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Supplemental Information

Smad7 Controls Immunoregulatory PDL2/1-PD1 Signaling in Intestinal Inflammation and Autoimmunity

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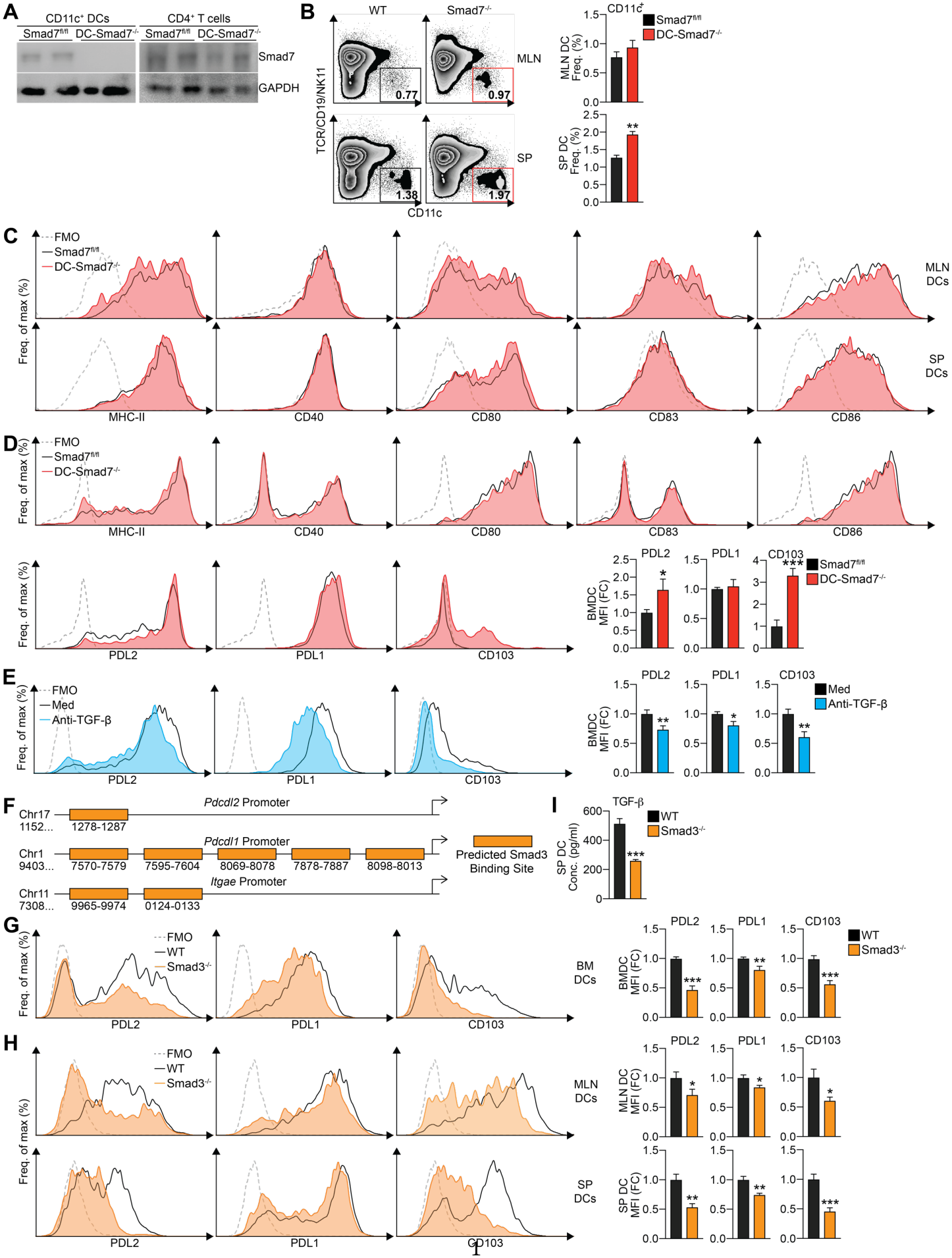
A
CD11c+ DCs
CD4+ T cells
Smad7fl/fl
DC-Smad7-/-
Smad7fl/fl
DC-Smad7-/-
Smad7
GAPDH
B
WT
Smad7-/-
MLN
SP
TCR/CD19/NK11
CD11c
0.77
0.97
1.38
1.97
CD11c+
Smad7fl/fl
DC-Smad7-/-
MLN DC Freq. (%)
SP DC Freq. (%)
**
C
FMO
Smad7fl/fl
DC-Smad7-/-
Freq. of max (%)
MHC-II
CD40
CD80
CD83
CD86
MLN DCs
SP DCs
D
FMO
Smad7fl/fl
DC-Smad7-/-
Freq. of max (%)
MHC-II
CD40
CD80
CD83
CD86
Freq. of max (%)
PDL2
PDL1
CD103
PDL2
PDL1
CD103
Smad7fl/fl
DC-Smad7-/-
BMDC MFI (FC)
*
***
E
FMO
Med
Anti-TGF-β
Freq. of max (%)
PDL2
PDL1
CD103
PDL2
PDL1
CD103
Med
Anti-TGF-β
BMDC MFI (FC)
**
*
**
F
Pdcdl2 Promoter
Chr17
1152...
