

# European Journal of Immunology

Supporting Information

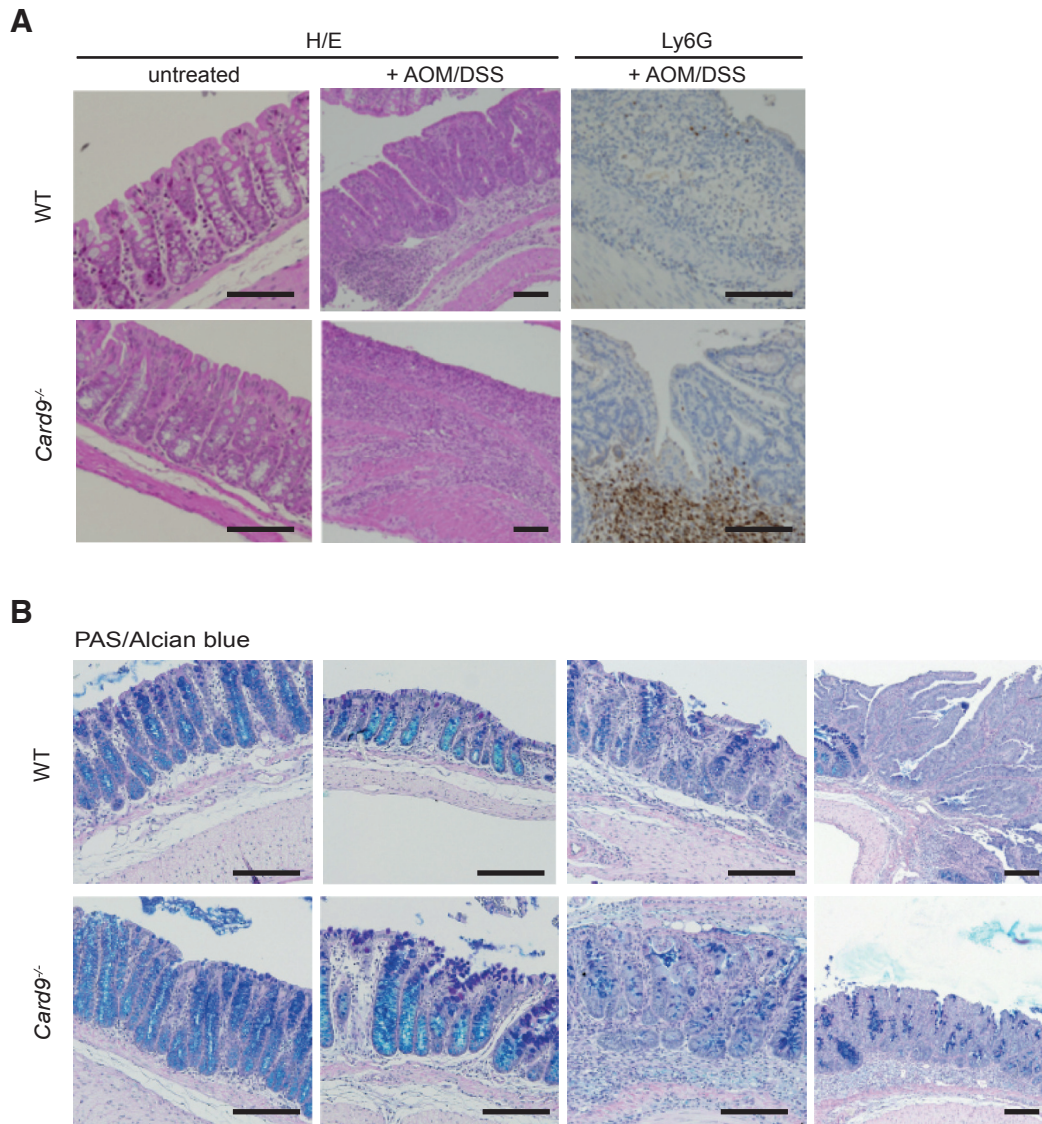
for

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**Card9-dependent IL-1 $\beta$  regulates IL-22 production from group 3 innate lymphoid cells and promotes colitis-associated cancer**

## Supporting Information Figure 1

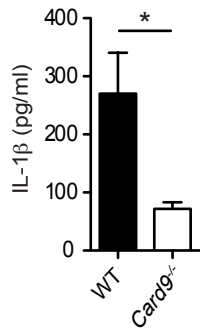


### Supporting Information Figure 1.

#### 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*<sup>-/-</sup> mice at day 68 (+AOM/DSS). (B) Representative images of PAS/Alcian blue stained colon sections from AOM/DSS-treated WT and *Card9*<sup>-/-</sup> mice at day 68. Depicted are increasing degrees of hyperplasia in WT and *Card9*<sup>-/-</sup> colons from left to right. (A,B) Representative data of at least two independent experiments are shown. Scale bars represent 100  $\mu$ m.

