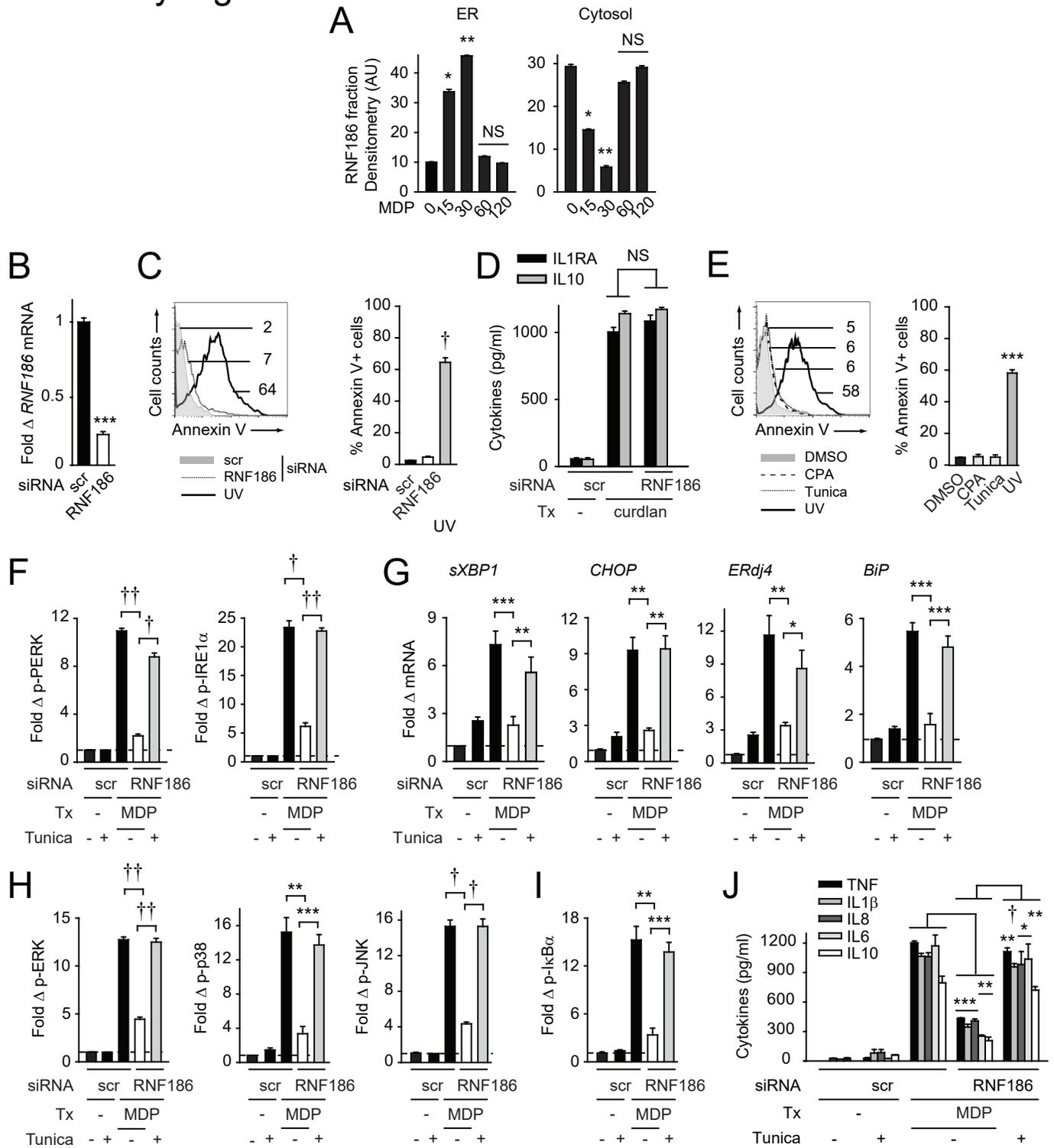
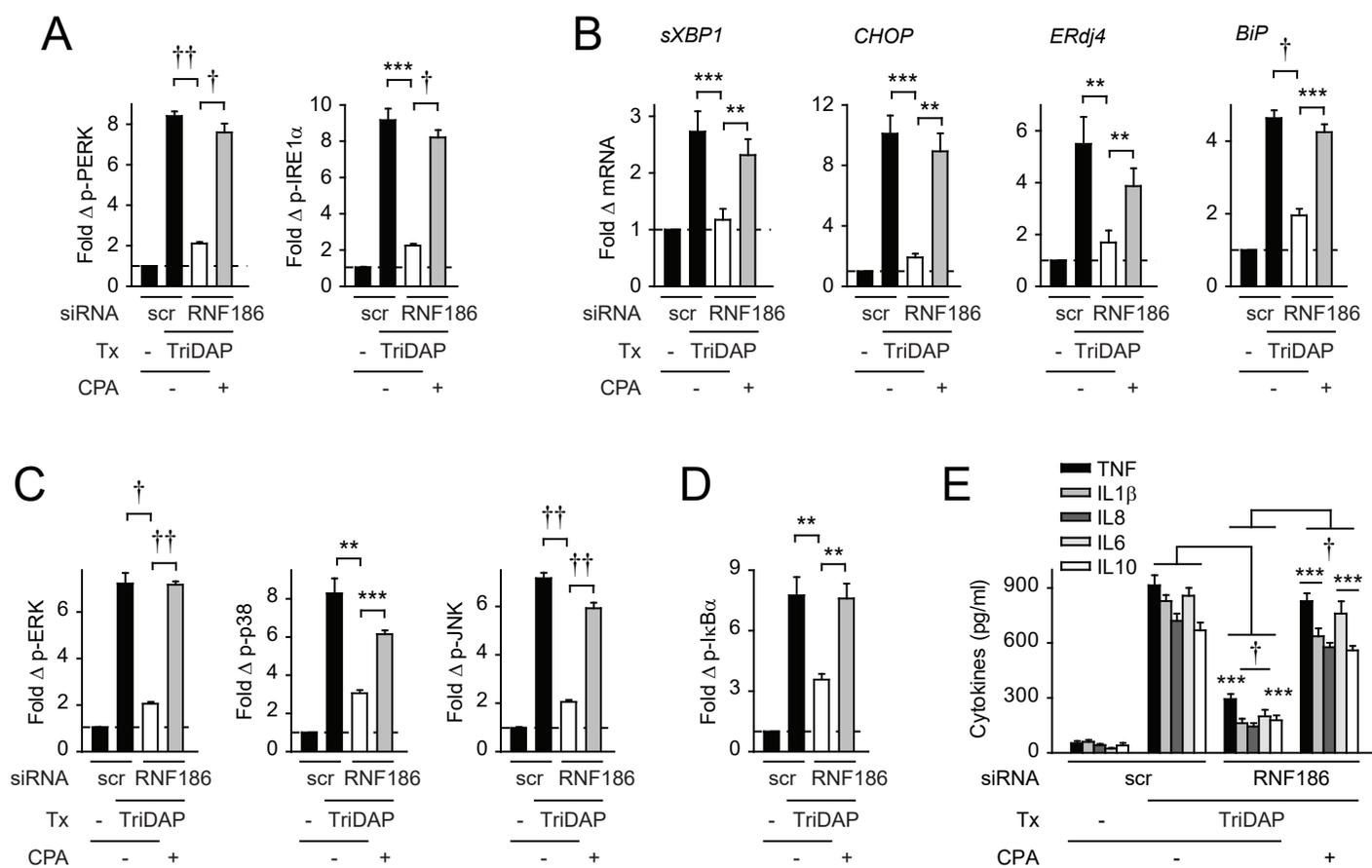


# Supplementary Figure 1



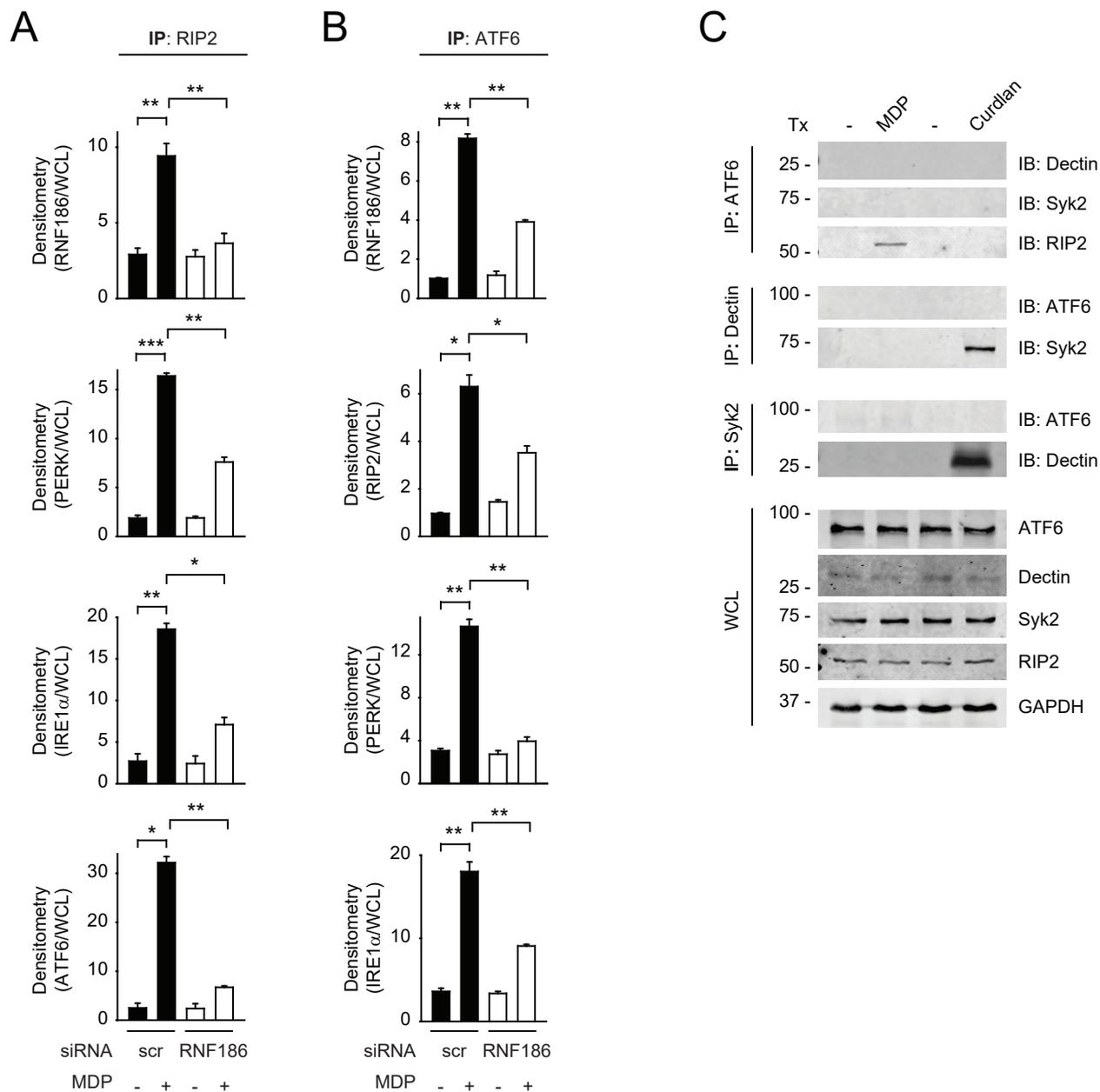
**Figure S1. Complementation of UPR signaling with tunicamycin restores NOD2-induced signaling and cytokines in RNF186-deficient MDMs.** (A) Quantification of RNF186 ER localization immunoblots from Figure 1A (2 independent experiments). (B-D) MDMs were transfected with scrambled or RNF186 siRNA. (B) Fold *RNF186* mRNA expression (n=6 donors; similar results were observed in an additional n=6). (C) Cell death was assessed by annexin V staining per flow cytometry (n=6). (D) MDMs (n=6) were treated with 100  $\mu$ g/ml curdlan and assessed for cytokines at 24h. (E) MDMs (n=6) were treated with vehicle control (DMSO), 10  $\mu$ M CPA or 3  $\mu$ g/ml tunicamycin for 24h. Cell death was assessed by annexin V staining. For 'C' and 'E' UV stimulation at 50-100 J/m<sup>2</sup> is shown as a positive control. (F-J) MDMs (n=6) were transfected with scrambled or RNF186 siRNA and then treated with 100 $\mu$ g/ml MDP  $\pm$  3 $\mu$ g/ml tunicamycin (Tunica). (F) Fold phospho-PERK and phospho-IRE1 $\alpha$  at 30min. (G) Fold mRNA expression at 6h. (H, I) Fold phospho-protein at 15min. (J) Cytokines at 12h. Mean + SEM. NS, not significant; scr, scrambled; Tx, treatment. \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>; ††, p<1 $\times$ 10<sup>-5</sup>.