1278-1287
Pdcdl1 Promoter
Chr1
9403...
7570-7579
7595-7604
8069-8078
7878-7887
8098-8013
Itgae Promoter
Chr11
7308...
9965-9974
0124-0133
Predicted Smad3 Binding Site
I
TGF-β
WT
Smad3-/-
SP DC Conc. (pg/ml)
***
G
FMO
WT
Smad3-/-
Freq. of max (%)
PDL2
PDL1
CD103
BM DCs
PDL2
PDL1
CD103
WT
Smad3-/-
BMDC MFI (FC)
***
**
***
H
FMO
WT
Smad3-/-
Freq. of max (%)
PDL2
PDL1
CD103
MLN DCs
SP DCs
PDL2
PDL1
CD103
MLN DC MFI (FC)
SP DC MFI (FC)
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Figure S1. Smad7$^{-/-}$ DCs present with a tolerogenic phenotype, in contrast to Smad3$^{-/-}$ DCs. Related to Figure 1. (**A**) Western blot of Smad7 in splenic CD11c^{+} DCs (**left**) and CD4^{+} T cells (**right**) isolated *ex vivo* from naïve Smad7$^{fl/fl}$ and DC-Smad7$^{-/-}$ mice. (**B**) Representative FACS plots (**left**) and frequencies (**right**) of CD11c^{+} DCs in the spleens and MLNs isolated *ex vivo* from naïve Smad7$^{fl/fl}$ and DC-Smad7$^{-/-}$ mice (n=6). (**C**) Representative FACS histograms of MHC-II, CD40, CD80, CD83, and CD86 in MLN (**top**) and splenic (**bottom**) DCs from these mice (n=11). (**D**) Representative FACS histograms of MHC-II, CD40, CD80, CD83, CD86, PDL2, PDL1, and CD103 (**top**), and FACS histograms and MFIs of PDL2, PDL1, and CD103 (**bottom**), in BMDCs differentiated from Smad7$^{fl/fl}$ and DC-Smad7$^{-/-}$ mice (n=6). (**E**) Representative FACS histograms (**left**) and MFIs (**right**) of PDL2, PDL1, and CD103 in BMDCs differentiated with or without TGF-β-neutralizing antibody (20 μg/mL) (n=12). (**F**) Schematic localization of putative Smad3 binding sites on *Pdcdl2* (PDL2), *Pdcdl1* (PDL1), and *Itgae* promoter regions from Alggen, a web-based algorithm for TFBS predictions. (**G**) Representative FACS histograms (**left**) and MFIs (**right**) of PDL2, PDL1, and CD103 in BMDCs differentiated from WT and Smad3$^{-/-}$ mice (n=11). (**H**) Representative FACS histograms (**left**) and MFIs (**right**) of PDL2, PDL1, and CD103 in MLN (**top**) and splenic (**bottom**) CD11c^{+} DCs from naïve Smad7$^{fl/fl}$ and DC-Smad7$^{-/-}$ mice (n=6). (**I**) ELISA quantification of secreted TGF-β by MLN DCs from these mice (n=4). MFI data reflective of CD11c^{+} population and expressed as fold change (FC) from (**D**) Smad7$^{fl/fl}$ condition, (**E**) media condition, or (**G-H**) WT condition. Data representative of ≥ 3 independent experiments. Mean ± SEM. *p<.05, **p<.01, ***p<.001, by unpaired T test.

