg/ml**;** and lipomannan (LM-MS), 10 ng/ml]. The different ligands have slightly different effects on TLR2 expression on PDCs (*left panel*; SH+CD11c+PDCA-1+ gating) and CDCs (*right panel*; SH−CD11c+ gating). Numbers in parentheses indicate frequency of TLR2+ cells. Dot plots represent 2 independent experiments. Around 5000 events in the CD11c+ gate were used for analysis. Average values for TLR2 expression on PDCs and CDCs under all conditions are shown in Figs. 6B and 6C. (**C**) Lack of protection by PSA in TLR2-knockout mice. The diminished cumulative clinical scores (not including histologic scores) documented in WT mice after PSA treatment (*second bar*) are not observed in TLR2−/− mice. For the TNBS + PBS WT, TNBS + PSA WT, TNBS + PBS TLR2−/−, and TNBS + PSA TLR2−/− groups, n = 13, 14, 6, and 6, respectively. Clinical scores were assessed for statistical significance by two-tailed nonparametric Mann-Whitney test. *p<0.05; ns, not significant.

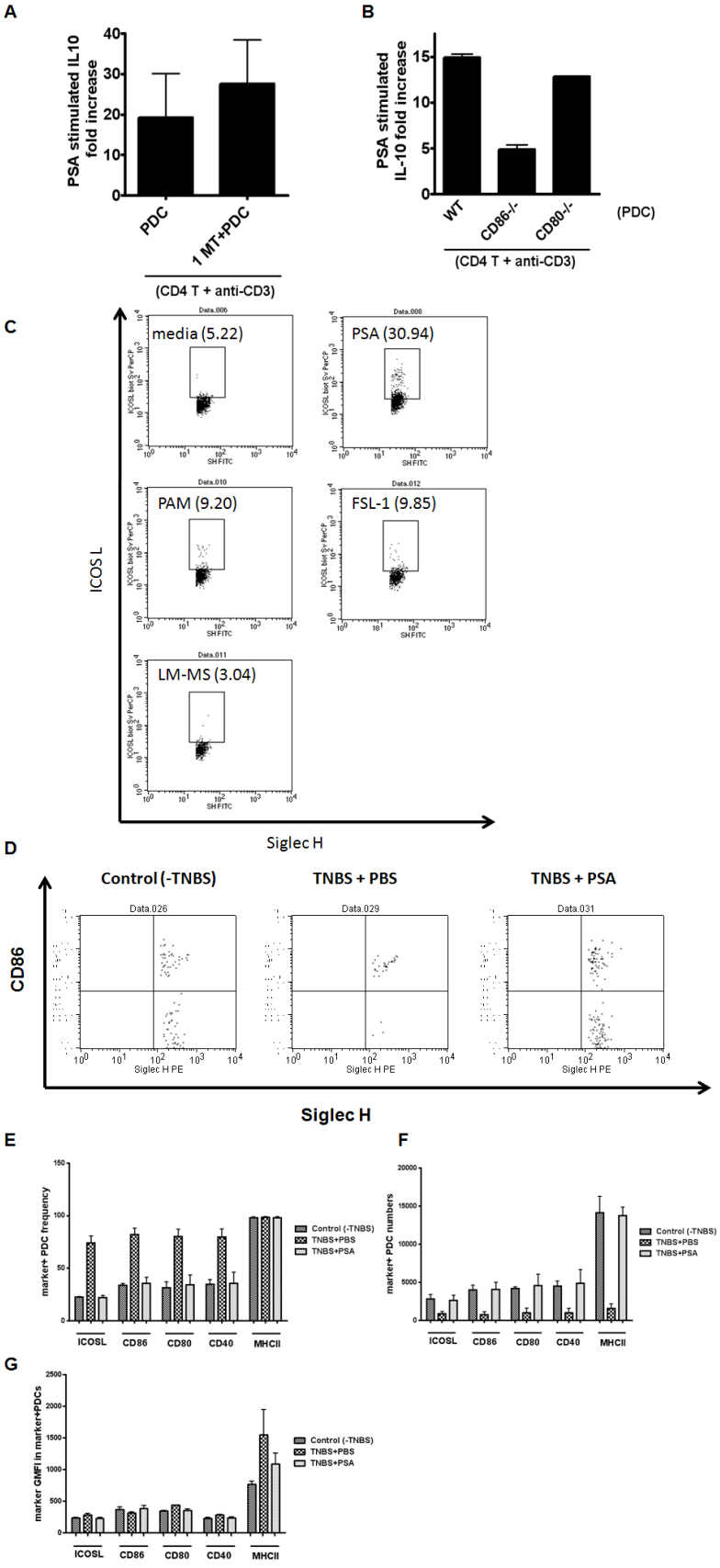
A
PSA stimulated IL10 fold increase
40
30
20
10
0
PDC
1 MT+PDC
(CD4 T + anti-CD3)
B
PSA stimulated IL-10 fold increase
15
10
5
0
WT
CD86-/-
CD80-/-
(PDC)
(CD4 T + anti-CD3)
C
media (5.22)
PSA (30.94)
PAM (9.20)
FSL-1 (9.85)
LM-MS (3.04)
ICOS L
Siglec H
D
Control (-TNBS)
TNBS + PBS
TNBS + PSA
CD86
Siglec H
E
marker+ PDC frequency
Control (-TNBS)
TNBS+PBS
TNBS+PSA
ICOSL
CD86
CD80
CD40
MHCII
F
marker+ PDC numbers
G
marker GMFI in marker+PDCs