# Supplementary Figure 2



**Figure S2. Complementation of UPR signaling restores NOD1-induced signaling and cytokines in RNF186-deficient MDMs.** MDMs (n=6) were transfected with scrambled or RNF186 siRNA and then treated with 100  $\mu$ g/ml TriDAP (L-Ala-gamma-D-Glu-mDAP; InvivoGen)  $\pm$  10  $\mu$ M CPA. **(A)** Fold change phospho-PERK and phospho-IRE1 $\alpha$  at 30min. **(B)** Fold change mRNA expression at 6h. **(C, D)** Fold change phospho-proteins at 15min. **(E)** Cytokines at 12h. Mean + SEM. Scr, scrambled; Tx, treatment. \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>; ††, p<1 $\times$ 10<sup>-5</sup>.

# Supplementary Figure 3



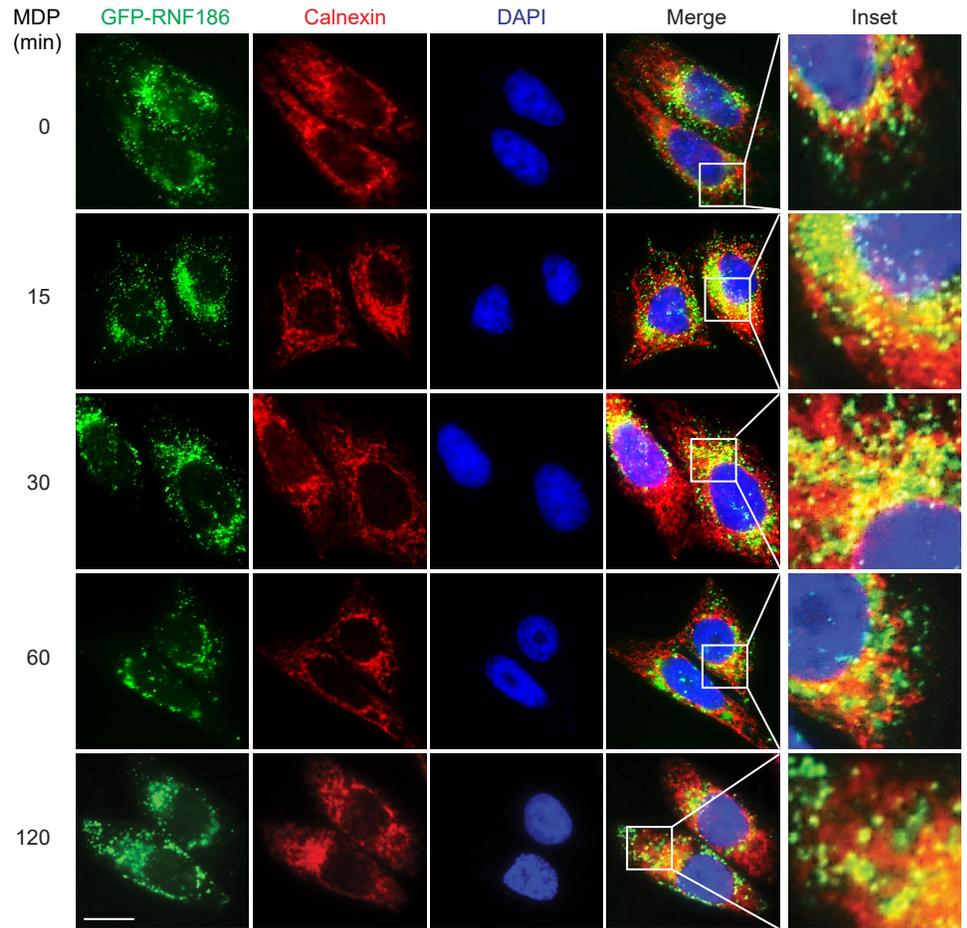
**Figure S3. RNF186 does not associate with the Dectin-1 signaling complex. (A)** Quantification of **Figure 2C** for IP: RIP2 (3 independent experiments). **(B)** Quantification of **Figure 2C** for IP: ATF6 (3 independent experiments). Mean + SEM. **(C)** Human MDMs were transfected with scrambled or RNF186 siRNA and then treated with 100 $\mu$ g/ml curdlan or 100 $\mu$ g/ml MDP (as a positive control for UPR-associated proteins) for 15min. ATF6, Dectin-1 or Syk2 was immunoprecipitated (IP) followed by immunoblotting (IB) for the indicated proteins. Curdlan-induced association of Dectin-1 and Syk2 was assessed as a positive control through reciprocal immunoprecipitation approaches. Expression of the respective proteins and GAPDH in whole cell lysates (WCL) served as loading controls. Marker positions are shown (kDa). Scr, scrambled; Tx, treatment. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ .

# Supplementary Figure 4

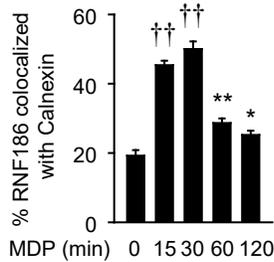
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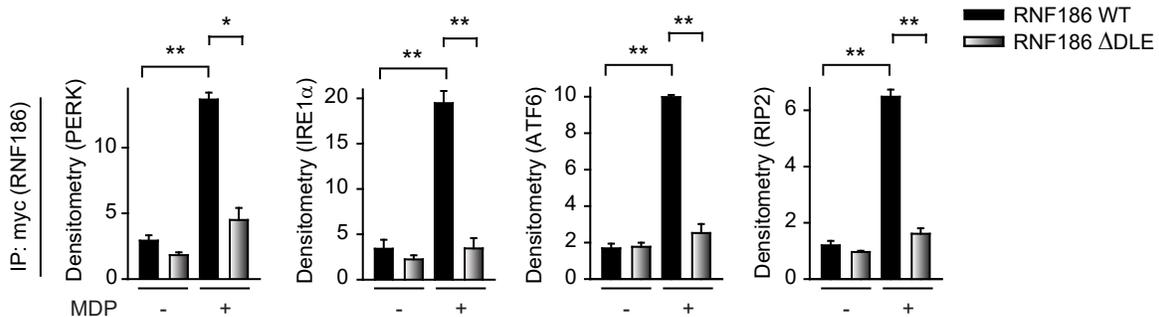
**B**



**C**

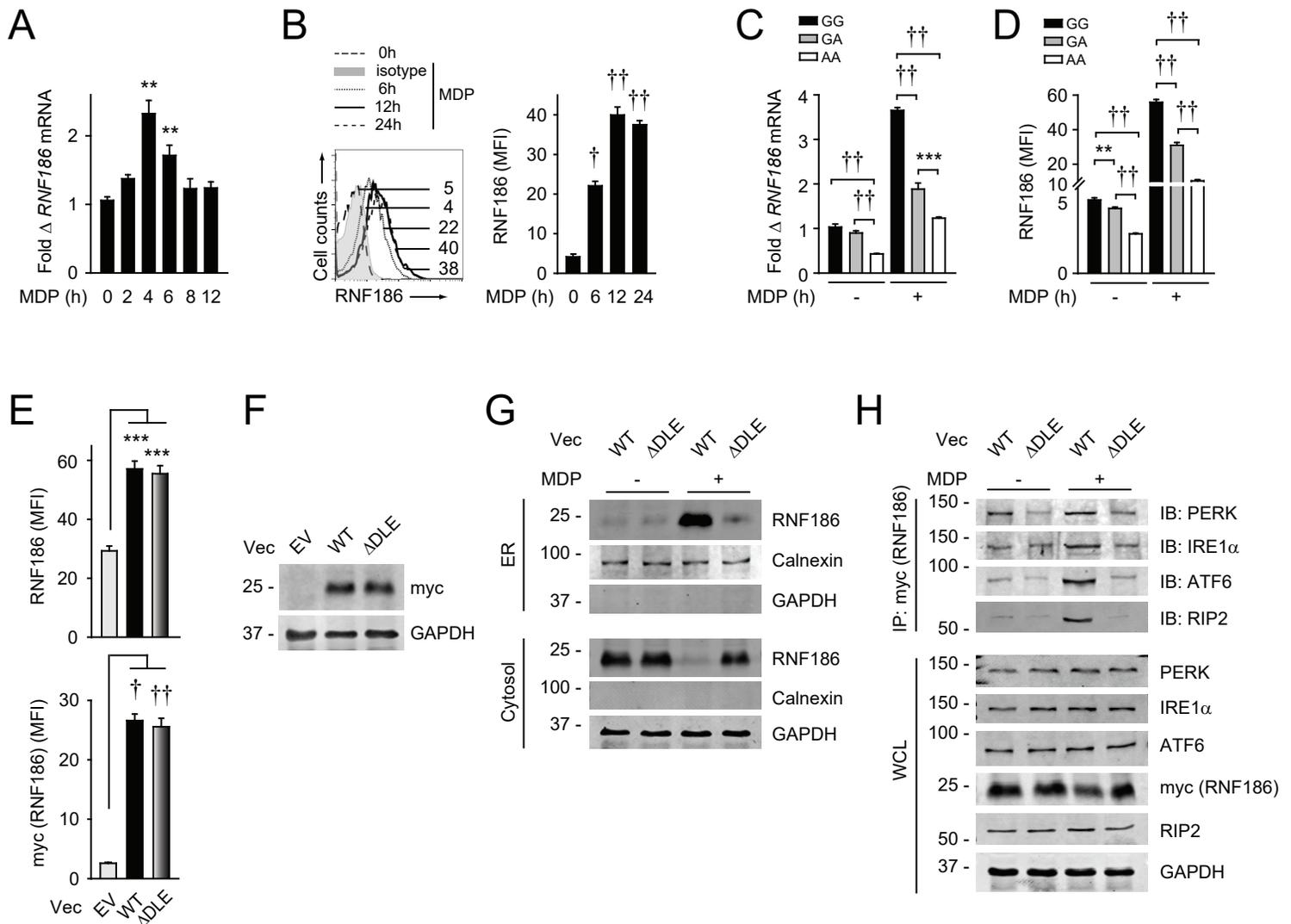


**D**



**Figure S4. A proximal region in RNF186 regulates ER localization in HeLa cells. (A)** RNF186 protein model representing wild type (WT) and  $\Delta$ DLE mutant (putative ER retention motif). **(B-C)** HeLa cells were transfected with GFP-tagged RNF186 and with NOD2 followed by treatment with 100 $\mu$ g/ml MDP for the indicated times. Cells were immunostained for calnexin (ER, red) and nucleus (DAPI, blue). **(B)** Representative micrographs with scale bar representing 10 $\mu$ m. Inset represents enlarged images from the merged panel. **(C)** Summary graph of percent RNF186 colocalized with calnexin (ER) (25 cells quantified). Representative of two independent experiments. **(D)** Quantification of **Figure 3C** for IP:RNF186 (summary 3 independent experiments). Mean + SEM. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; ††,  $p < 1 \times 10^{-5}$ .

