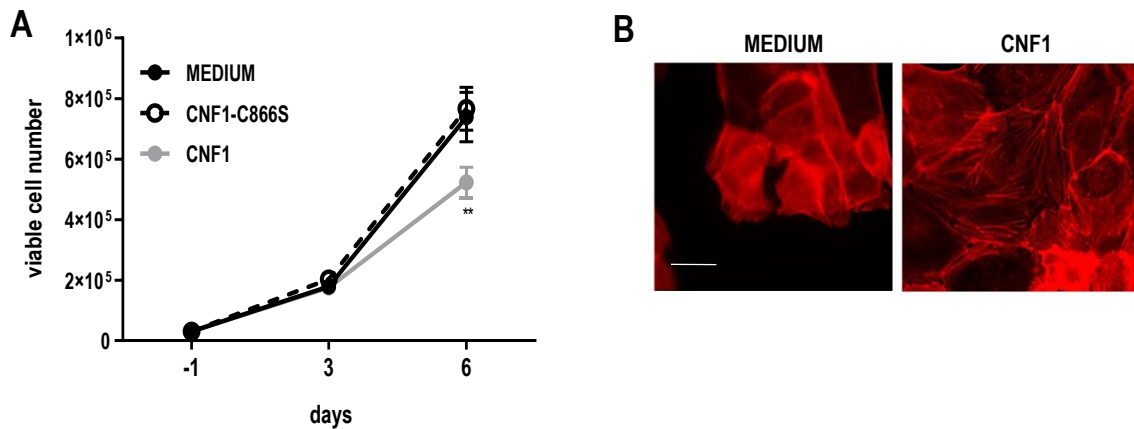
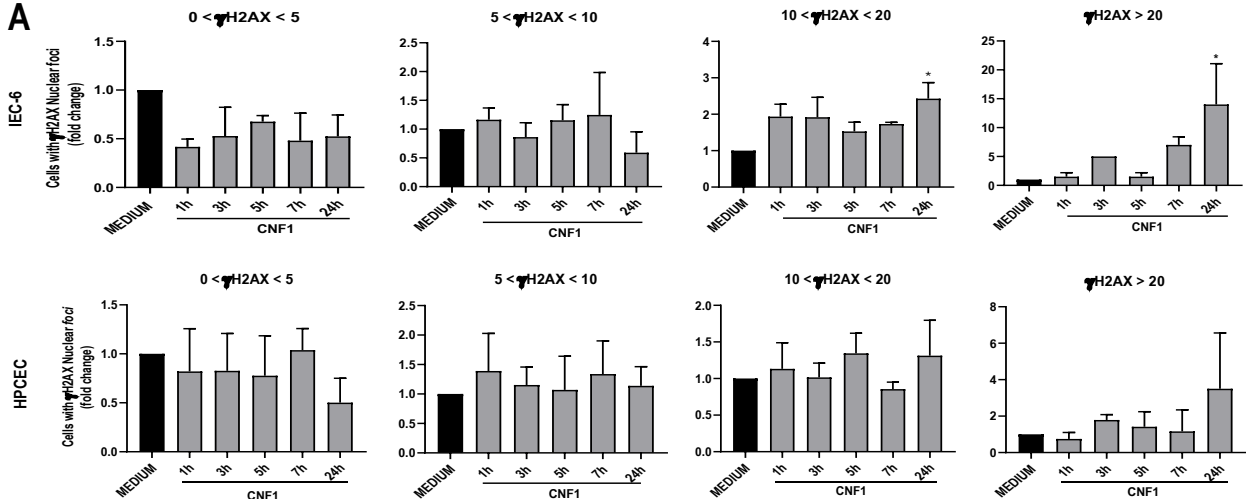
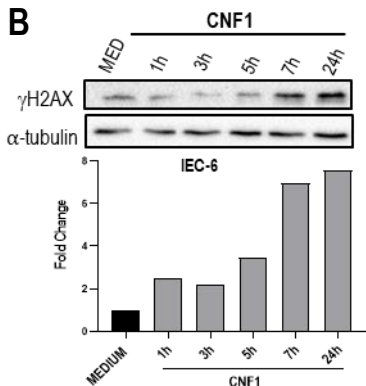
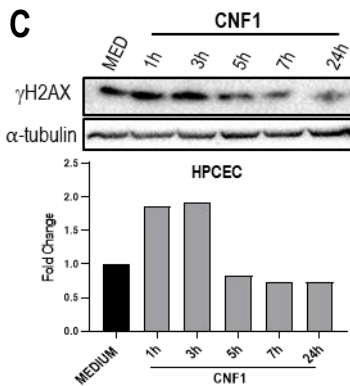
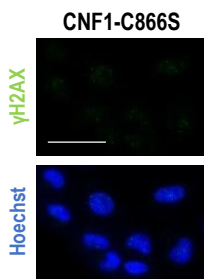
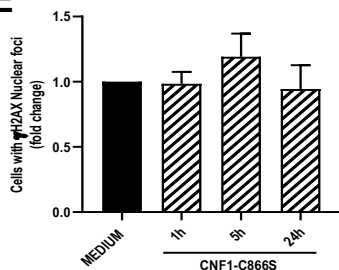
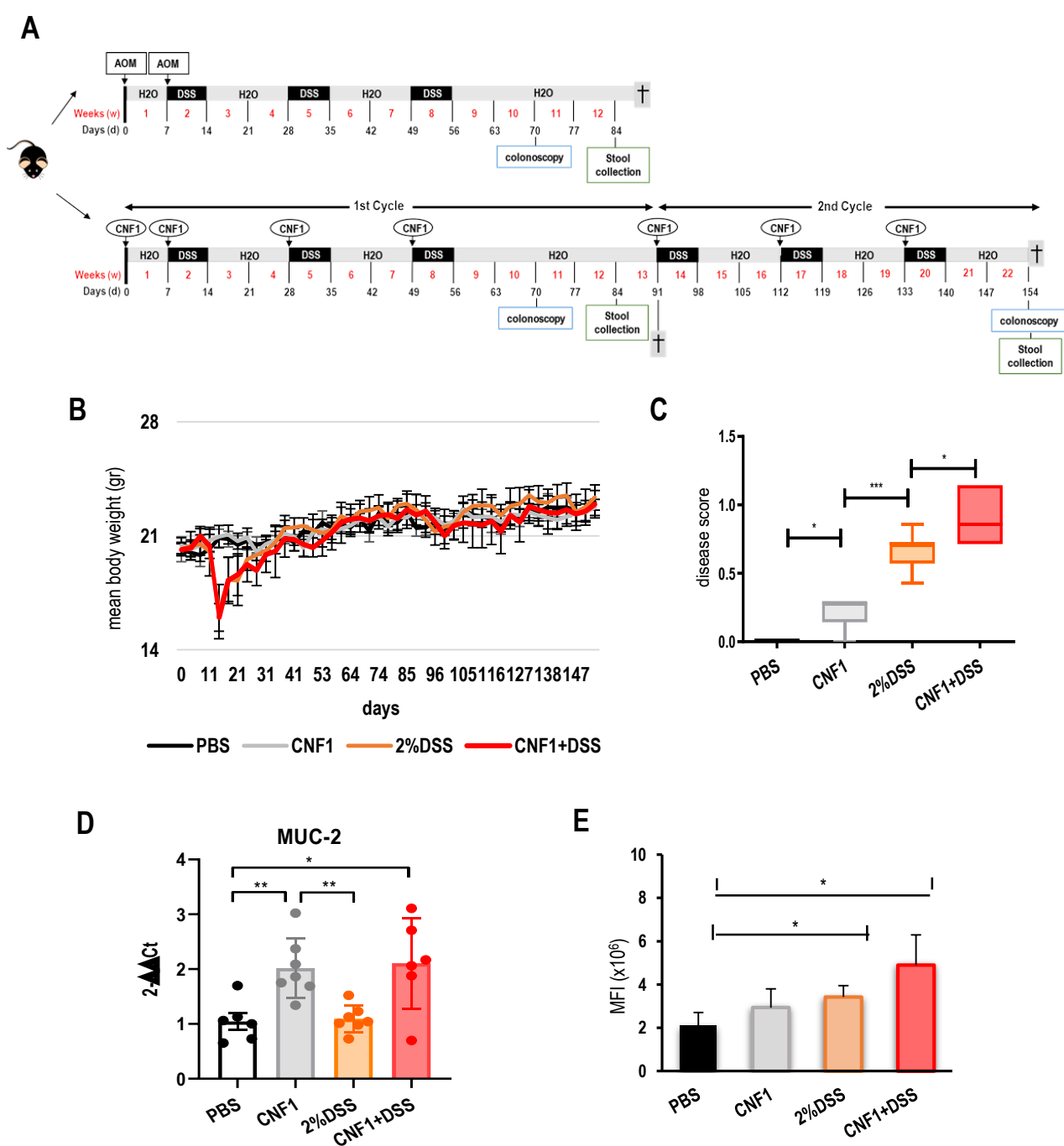




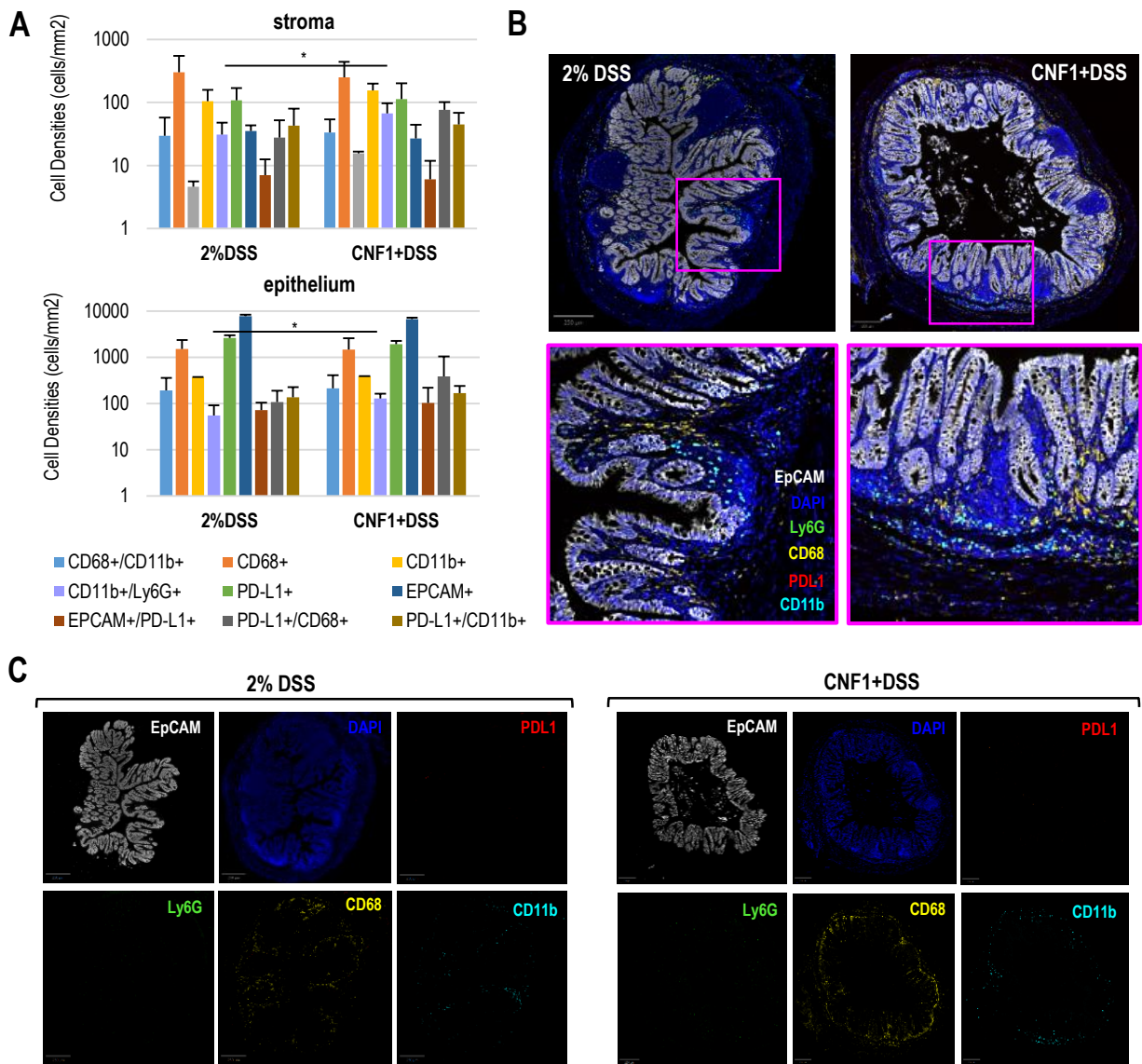
Supplementary Figure 1. CNF1 effects on HPCEC cells. (A) Trypan blue exclusion count of HPCEC cells after 3 and 6 days of culture in the presence of CNF1 or CNF1-C866S (25 pM, N=3). (** $p < 0.01$ vs CNF1-C866S and PBS). **(B)** Representative immunofluorescence micrographs of phalloidin staining of HPCEC cells treated with 25 pM CNF1 for 24 h or untreated controls. Scale bar: 10 μ m.