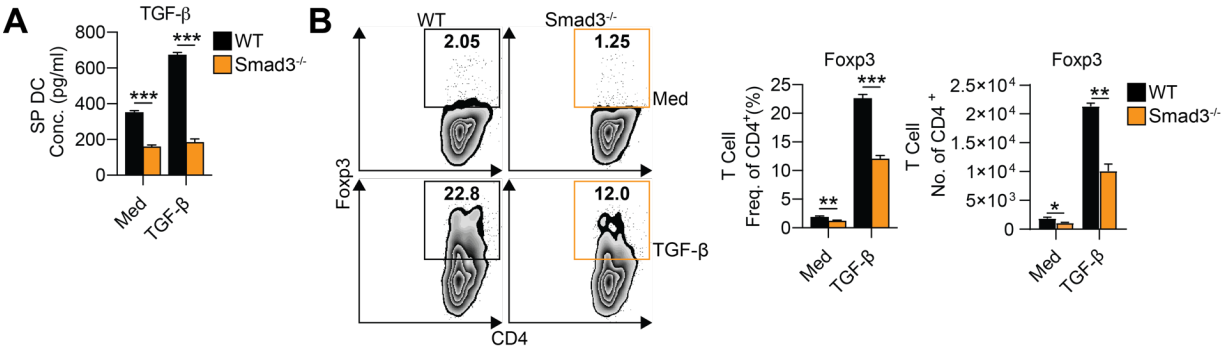

Figure S2. Smad3 promotes DC responsiveness to TGF-β and their ability to induce Tregs. Related to Figure 2. (**A**) ELISA quantification of secreted TGF-β from splenic WT and Smad3$^{-/-}$ CD11c^{+} DCs stimulated with or without TGF-β (0.5 ng/mL) for 12h, washed and cultured for another 48 h (n=6). (**B**) Representative FACS plots (**left**), frequencies (**middle**), and cell count per well (**right**) of Foxp3-GFP^{+} populations in naïve CD4^{+} Foxp3-GFP T cells co-cultured with splenic CD11c^{+} DCs from Smad7$^{fl/fl}$ or DC-Smad7$^{-/-}$ mice at a 1:3 DC:T cell ratio, with or without TGF-β (0.5 ng/mL), for 4 d (n=4). Data representative of ≥ 3 independent experiments. Mean ± SEM. *p<.05, **p<.01, ***p<.001, by unpaired T test.

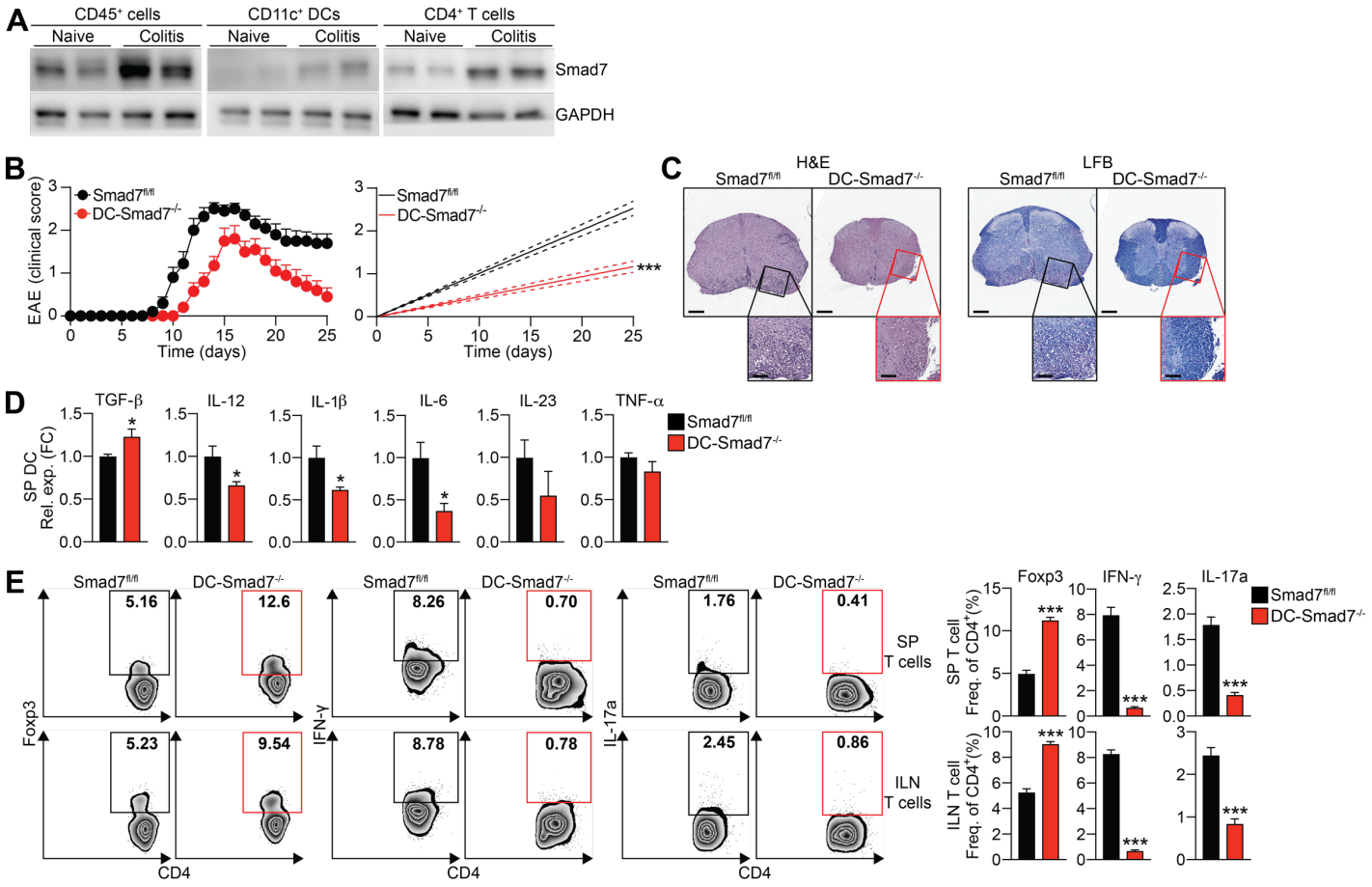

Figure S3. Smad7 is increased during colitis, and Smad7-deficiency in DCs mitigates EAE. Related to Figure 3. (**A**) Western blot of Smad7 in MLN CD45$^+$ cells (**left**), CD11c$^+$ DCs (**middle**), and CD4$^+$ T cells (**right**) isolated *ex vivo* from naïve and colitic WT mice after 7 days of 3% DSS in drinking water. (**B**) Clinical EAE scores (**left**) and linear regression analysis (**right**) of Smad7$^{fl/fl}$ and DC-Smad7$^{-/-}$ mice immunized with MOG/CFA (n=10). (**C**) Representative histopathological sections of spinal cords stained with H & E (**left**) showing inflammatory infiltrates and Luxol Fast Blue (LFB) (**right**) showing demyelination of these mice at peak disease. Scale bars represent ~250μm (**top**) and ~100μm (**bottom**). (**D**) qRT-PCR of TGF-β, IL-12, IL-1β, IL-6, IL-23, and TNF-α of splenic DCs in these mice at disease onset (n=3-5). Data expressed as fold change from Smad7$^{fl/fl}$ media condition. (**E**) Representative FACS plots (**left**) and frequencies (**right**) of Foxp3$^+$, IFN-γ$^+$, and IL-17a$^+$ populations in splenic (**top**) and draining inguinal lymph node (**bottom**) at disease onset (n=10). Data representative of ≥ 3 independent experiments. Mean ± SEM. *p<.05, ***p<.001 by unpaired T test.