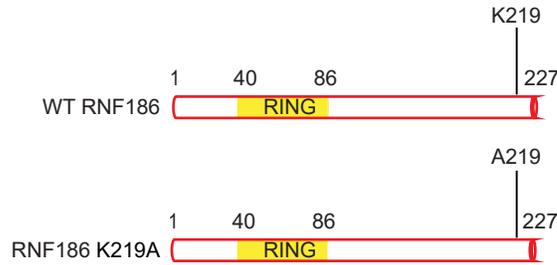
# Supplementary Figure 5



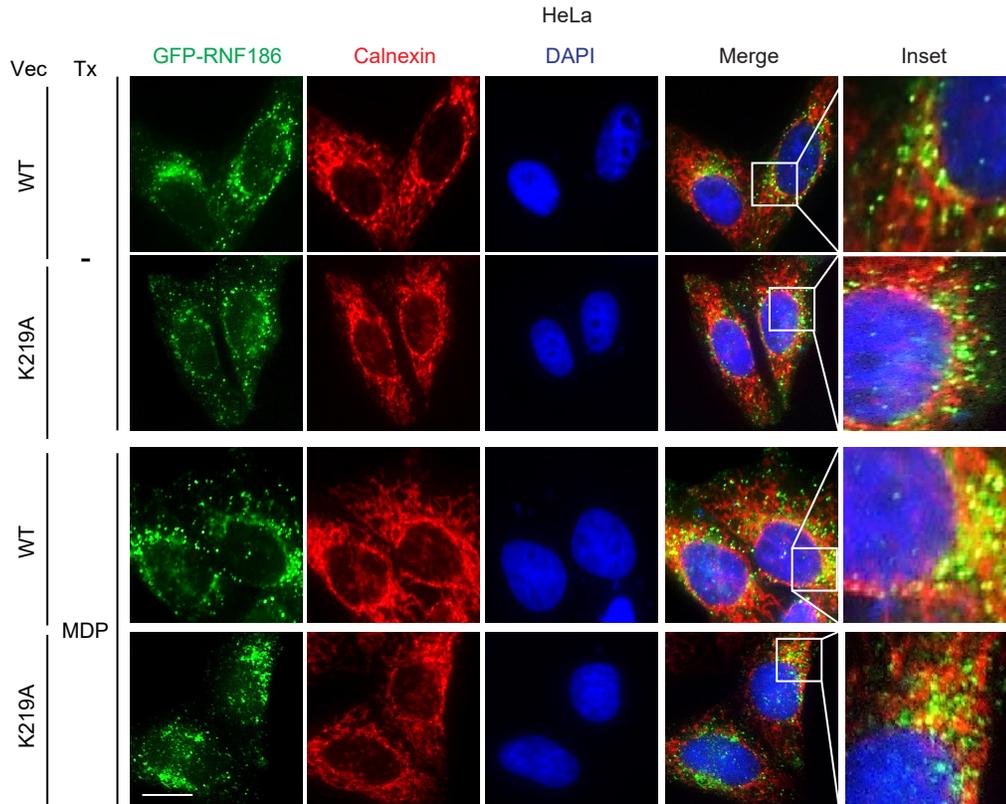
**Figure S5. A proximal region in RNF186 regulates localization to the ER in MDMs.** (A-B) MDMs were treated with 100 $\mu$ g/ml MDP. (A) *RNF186* mRNA expression at the indicated times (n=6 donors). (B) RNF186 protein expression by flow cytometry at the indicated times. (Left) Representative histogram with mean fluorescence intensity (MFI) values. Isotype control is from 24h treated cells. (Right) Summary graph of MFI (n=6 donors). (C-D) MDMs from rs6426833 GG, GA or AA carriers (n=10 donors/genotype) were treated with 100  $\mu$ g/ml MDP. (C) *RNF186* mRNA at 4h. (D) RNF186 protein expression at 24h by flow cytometry. (E-H) MDMs (rs6426833 AA low RNF186-expressing carriers) were transfected with empty vector (EV) or myc-tagged RNF186 WT or  $\Delta$ DLE. (E-F) RNF186 protein expression as detected with anti-RNF186 or anti-myc antibody by: (E) flow cytometry (n=6) or (F) Western blot. (G) MDMs were then treated with 100 $\mu$ g/ml MDP for 30min. ER and cytosolic fractions were assessed for myc (RNF186) expression by Western blot. Markers for ER (calnexin) and cytosolic (GAPDH) fractions are shown. Representative of 2 independent experiments. (H) MDMs were treated with 100 $\mu$ g/ml MDP for 30min. Myc (RNF186) was immunoprecipitated (IP) followed by immunoblotting (IB) of the indicated proteins. Representative of 2 independent experiments. Mean + SEM. Vec, vector. \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>; ††, p<1 $\times$ 10<sup>-5</sup>.

# Supplementary Figure 6

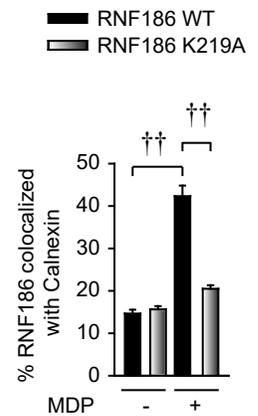
## A



## B

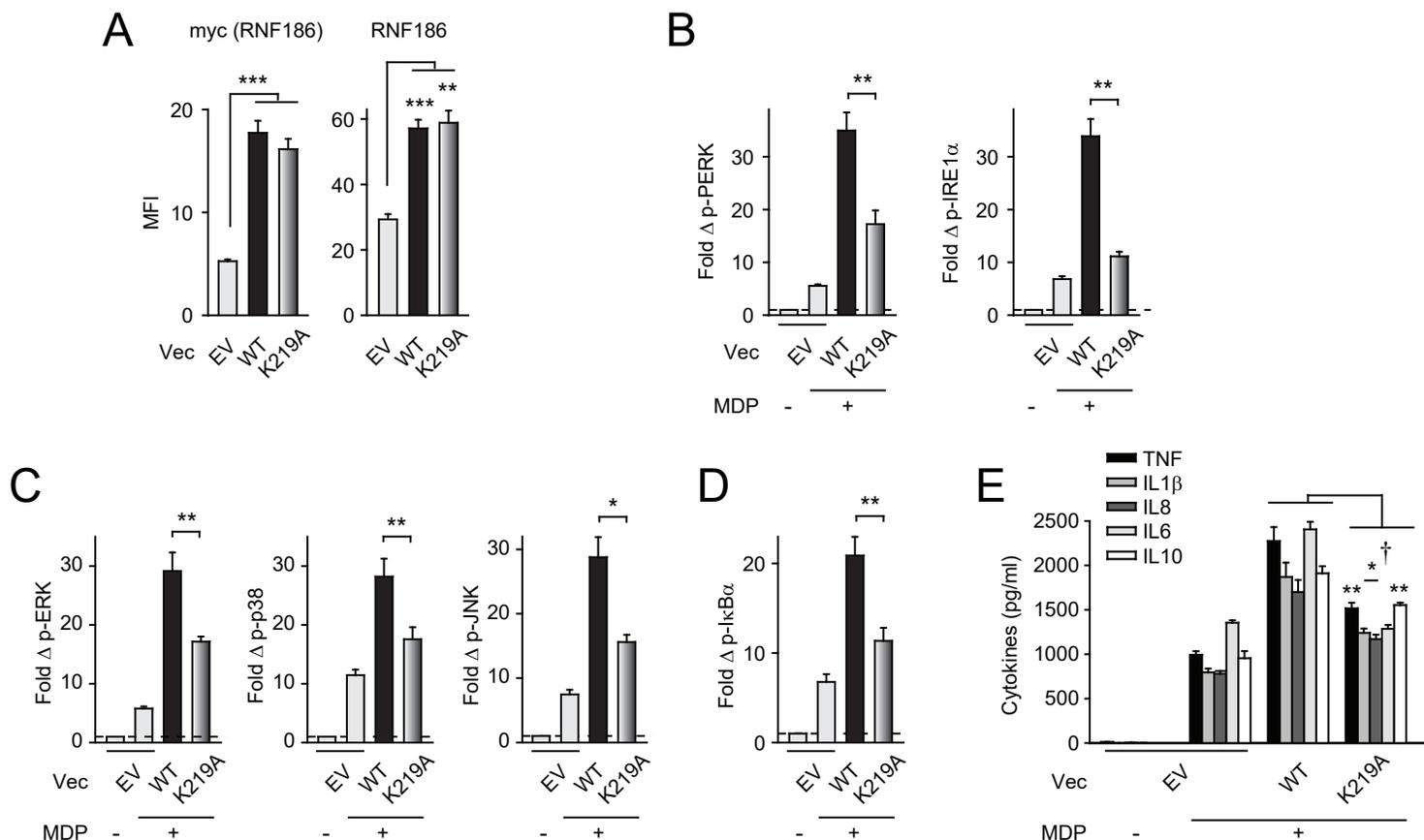


## C



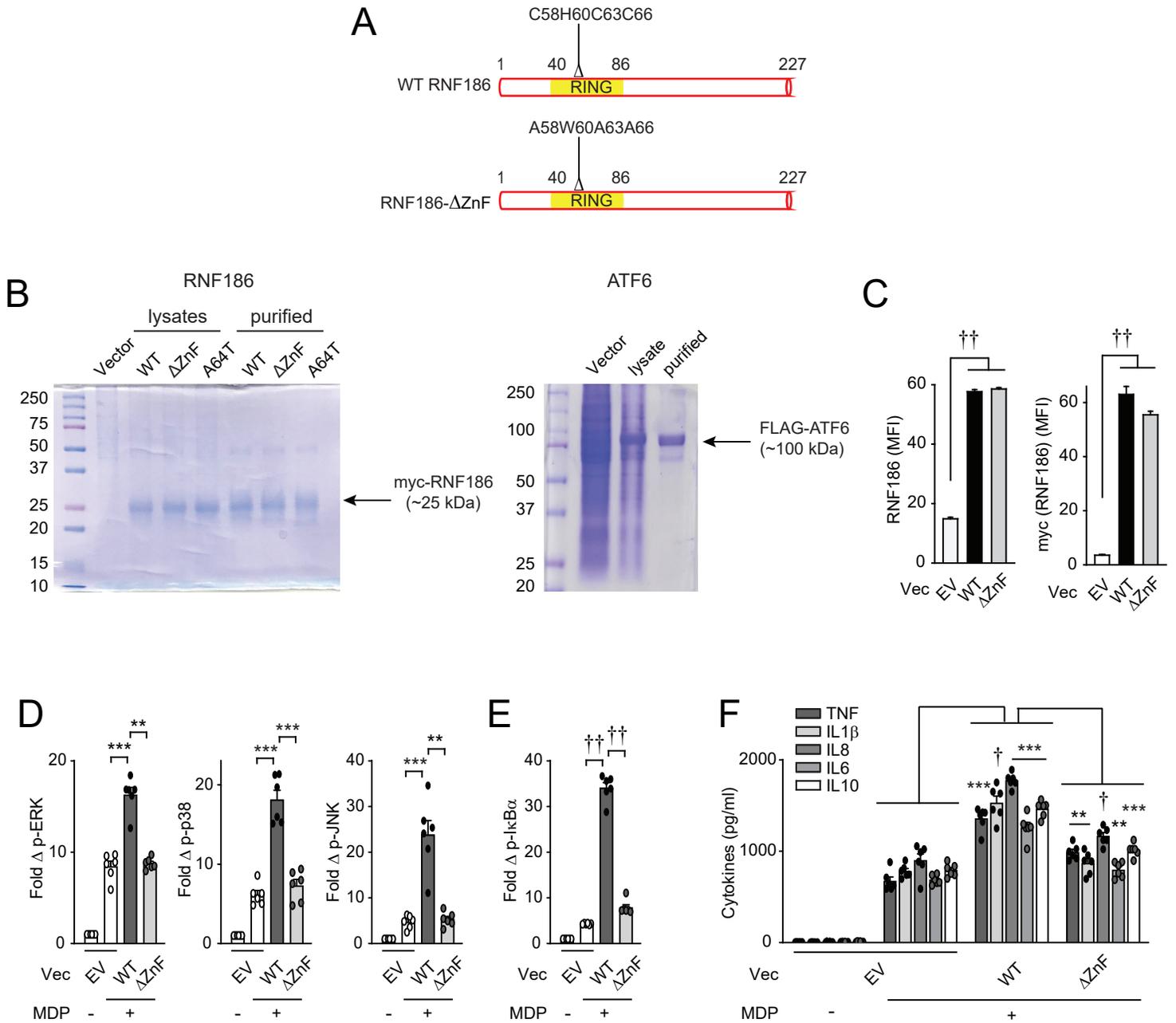
**Figure S6. A C-terminal region in RNF186 regulates localization to the ER. (A)** RNF186 protein model representing wild type (WT) and K219A mutant (predicted ER localization). **(B-C)** HeLa cells were transfected with GFP-tagged RNF186-WT or RNF186-K219A and NOD2 and then treated with 100µg/ml MDP for 30min. Cells were immunostained for calnexin (ER, red) and nucleus (DAPI, blue). **(B)** Representative micrographs with scale bar representing 10µm. Inset represents enlarged images from the merged panel. **(C)** Summary graph of percent RNF186 variants colocalized with calnexin (ER) (25 cells quantified). Representative of two independent experiments. ††,  $p < 1 \times 10^{-5}$ .

