

European Journal of Immunology

Supporting Information

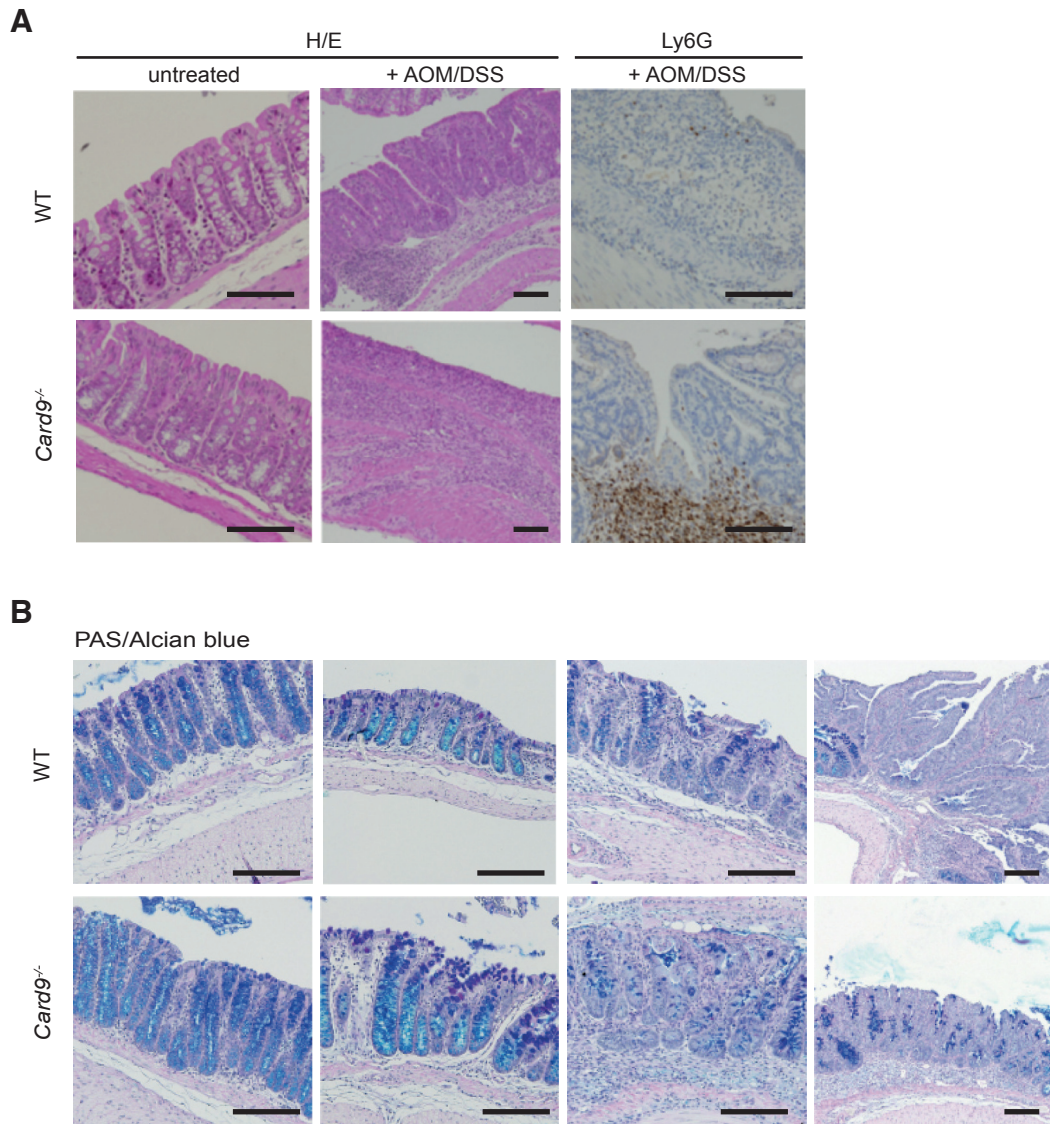
for

DOI 10.1002/eji.201646765

Hanna Bergmann, Susanne Roth, Konstanze Pechloff, Elina A. Kiss, Sabine Kuhn, Mathias Heikenwälder, Andreas Diefenbach, Florian R. Greten and Jürgen Ruland

Card9-dependent IL-1 β regulates IL-22 production from group 3 innate lymphoid cells and promotes colitis-associated cancer

Supporting Information Figure 1

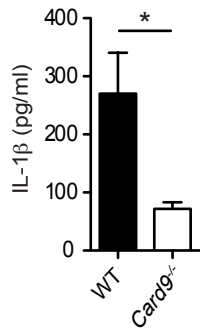


Supporting Information Figure 1.

Enhanced epithelial damage and inflammation in the colon of *Card9*-deficient mice during chronic colitis.