## Supporting Information Figure 2

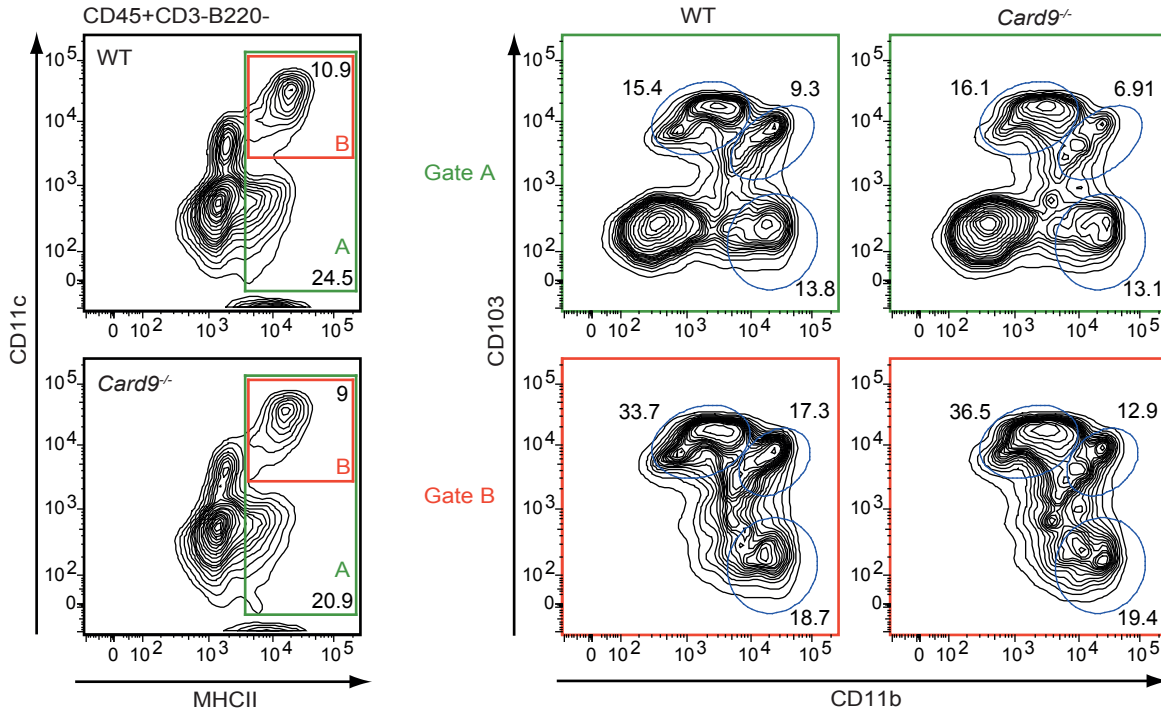


### Supporting Information Figure 2.

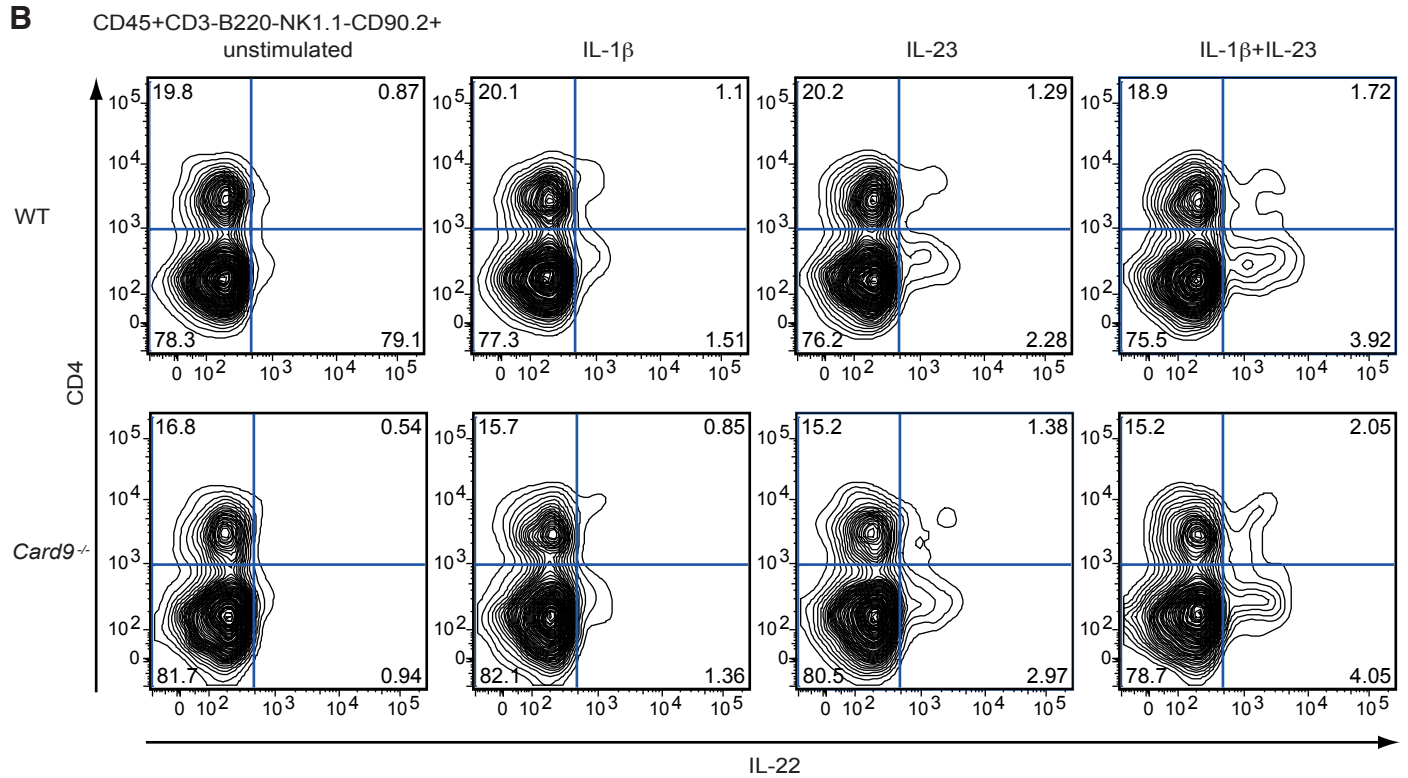
**Impaired IL-1 $\beta$  production in colons of *Card9*<sup>-/-</sup> mice in the AOM/DSS colitis-associated cancer model.** IL-1 $\beta$  protein levels were determined in supernatants of ex vivo cultured distal colons from WT (n=6) and *Card9*<sup>-/-</sup> (n=7) mice after AOM/DSS treatment at day 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



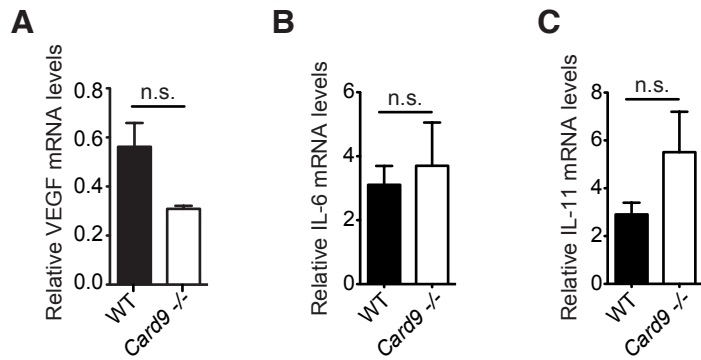
### B



### Supporting Information Figure 3.

**Representative FACS blots and gating strategies.** (A) Representative FACS plots showing the frequencies of MHCII<sup>hi</sup> (Gate A) and MHCII<sup>hi</sup>CD11c<sup>-</sup> (Gate B) DCs in the colonic lamina propria of WT and *Card9*<sup>-/-</sup> mice that were further subdivided into CD103<sup>-</sup>CD11b<sup>+</sup>, CD103<sup>+</sup>CD11b<sup>-</sup>, and CD103<sup>+</sup>CD11b<sup>+</sup> DCs. (B) Representative FACS plots showing the frequencies of CD4<sup>+</sup> or CD4<sup>-</sup> IL-22<sup>+</sup> ILCs in isolated whole mesenteric lymph node (MLN) cells of WT (n=3) and *Card9*<sup>-/-</sup> (n=3) mice left untreated, or after in vitro stimulation with IL-1 $\beta$ , IL-23, or IL-1 $\beta$  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*<sup>-/-</sup> (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  $\beta$ -actin transcript levels. The data of one experiment are shown as the mean + s.e.m. n.s., not significant, Student's t-test.