# Supplementary Figure 7



**Figure S7. A C-terminal region in RNF186 regulating ER localization regulates PRR-induced UPR signaling, downstream signaling and cytokine secretion in MDMs.** MDMs (rs6426833 AA low RNF186-expressing carriers) were transfected with empty vector (EV) or myc-tagged RNF186 WT or K219A. **(A)** RNF186 protein expression as detected by flow cytometry (mean fluorescence intensity [MFI]) (n=6). **(B-E)** Transfected MDMs (n=6) were treated with 100 $\mu$ g/ml MDP. **(B)** Fold phospho-PERK and phospho-IRE1 $\alpha$  at 30min. **(C-D)** Fold phospho-protein induction at 15min. **(E)** Cytokines at 24h. Mean + SEM. Vec, vector. \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>.

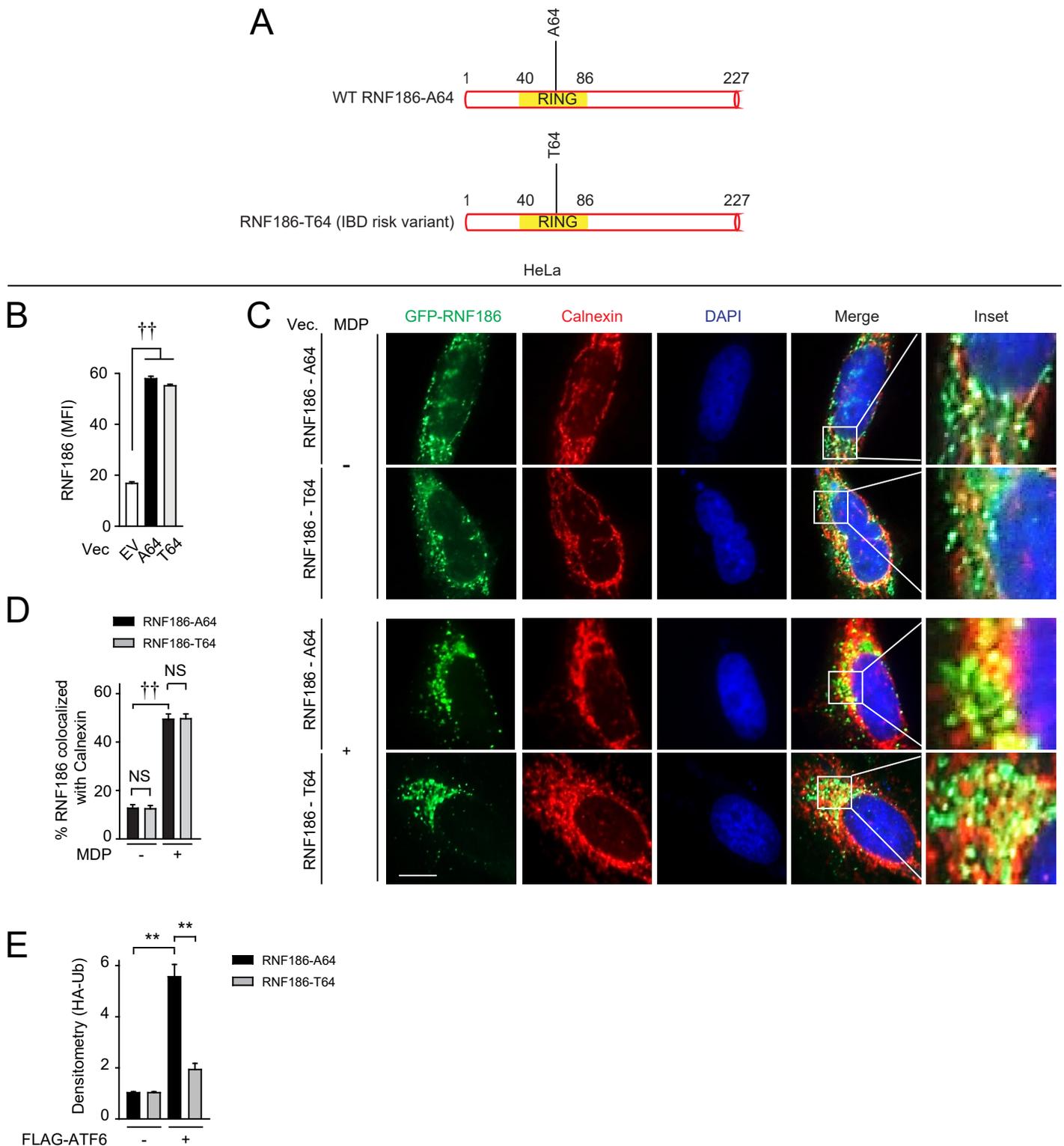
# Supplementary Figure 8



**Figure S8. RNF186 E3 ubiquitin ligase activity is required for NOD2-induced, UPR-dependent outcomes.** (A) RNF186 protein model representing wild type (WT) and  $\Delta$ ZnF mutant. (B) Purified myc-tagged RNF186 (WT or  $\Delta$ ZnF or A64T) and FLAG-tagged ATF6 proteins were assessed by Coomassie staining. Lysates prior to purification are included as controls to show relatively enriched purified bands and contrast with singular band after purification. (C-F) MDMs (low RNF186-expressing rs6426833 AA carriers) were transfected with empty vector (EV) or myc-tagged RNF186 WT or  $\Delta$ ZnF. (C) RNF186 protein expression as detected with anti-RNF186 and anti-myc antibodies by flow cytometry (n=6). (D-F) Transfected MDMs (n=6) were treated with 100 $\mu$ g/ml MDP. (D-E) Fold phospho-protein at 15min. (F) Cytokines at 24h. Mean + SEM. Vec, vector. \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>; ††, p<1 $\times$ 10<sup>-5</sup>.

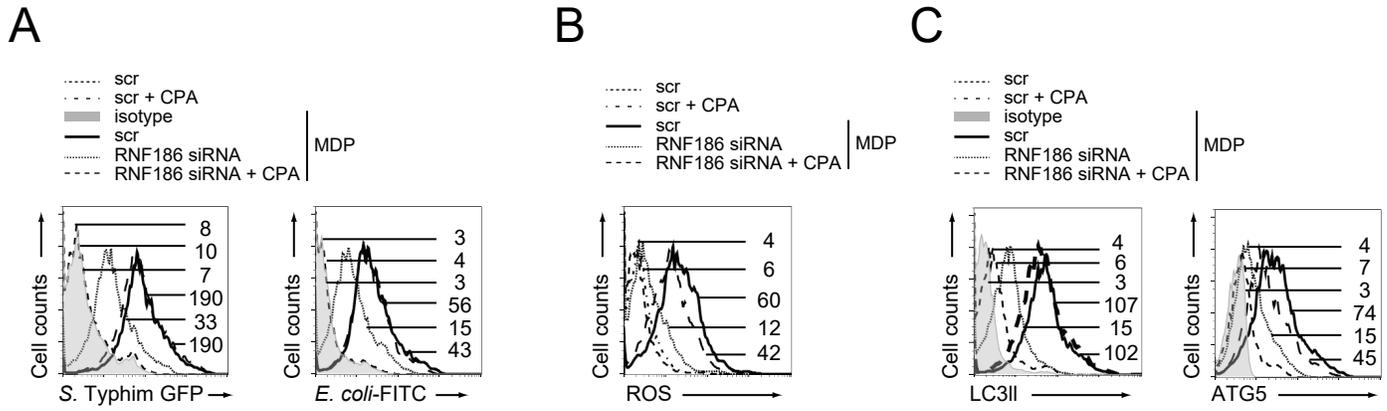


# Supplementary Figure 10



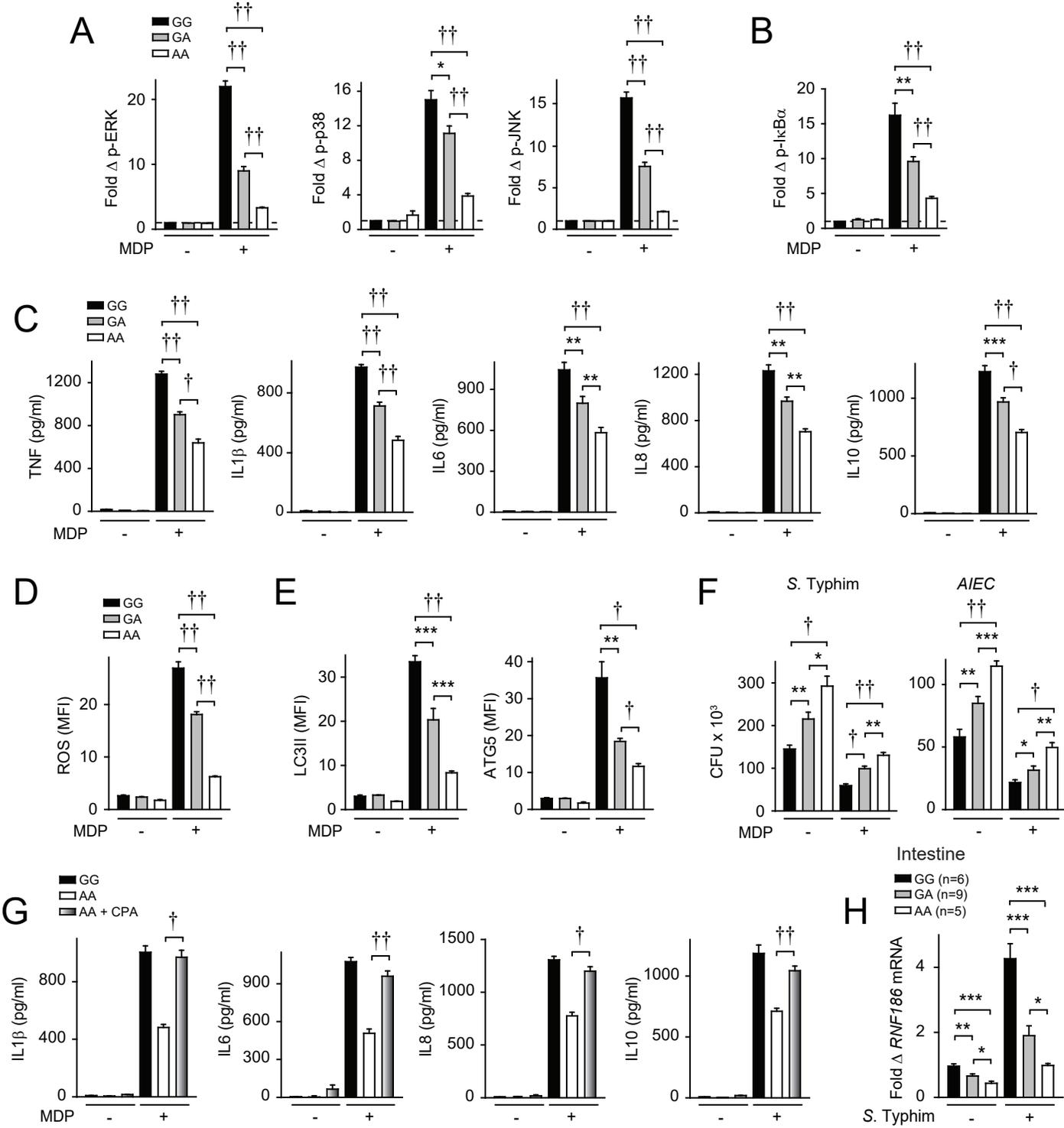
**Figure S10. The rare RNF186-A64T disease-risk variant expresses at levels equal to WT RNF186 and localizes normally to the ER. (A)** RNF186 protein model representing RNF186 A64 wild type (WT) and T64 mutant (rare variant). **(B-D)** HeLa cells were transfected with GFP-tagged RNF186 A64 or T64 and with NOD2. **(B)** RNF186 protein expression by flow cytometry (6 replicates). **(C-D)** Transfected cells were treated with 100µg/ml MDP for 30min and then immunostained for calnexin (ER, red) and nucleus (DAPI, blue). **(C)** Representative micrographs with scale bar representing 10µm. Inset represents enlarged images from the merged panel. **(D)** Summary graph indicating percent RNF186 colocalized with calnexin (ER) (25 cells quantified). Representative of two independent experiments. **(E)** Quantification of **Figure 6A** for in vitro ubiquitination of ATF6 (2 independent experiments). Mean + SEM. NS, not significant. Vec, vector. \*\*, p<0.01; ††, p<1×10<sup>-5</sup>.