(A) Representative H&E-stain and Ly6G-IHC of colon sections from untreated or AOM/DSS-treated WT and *Card9*^{-/-} mice at day 68 (+AOM/DSS). (B) Representative images of PAS/Alcian blue stained colon sections from AOM/DSS-treated WT and *Card9*^{-/-} mice at day 68. Depicted are increasing degrees of hyperplasia in WT and *Card9*^{-/-} colons from left to right. (A,B) Representative data of at least two independent experiments are shown. Scale bars represent 100 μ m.

Supporting Information Figure 2

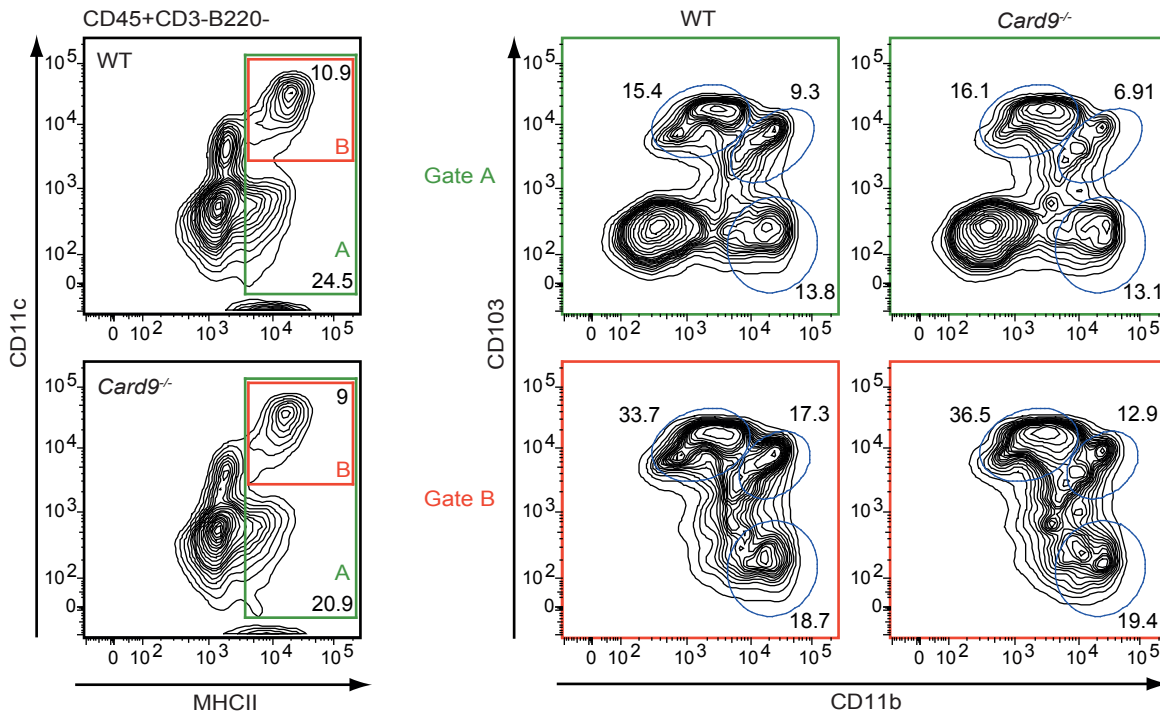


Supporting Information Figure 2.