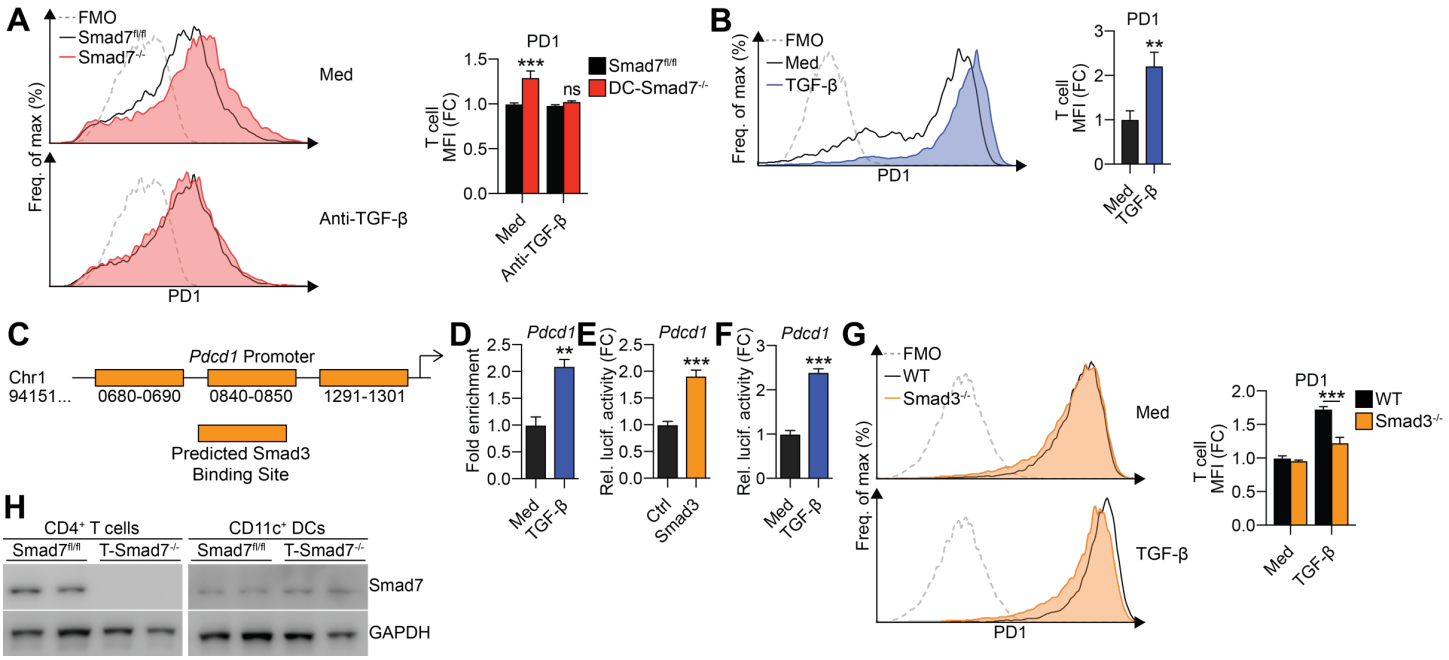

Figure S4. Smad3 promotes CD4$^+$ T cell responsiveness to TGF-β and ability to upregulate PD1. Related to Figure 4. (**A**) Representative FACS histograms (**left**) and frequencies (**right**) of PD1 in naïve CD4$^+$ T cells co-cultured with MLN CD11c$^+$ DCs from either Smad7$^{fl/fl}$ or DC-Smad7$^{-/-}$ mice with or without anti-TGF-β (10 mg/mL) at a 1:3 DC:T cell ratio, for 18 h (n=6). (**B**) Representative FACS histograms (**left**) and MFIs (**right**) of PD1 in naïve CD4$^+$ T cells stimulated with low-dose anti-CD3 (0.5µg/mL) and anti-CD28 (0.5µg/mL) with or without TGF-β (2.5 ng/mL) for 18 h (n=10). (**C**) Schematic localization of putative Smad3 binding sites on *Pdcd1* (PD1) promoter region from Alggen, a web-based algorithm for TFBS predictions. (**D**) ChIP analysis of Smad3 binding promoter region of *Pdcd1* in CD4$^+$ T cells stimulated with or without TGF-β (10 ng/mL) (n=3). ChIP data expressed as percent fold enrichment as compared to input control. (**E**) Luciferase reporter activity of *Pdcd1* promoter in HEK-293 cells stimulated with control or Smad3 construct or (**F**) with or without TGF-β (2 ng/mL) (n=6). Luciferase activity represented as relative activity fold change from control or media condition. (**G**) Representative FACS histograms (**left**) and frequencies (**right**) of PD1 in naïve CD4$^+$ T cells from WT and Smad3$^{-/-}$ mice with or without TGF-β (2.5 ng/mL) at a 1:3 DC:T cell ratio, for 18 h (n=6). (**H**) Western blot of Smad7 in splenic CD4$^+$ T cells (**left**) and CD11c$^+$ DCs (**right**) isolated *ex vivo* from naïve Smad7$^{fl/fl}$ and T-Smad7$^{-/-}$ mice. Data representative of ≥ 3 independent experiments. MFI data reflective of CD4$^+$ population and expressed as FC from (**A**) Smad7$^{fl/fl}$ media condition, (**B**) media condition, or (**G**) WT media condition. Mean ± SEM. ns, **p<.01, ***p<.001 by unpaired T test.