# Supplementary Figure 11



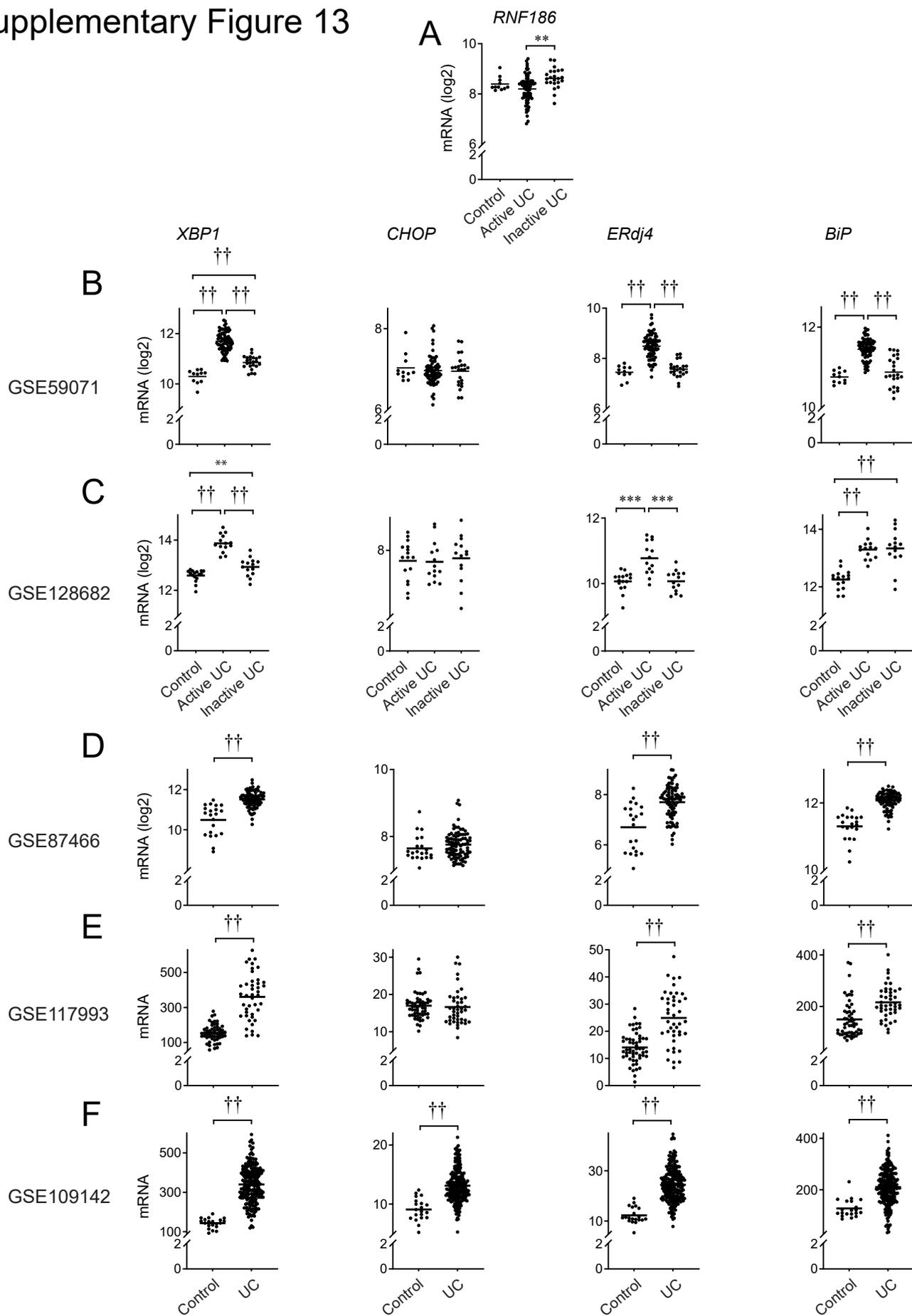
**Figure S11. RNF186-dependent UPR signaling regulates NOD2-induced antimicrobial pathways.** MDMs (n=6) were transfected with scrambled or RNF186 siRNA, then treated with 100  $\mu$ g/ml MDP for 48h  $\pm$  10 $\mu$ M CPA and analyzed as per **Figure 7** by flow cytometry with representative histograms for: **(A) Figure 7A**, **(B) Figure 7C**, and **(C) Figure 7D**. Scr, scrambled.

# Supplementary Figure 12



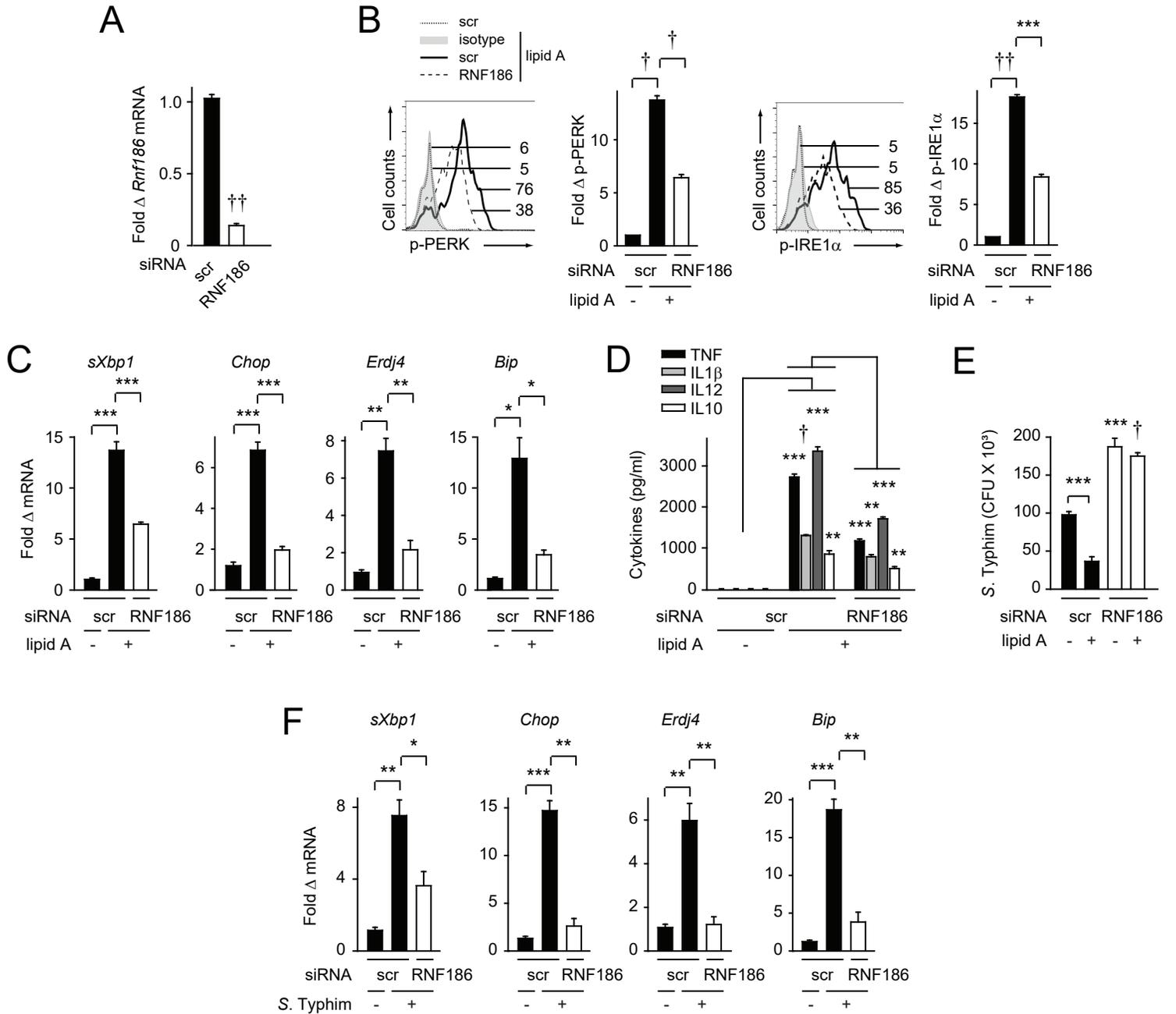
**Figure S12. MDMs from IBD-risk rs6426833 A carriers show reduced NOD2-induced signaling, cytokines and antimicrobial pathways. (A-F)** MDMs from rs6426833 GG, GA and AA carriers (n=10 donors/genotype) were left untreated or treated with 100µg/ml MDP. **(A-B)** Fold phospho-protein induction at 15min. **(C)** Cytokines at 12h. **(D)** ROS at 48h. **(E)** LC3II & ATG5 expression at 48h. **(F)** After 48h, intracellular bacterial clearance was assessed. **(G)** MDMs from rs6426833 GG and AA carriers (n=10 donors/genotype) were left untreated or treated with 100µg/ml MDP ± 10µM CPA and assessed for cytokines at 12h. **(H)** Human intestinal myeloid cells were co-cultured with *S. Typhimurium* for 2h. *RNF186* mRNA expression stratified on rs6426833 genotype. Mean + SEM. \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †, p<1×10<sup>-4</sup>; ††, p<1×10<sup>-5</sup>.

# Supplementary Figure 13



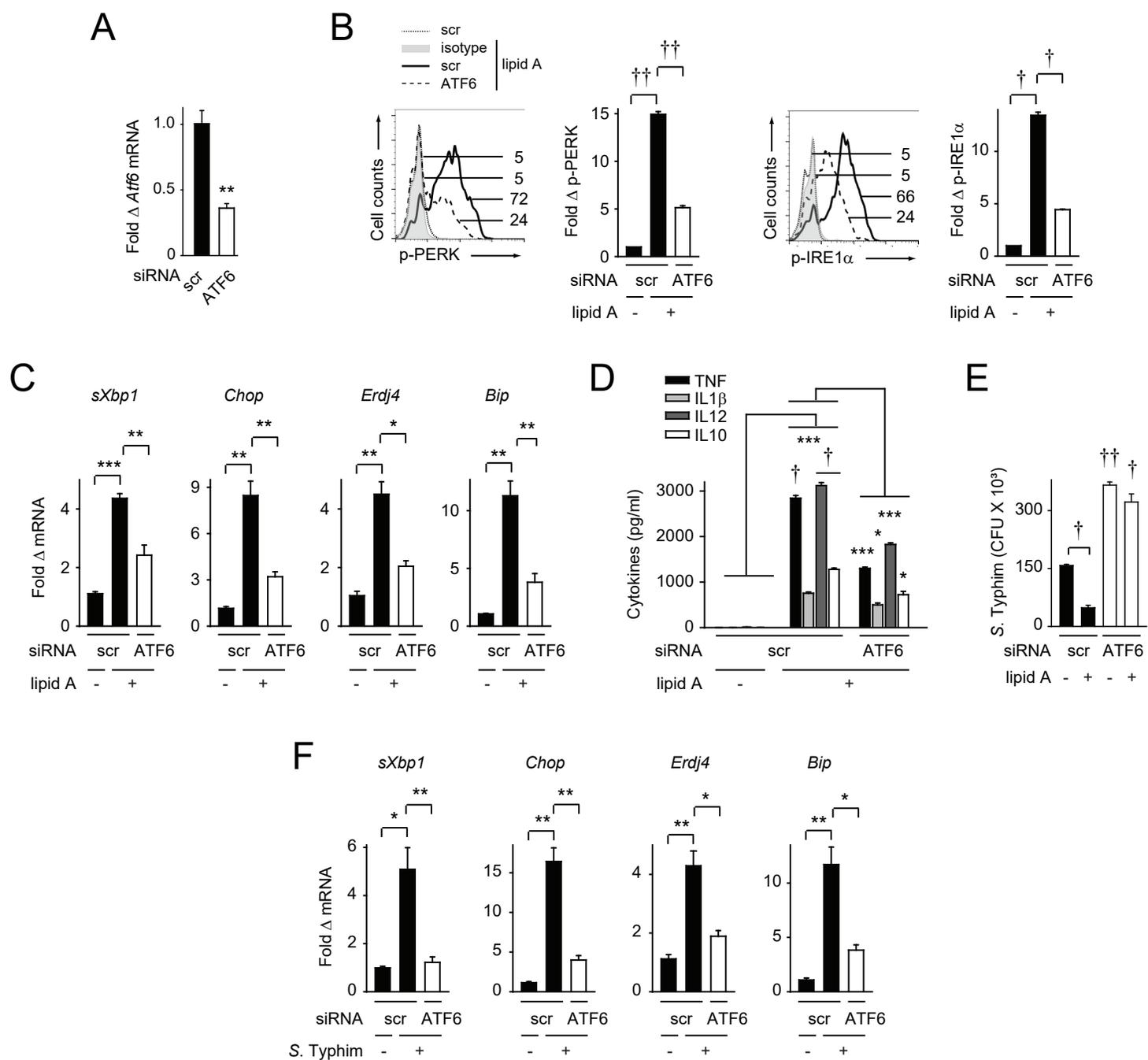
**Figure S13. UPR pathways are upregulated in colonic tissues from UC patients.** Colonic mRNA expression for the indicated genes was analyzed from the following datasets: **(A-B)** GSE59071 (n=11 control; n=74 active UC; n=23 inactive UC). **(C)** GSE128682 (n=16 control; n=14 active UC; n=14 inactive UC). **(D)** GSE87466 (n=21 control; n=87 UC). **(E)** GSE117993 (RISK pediatric cohort: n=55 control; n=43 UC). **(F)** GSE109142 (PROTECT pediatric cohort: n=20 control; n=206 UC). \*\*, p<0.01; \*\*\*, p<0.001; ††, p<1×10<sup>-5</sup>.