Figure S5, related to Figure 7. Co-stimulatory molecules in PSA function. (**A** and **B**) IDO and CD80 do not play a role in PSA-stimulated IL-10 production *in vitro*. Fold-increase in IL-10 secretion (as measured by ELISA) in PSA-treated wells relative to that in corresponding PSA-untreated conditions is shown. WT CD4+ T cells were cultured either with PDCs from WT mice in the presence or absence of 1-methyl-D-tryptophan (1 MT; 250 µM) (**A**) or with PDCs from WT, CD86–/–, or CD80–/– mice (**B**). (**C**) Expression of ICOSL by PDCs *in vitro*. Representative dot plots show bone marrow–derived PDCs (gated on SH+PDCA-1+CD11c+) stimulated with medium alone or various TLR2 ligands [PSA, 60 µg/ml; Pam3CSK4 (PAM), 0.1 µg/ml; FSL-1, 0.1 µg/ml**;** and lipomannan (LM-MS), 10 ng/ml]. Boxes in the plot indicate ICOSL+PDCs, whose frequency is indicated by the numbers in parentheses. (**D**, **E, F** and **G**) Antigen presentation and co-stimulatory molecules in MLNs of mice with TNBS-induced colitis. MLN cells obtained from mice 3 days after intrarectal administration of TNBS or control buffer in the TNBS model of colitis were labeled for several cell surface–associated antigen presentation and co-stimulatory markers (MHCII and ICOSL, CD86, CD80, CD40) and were analyzed by flow cytometry (**D**). Dot plots show representative marker CD86+ cells (*upper right quadrant*) in the SH+B220+CD11c+ (PDC) gating for different treatment groups. Bar graph shows percentages of PDCs positive for different markers among total PDCs for each treatment group (**E**). Bar graph shows absolute numbers of PDCs positive for different markers for each treatment group (**F**). Bar graph shows geometric mean fluorescence intensities of individual marker in PDCs positive for different markers for each treatment group (**G**). For each treatment group, n = 3 or 4. Error bars indicate SEM values.

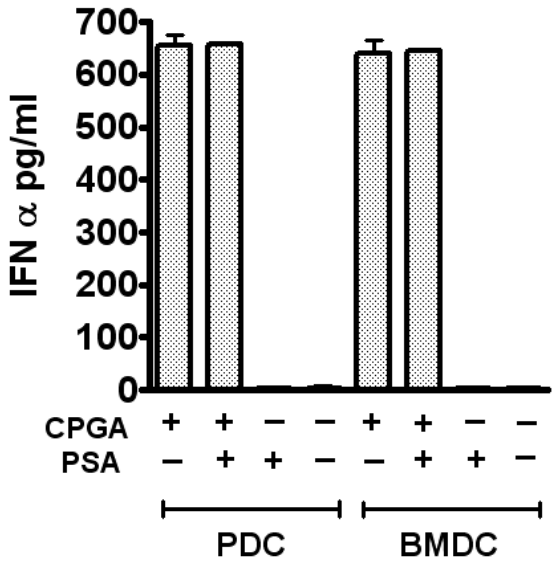

Figure S6, **related to Figure 5.** PSA treatment does not induce IFNα production by DCs. Isolated PDCs (5×10^4/well) or BMDCs (2×10^5/well) did not liberate IFNα when cultured in the presence of PSA for 44 hours. Similar results were obtained after 20 hours of culture. CpGA, a ligand for TLR9, induced high levels of IFNα, and this effect was not perturbed in the presence of PSA.