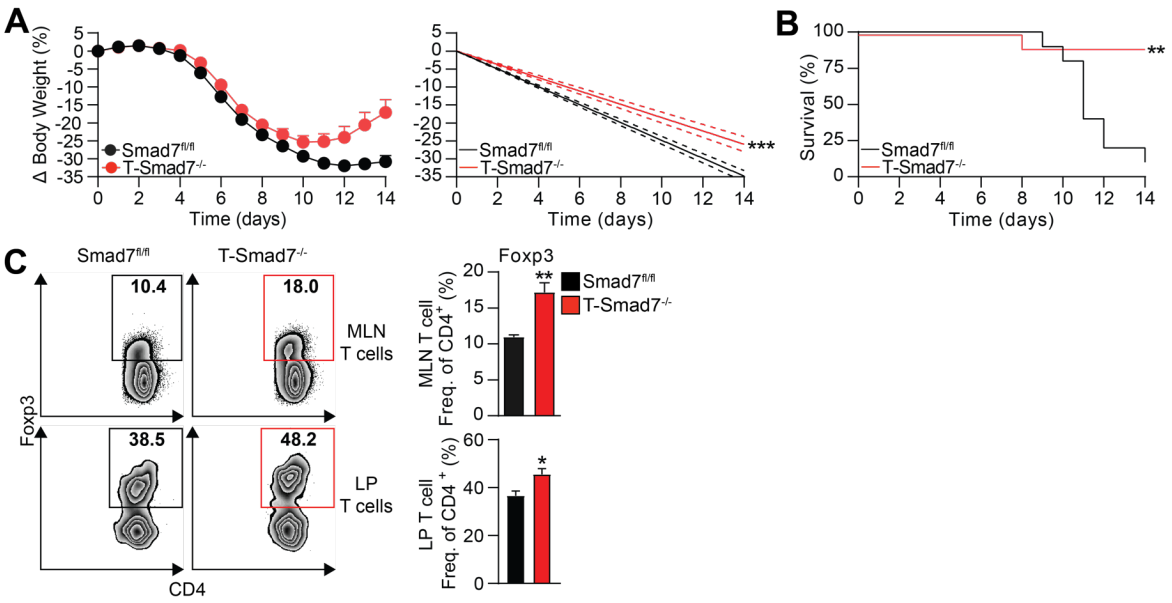

Figure S5. Smad7 deficiency in T cells protects against DSS-induced colitis. Related to Figure 4. (**A**) Percent body weight changes (**left**) and linear regression analysis (**right**) of Smad7$^{fl/fl}$ and T-Smad7$^{-/-}$ mice treated with 3% DSS in drinking water for 7 d, followed by untreated water for 7 d (n=10). The lowest final body weight score for mice that did not survive treatment was recorded for subsequent days until cessation of monitoring. (**B**) Survival rate of these colitis mice. (**C**) Representative FACS plots (**left**) and frequencies (**right**) of Foxp3^{+} populations in MLN (**top**) and LP (**bottom**) CD4^{+} T cells in these mice after 7 days of DSS (n=5). Data representative of ≥ 3 independent experiments. Mean ± SEM. *p<.05, **p<.01, by unpaired T test.