# Supplementary Figure 14



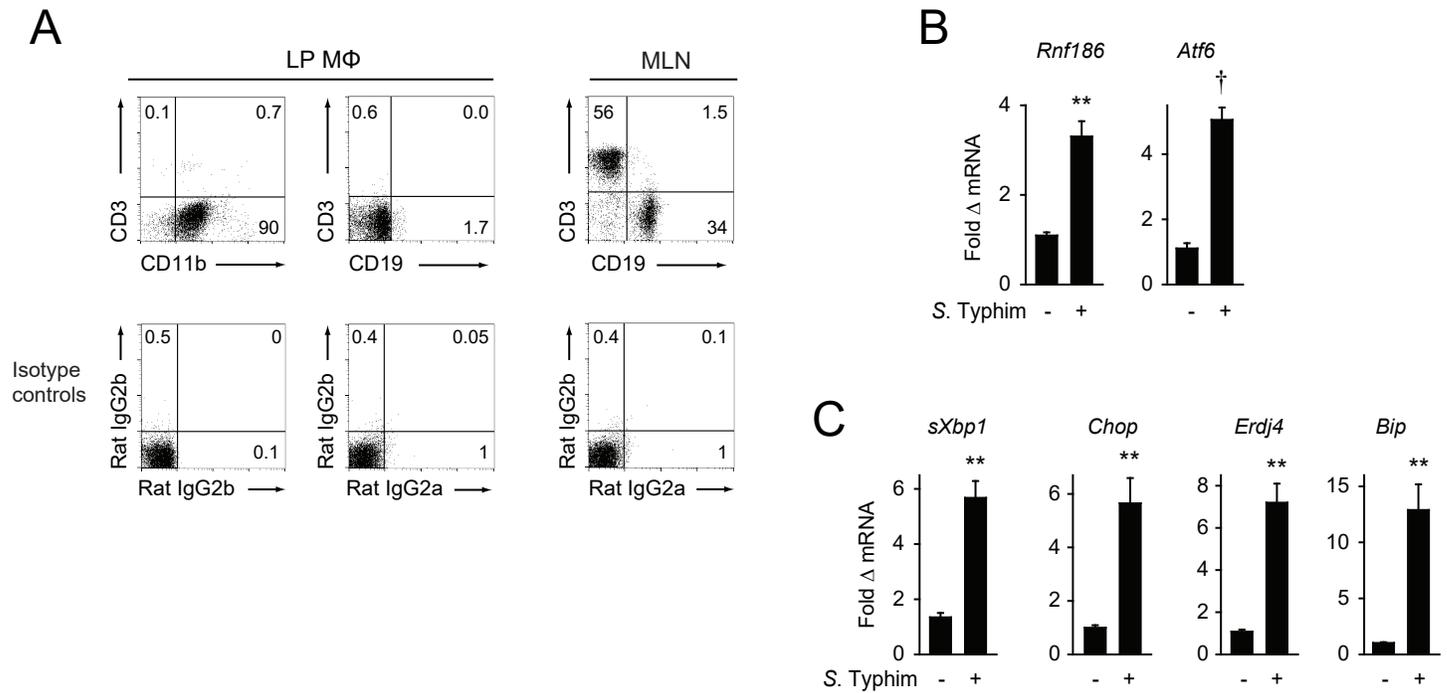
**Figure S14. RNF186 promotes PRR-induced UPR signaling, cytokines and bacterial clearance in mouse BMMs.** Mouse bone marrow-derived macrophages (BMMs) were transfected with scrambled or RNF186 siRNA. **(A)** Fold mRNA expression (5 replicates). **(B-E)** Cells were then treated with 0.1  $\mu$ g/ml lipid A. **(B)** Fold phospho-protein induction at 30min (5 replicates; representative of 2 independent experiments). **(C)** Fold mRNA expression at 4h (5 replicates). **(D)** Cytokines at 24h (5 replicates). **(E)** After 48h intracellular bacterial clearance was assessed (5 replicates). Significance is between RNF186 siRNA-transfected and scrambled siRNA-transfected cells for the corresponding condition or as indicated. **(F)** Cells were co-cultured with *S. Typhimurium*. Fold mRNA expression at 4h (5 replicates). Mean + SEM. Scr, scrambled. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; †,  $p < 1 \times 10^{-4}$ ; ††,  $p < 1 \times 10^{-5}$ .

# Supplementary Figure 15



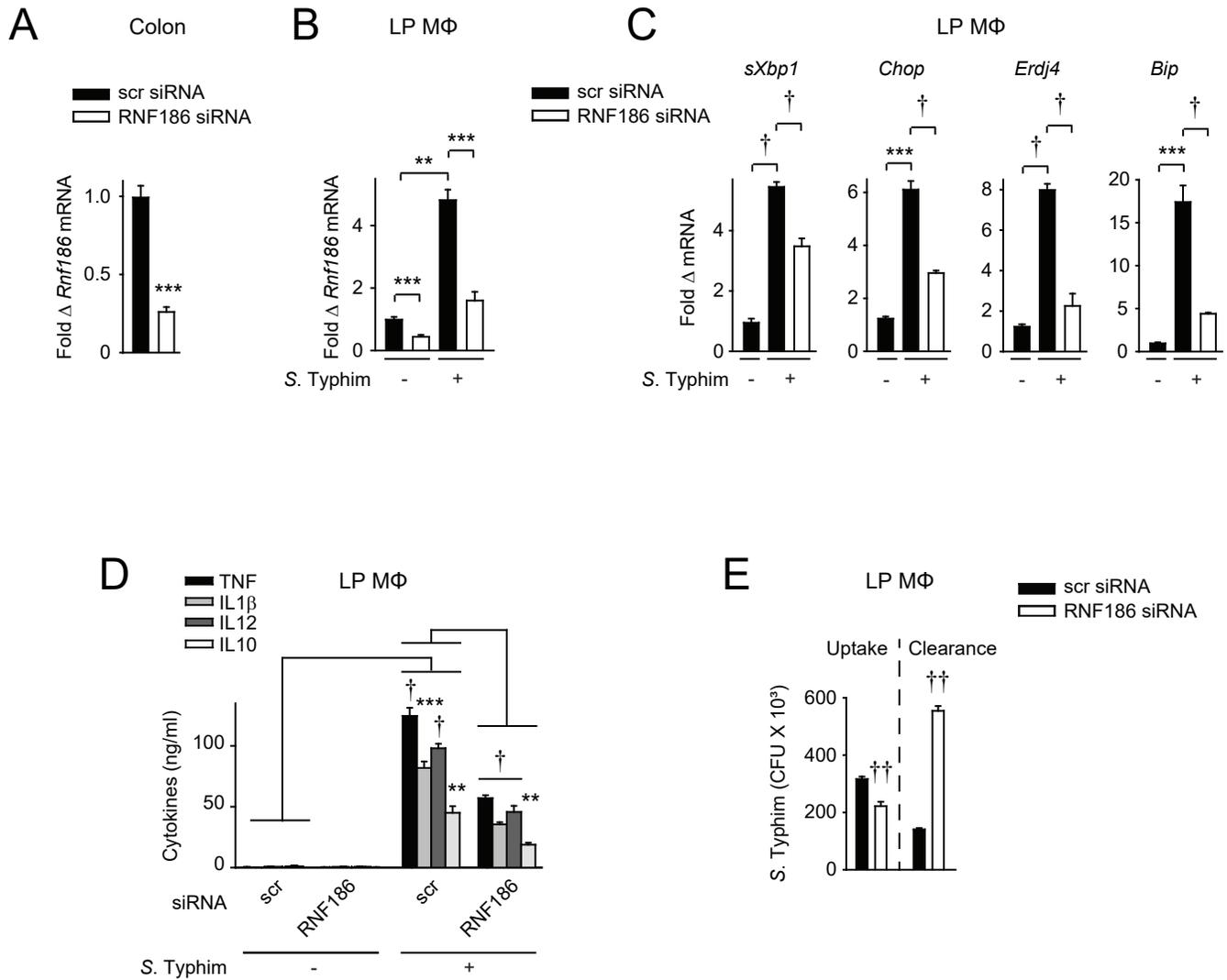
**Figure S15. ATF6 promotes PRR-induced UPR signaling, cytokines and bacterial clearance in mouse BMMs.** Mouse BMMs were transfected with scrambled or ATF6 siRNA. **(A)** Fold mRNA expression (5 replicates). **(B-E)** Cells were then treated with 0.1 $\mu$ g/ml lipid A. **(B)** Fold phospho-protein induction at 30min (5 replicates; representative of 2 independent experiments). **(C)** Fold mRNA expression at 4h (5 replicates). **(D)** Cytokines at 24h (5 replicates). **(E)** After 48h intracellular bacterial clearance was assessed (5 replicates). Significance is between ATF6 siRNA-transfected and scrambled siRNA-transfected cells for the corresponding condition or as indicated. **(F)** Cells were co-cultured with *S. Typhimurium*. Fold mRNA expression at 4h (5 replicates). Mean + SEM. Scr, scrambled. \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; †,  $p < 1 \times 10^{-4}$ ; ††,  $p < 1 \times 10^{-5}$ .

