Figure S1. SMAD3-KO and WT mice present no differences in hematopoietic and lymphoid cell subsets. (A) Mean BM cellularity (2 tibiae and 2 femurs). (B) Percentage of granulocytes (CD11b+/Gr-1+) and monocytes (CD11b+/Gr-1-) in the BM. (C) Spleen cellularity. (D) Percentage of CD4 and CD8 cells in the spleen and (E) proportion of naive (CD62L+/CD44-), central memory (CD62L+/CD44+) and effector memory (CD62L-/CD44+) subsets. (F) Percentage of granulocytes and monocytes in the spleen. (G) Percentage of CD19+ (B lymphocytes), TCRγδ+ and NK (DX5+/TCRβ-) cells in the spleen. (H-J) Proportion of lymphoid cell subsets in mesenteric lymph nodes. All histograms represent the mean and SEM for 4 to 9 mice per genotype.
**Figure S2. Effect of SMAD3 genotype and TGF-β on T-cell proliferation.** (A) Effect of low dose (0.1 µg/ml) and (B) high dose (1 µg/ml) of soluble anti-CD3ε and anti-CD28 (5 µg/ml) on sorted naive CD8 T cells from either WT or SMAD3-KO mice. (C) Effect of low dose soluble anti-CD3ε (0.1 µg/ml) with anti-CD28 (5 µg/ml) on proliferation of naive CD4 T cells from either WT or SMAD3-KO mice. TGF-β was added to cell cultures at a concentration of 2.5 ng/ml where indicated. Histograms represent the mean of 4 independent experiments and error bars represent the SEM. Intragenotype and intergenotype differences were evaluated by the Student’s t test. All significant differences are shown (*=P < 0.05 and **=P < 0.01).
Figure S3. Histological examination of mouse organs. (A) Representative photographs of skin, lung, liver, spleen and small intestine at day 40 post-AHCT obtained from recipients of either WT or SMAD3-KO grafts (at least 3 animals per condition) and (B) representative photographs of the same organs plus colon in recipient of SMAD3-KO grafts at day 60 (at least 3 animals). (20X objective, 100 µm scale unit). (C) Pathological grading (maximum grade = 4) of small bowel GVHD (n=6).
Figure S4. Evidence of systemic T-cell activation without Th1 bias. (A) Percentages of CD43i+ CD4+ T-cells in the spleen (n=8) and mesenteric lymph nodes (n = 5) on d 40 post-AHCT. (B) Percentages of T-bet+ CD4+ T cells in the spleen on d 20 and d 40 post-AHCT (n = 5). (C) IFN-γ concentration in spleen extracts on d 20 and d 40 post-AHCT (n = 5). Histograms represent the mean and SEM. Statistics performed with Student's t test (*=P < 0.05, **=P < 0.01).
Figure S5. Neutrophil depletion efficacy in the colon. One representative of three contour plots of neutrophil staining in the colon of KO-recipient after I.P. treatment with either isotype or anti-Ly6G antibody.