A**B****C****D****E**

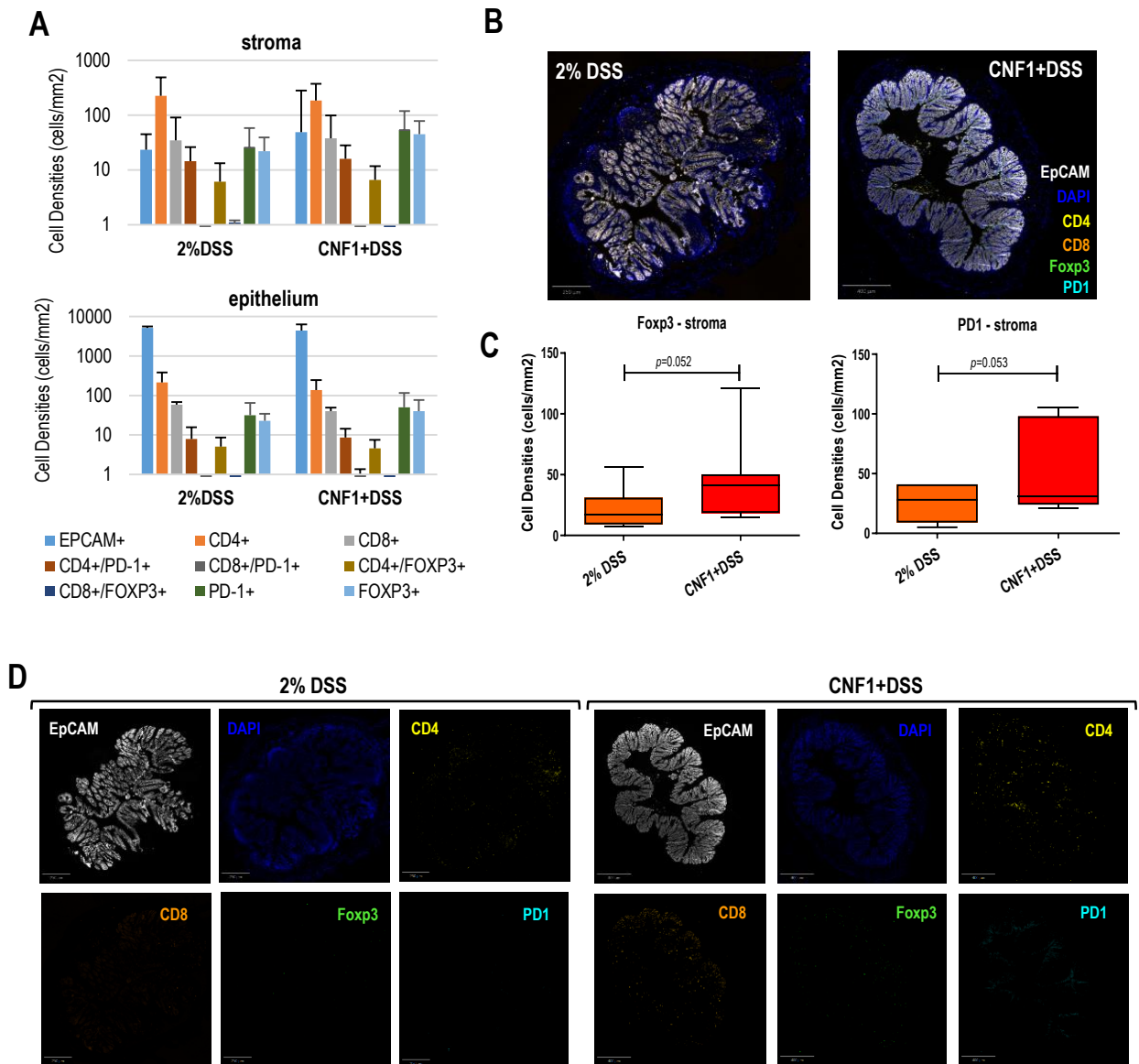
Supplementary Figure 2. CNF1-induced DDR in IEC-6 and HPCEC cells. (A) Classes of nuclear *foci* number of γ H2AX in IEC-6 and HPCEC cells. (* p <0.05). (B-C) Expression level of γ H2AX in IEC-6 and HPCEC cells by western blot. Representative micrographs and relative densitometry bar plots showing the fold change of γ H2AX in IEC-6 and HPCEC cells at each indicated time-point as compared to untreated cells. One representative experiment out of three with similar results is shown. (D) Representative fluorescence micrographs of IEC-6 cells stained with anti- γ H2AX (green). Nuclei were counterstained with Hoechst (blue). Scale bar: 10 μ m. (E) Bar plot showing