# Supplementary Figure 16



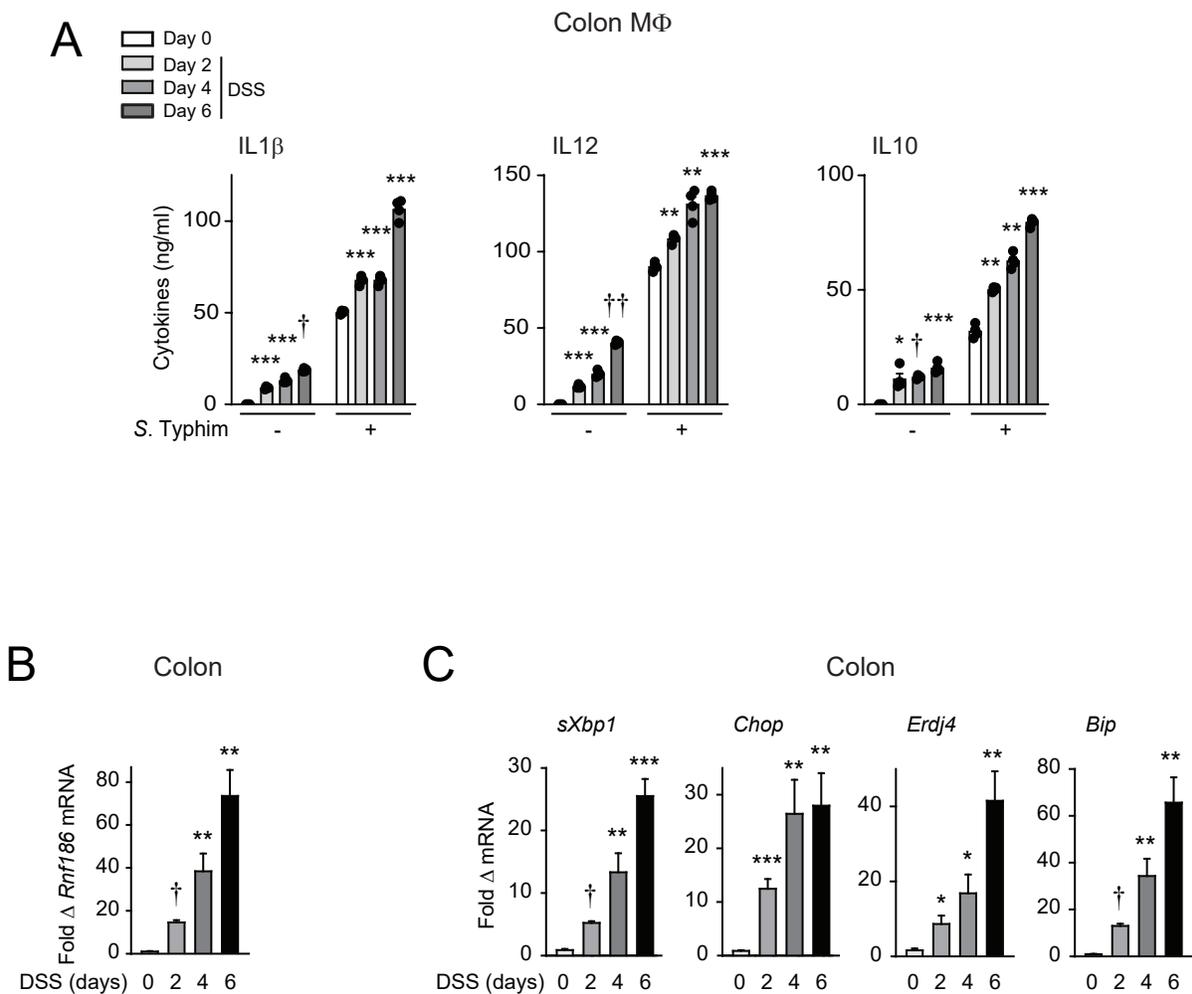
**Figure S16. Mouse colonic lamina propria macrophages co-cultured ex vivo with *S. Typhimurium* upregulate the UPR.** Colonic lamina propria macrophages (LP M $\Phi$ ) were isolated from WT mice. **(A)** Purity of colonic LP macrophages by flow cytometry. Mesenteric lymph nodes (MLN) were assessed as a positive control for CD3 and CD19 cells. **(B-C)** Cells were co-cultured with *S. Typhimurium*. **(B)** Fold *Rnf186* and *Atf6* mRNA at 4h (5 replicates). **(C)** Fold mRNA expression at 4h (5 replicates). Mean + SEM. \*\*,  $p < 0.01$ ; †,  $p < 1 \times 10^{-4}$ .

# Supplementary Figure 17



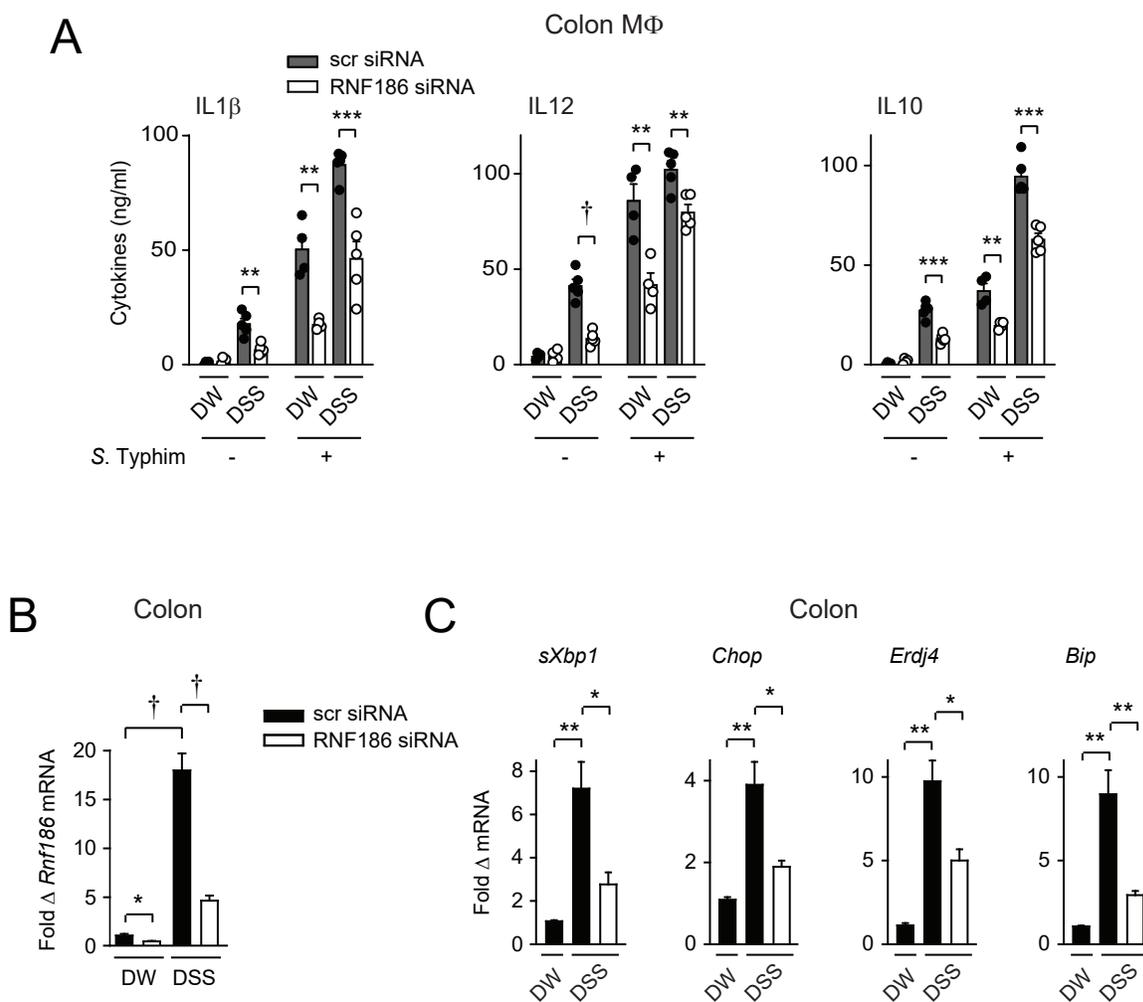
**Figure S17. RNF186 promotes the UPR, cytokines, bacterial uptake and bacterial clearance in colonic lamina propria macrophages.** Mice (n=5) were administered scrambled or RNF186 siRNA i.p. **(A)** Colon *Rnf186* mRNA expression. **(B-E)** Colonic lamina propria macrophages were isolated and co-cultured with *S. Typhimurium*. **(B)** *Rnf186* mRNA at 4h. **(C)** Fold mRNA expression at 4h. **(D)** Cytokines at 24h. **(E)** Bacterial uptake at 20min and gentamicin was then added and intracellular bacterial clearance was assessed after a total of 4h of bacterial co-culture. Mean + SEM. LP, lamina propria; M $\Phi$ , macrophage; scr, scrambled. \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times 10^{-4}$ ; ††, p<1 $\times 10^{-5}$ .

# Supplementary Figure 18



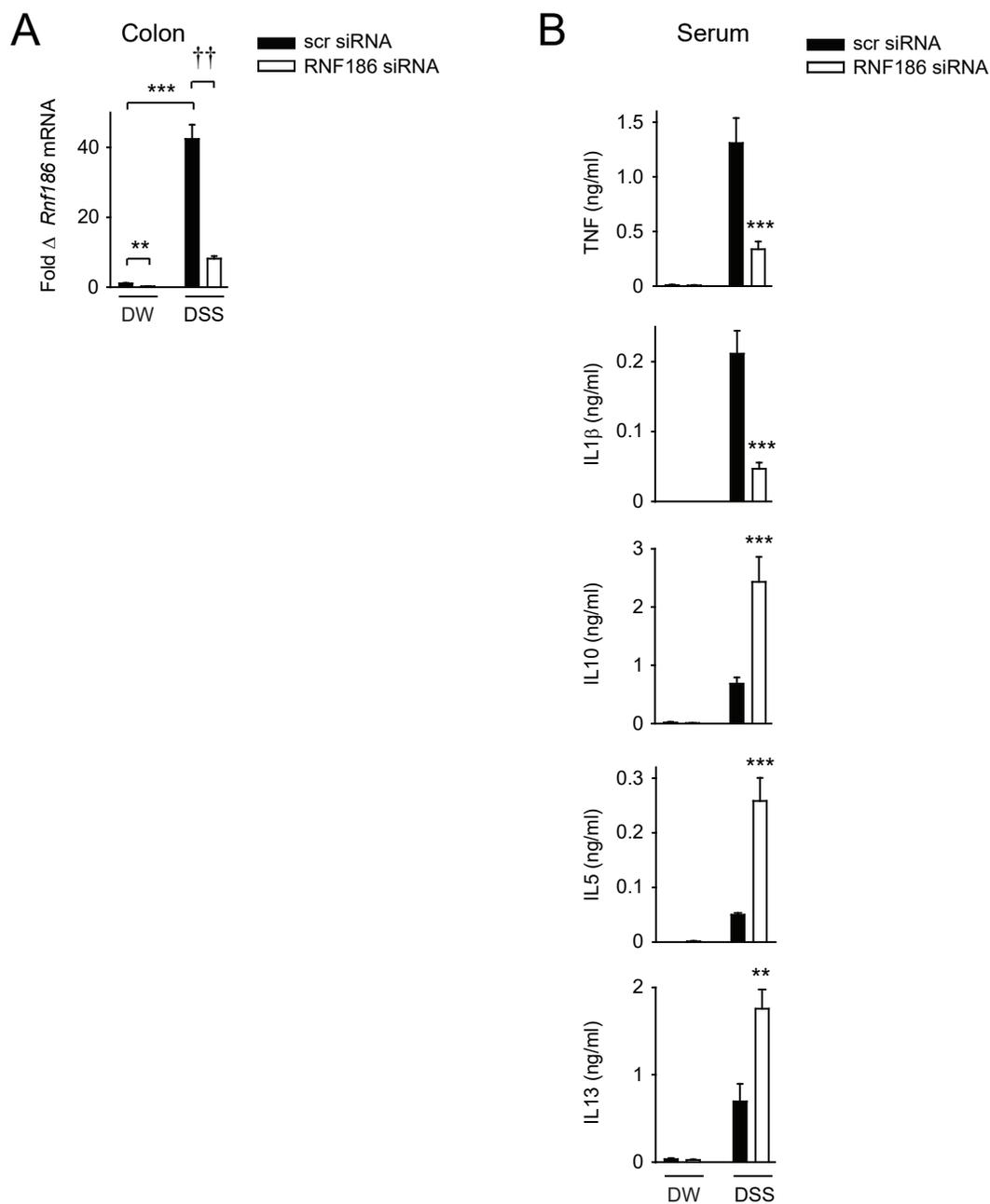
**Figure S18. RNF186 promotes the UPR in colonic tissues upon DSS administration.** Mice (n=4/time point) were given 2.5% DSS in drinking water (DW) for the indicated times. **(A)** Cytokine secretion from colonic macrophages from **Figure 10C**. **(B-C)** Colonic mRNA expression. Macrophage, MΦ. \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †, p<1×10<sup>-4</sup>; ††, p<1×10<sup>-5</sup>.