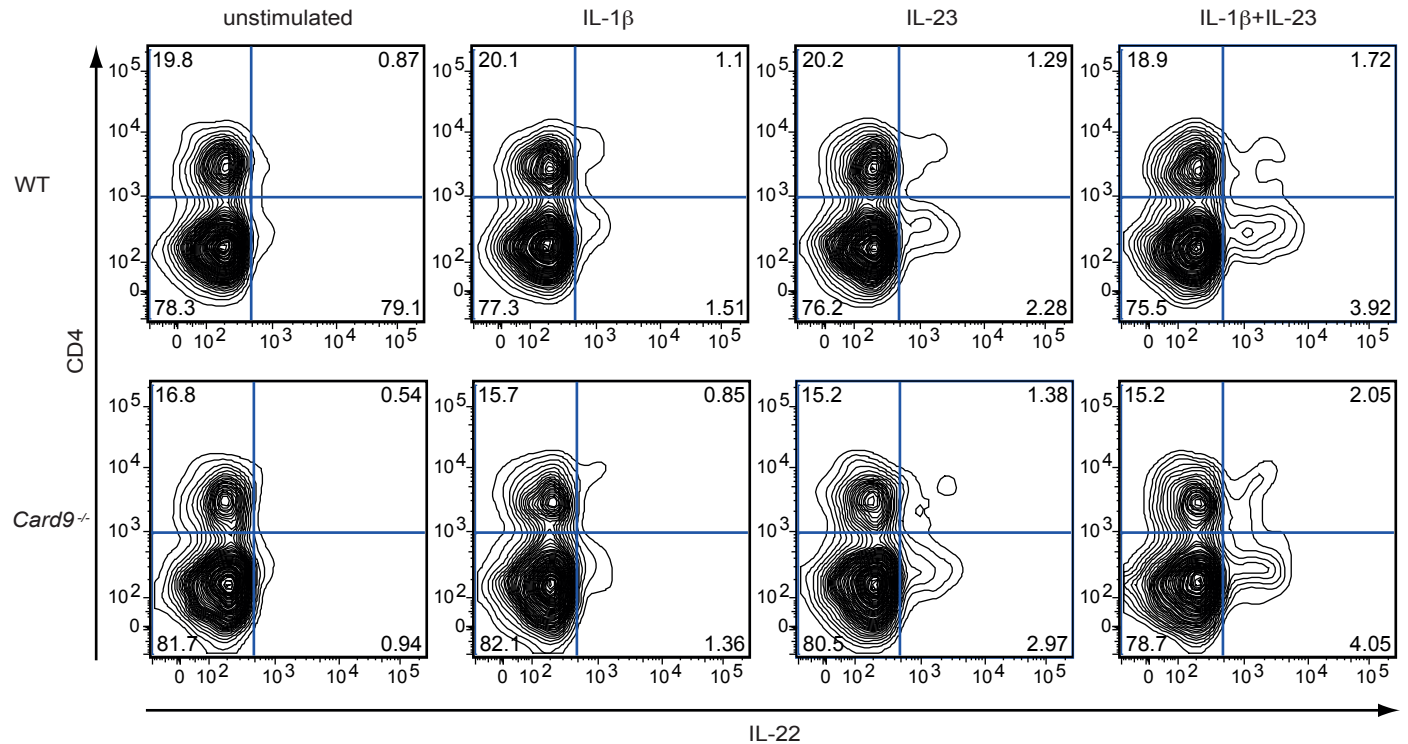
Impaired IL-1 β production in colons of *Card9*^{-/-} mice in the AOM/DSS colitis-associated cancer model. IL-1 β protein levels were determined in supernatants of ex vivo cultured distal colons from WT (n=6) and *Card9*^{-/-} (n=7) mice after AOM/DSS treatment at day 68. Data of one experiment are shown as the mean + s.e.m. *p < 0.05, Student's t-test.

Supporting Information Figure 3

A Lamina propria



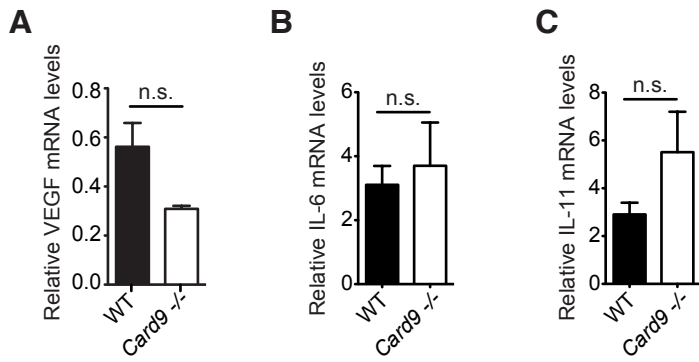
B CD45+CD3-B220-NK1.1-CD90.2+ unstimulated



Supporting Information Figure 3.

Representative FACS blots and gating strategies. (A) Representative FACS plots showing the frequencies of MHCII^{hi} (Gate A) and MHCII^{hi}CD11c⁺ (Gate B) DCs in the colonic lamina propria of WT and *Card9*^{-/-} mice that were further subdivided into CD103⁻CD11b⁺, CD103⁺CD11b⁻, and CD103⁺CD11b⁺ DCs. (B) Representative FACS plots showing the frequencies of CD4⁺ or CD4⁻ IL-22⁺ ILCs in isolated whole mesenteric lymph node (MLN) cells of WT (n=3) and *Card9*^{-/-} (n=3) mice left untreated, or after in vitro stimulation with IL-1 β , IL-23, or IL-1 β in combination with IL-23.

Supporting Information Figure 4



Supporting Information Figure 4.

Expression of STAT3 activators in the colon of AOM/DSS-treated mice. (A-C) mRNA was isolated from colonic tissue of AOM/DSS-treated WT (n=8) and *Card9*^{-/-} (n=5) mice at day 68. Relative expression of VEGF (A), IL-6 (B), and IL-11 mRNAs were determined by quantitative real-time PCR and normalized to β -actin transcript levels. The data of one experiment are shown as the mean + s.e.m. n.s., not significant, Student's t-test.