the fold change of γ H2AX-positive nuclei at different time points following exposure to 25 pM CNF1-C866S mutant toxin.



Supplementary Figure 3. Body weight and disease monitoring. (A) Treatment schedule of the AOM/DSS model and of the experimental model in which AOM/DSS was replaced by three cycles of i.r. CNF1 administration. (B) Mean body weight of mice (N=10) in each experimental group of the six cycles of i.r. CNF1 administration. One representative experiment out of two with similar results is shown. (C) Boxplot depicting the disease score generated at colonoscopy. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers represent Min and Max values. (* $p < 0.05$; *** $p < 0.001$). (D) Gene expression analysis of MUC-2 by real-time PCR in colon tissue. (E) Dextran-FITC Fluorescence intensity in plasma samples from the indicated treatment groups (N=5 per group). (* $p < 0.05$).



Supplementary Figure 4. Six-channel multiplexed IF imaging of myeloid infiltrates in 2% DSS vs CNF1+DSS treated animals. (A) Bar plots showing the density of the indicated marker or marker combination in the epithelium or stroma compartments of colon tissue. (* $p < 0.05$). **(B)** Representative images of colons from the indicated treatment groups (N=7 per group) multiple stained for EpCAM (white), CD11b (light blue) Ly6G (green), CD68 (yellow) PDL1 (red), and DAPI (blue). **(C)** Representative images of each single channel.



Supplementary Figure 5. Six-channel multiplexed staining of T lymphocyte infiltrates in 2% DSS vs CNF1+DSS- treated animals. (A) Bar plots showing the density of the indicated marker or marker combination in the epithelium or stroma compartments of colon tissue. **(B)** Representative images of colons from the indicated treatment groups (N=7 per group) multiple stained for EpCAM (white), CD4 (yellow), CD8 (orange), PD1 (light blue), Foxp3 (green) and DAPI (blue). **(C)** Boxplot of Foxp3 and PD1 expression in the stromal compartment of colon sections. Center lines show the medians; box limits indicate the 25th and 75th percentiles; whiskers represent Min and Max values. **(D)** Representative images of each single channel.