# Supplementary Figure 19



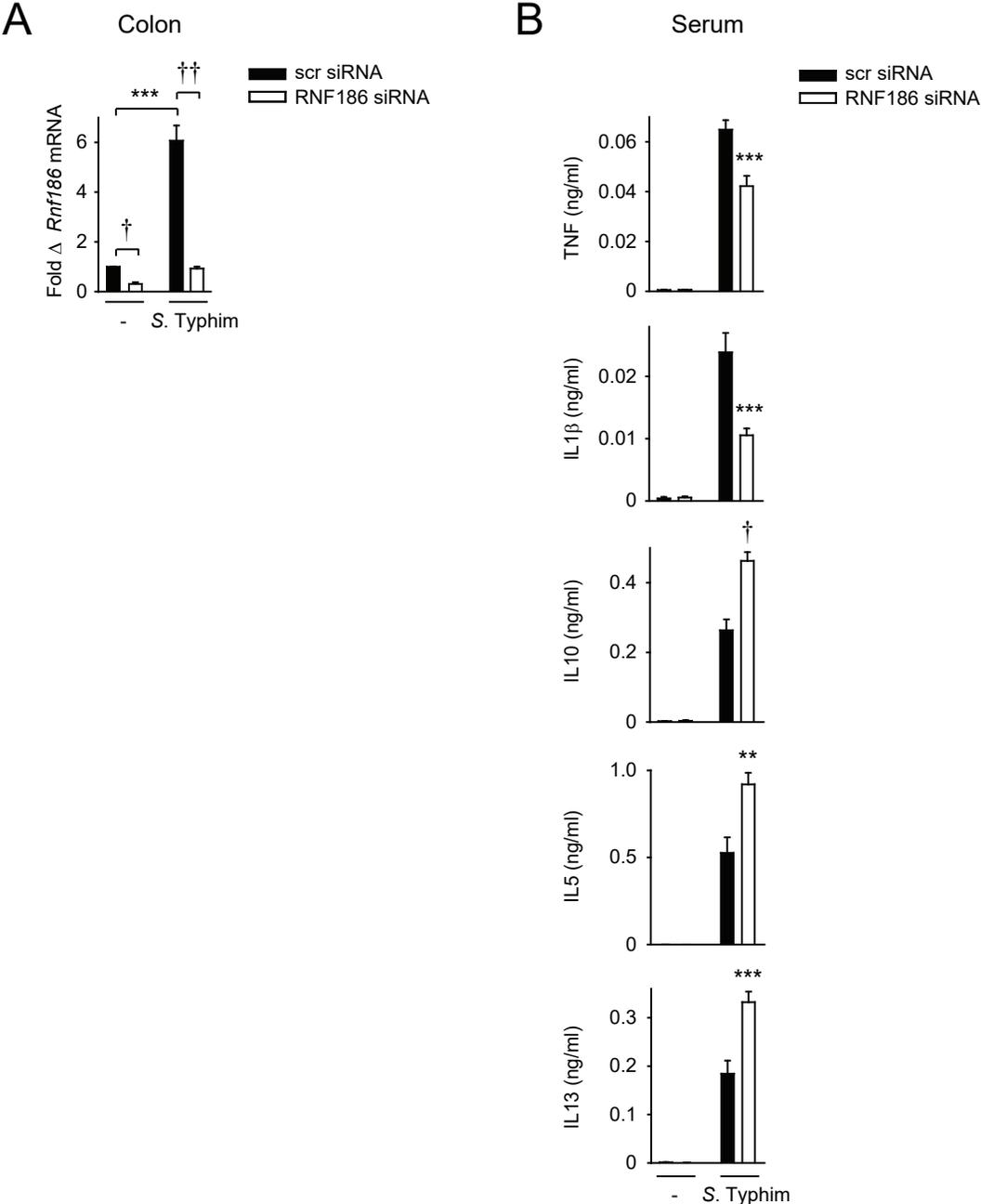
**Figure S19. RNF186 promotes the UPR in colonic tissues upon DSS administration.** Mice (n=5) were administered scrambled or RNF186 siRNA i.p. Mice were then given 2.5% DSS in DW for 4 days. **(A)** Cytokine secretion from colonic lamina propria macrophages from **Figure 10G**. **(B-C)** Colonic mRNA expression. Mean + SEM. Macrophage, M $\Phi$ ; scr, scrambled. \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>.

# Supplementary Figure 20



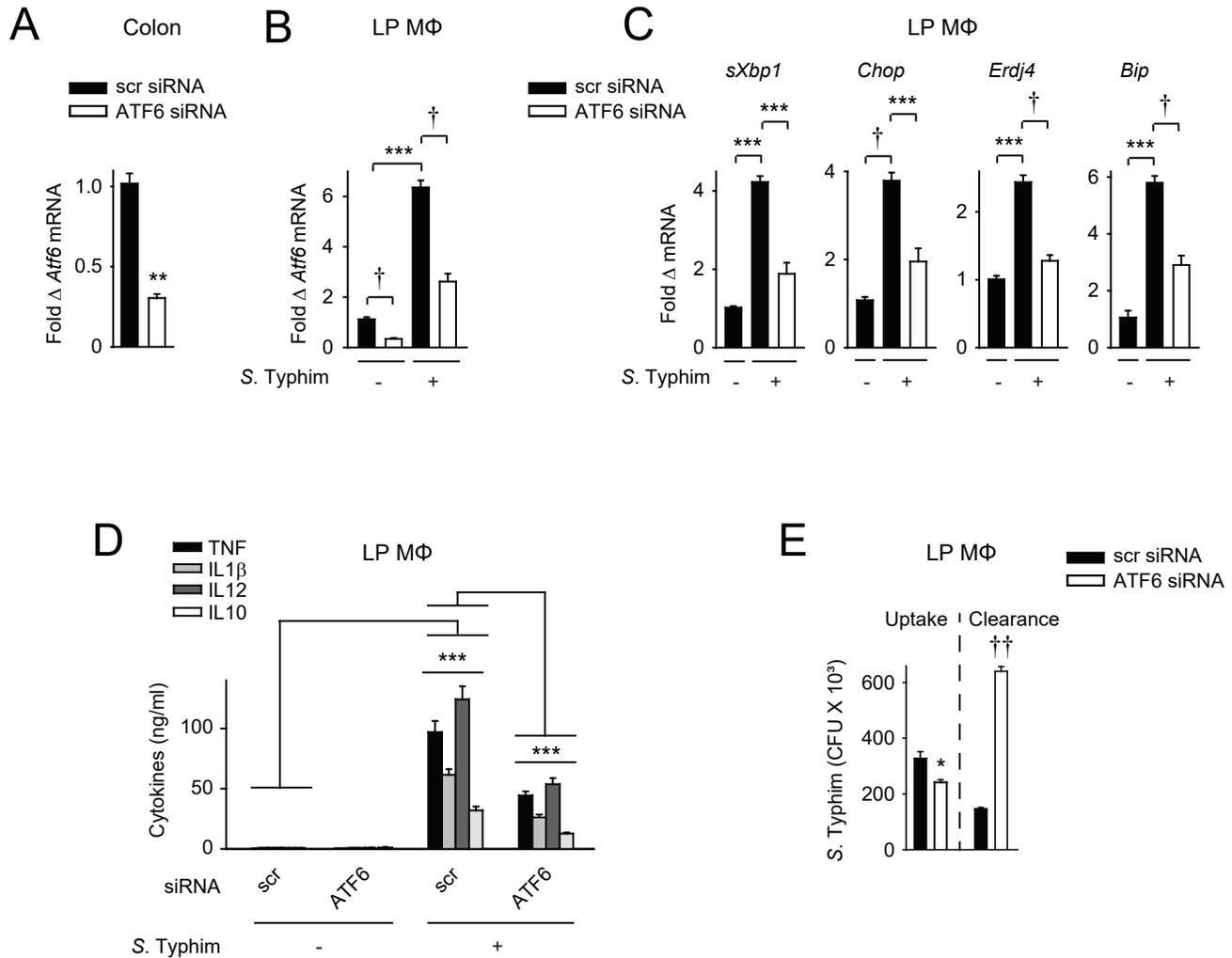
**Figure S20. RNF186 regulates serum cytokines in mice administered DSS.** Mice in **Figure 11** were assessed for: **(A)** Colon *Rnf186* mRNA expression. **(B)** Serum cytokines. Mean + SEM. Scr, scrambled. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; ††,  $p < 1 \times 10^{-5}$ .

# Supplementary Figure 21



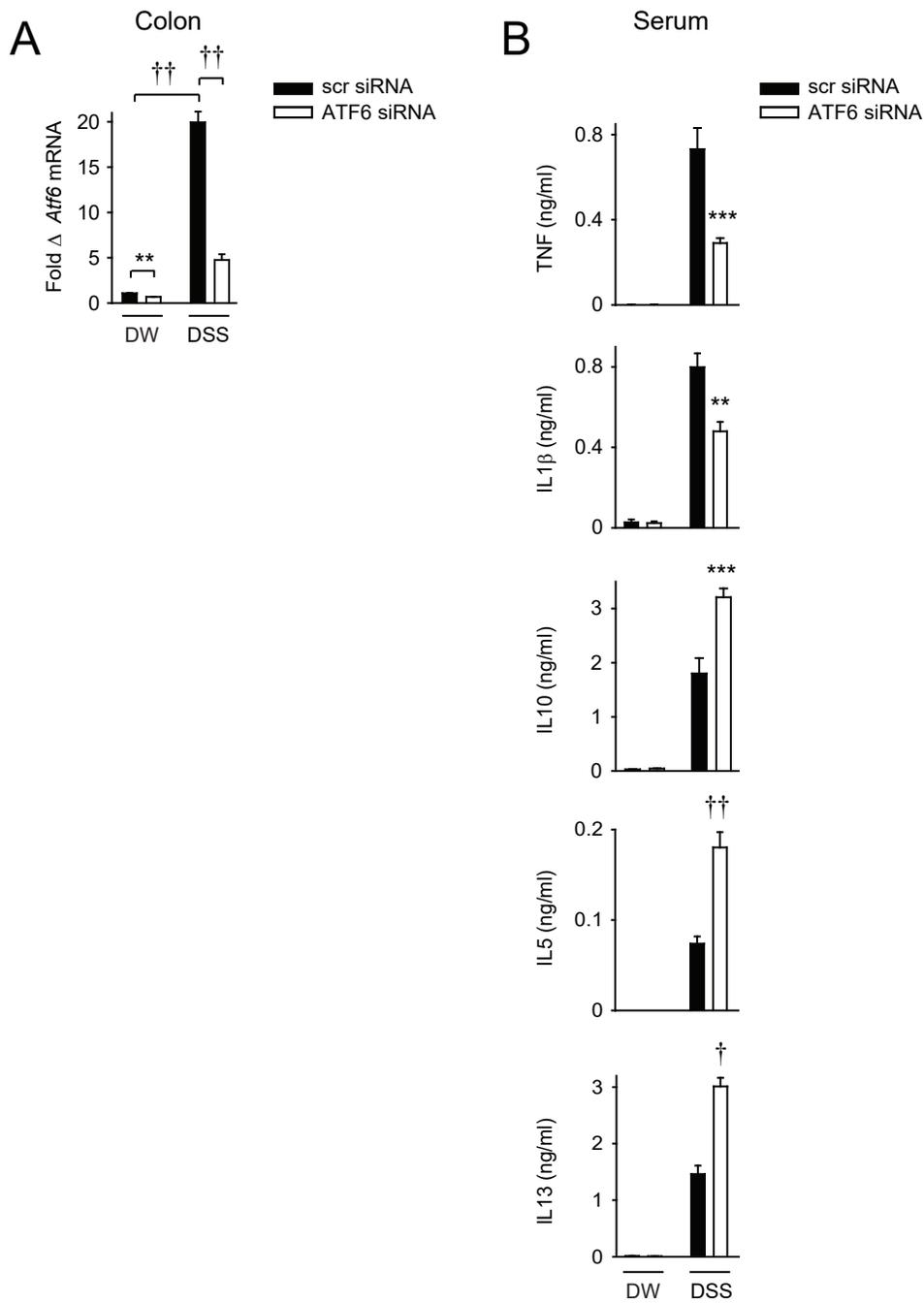
**Figure S21. RNF186 regulates serum cytokines in mice orally infected with *S. Typhimurium*.** Mice in **Figure 12** were assessed for: **(A)** Colon *Rnf186* mRNA expression. **(B)** Serum cytokines. Mean + SEM. Scr, scrambled. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; †,  $p < 1 \times 10^{-4}$ ; ††,  $p < 1 \times 10^{-5}$ .

# Supplementary Figure 22



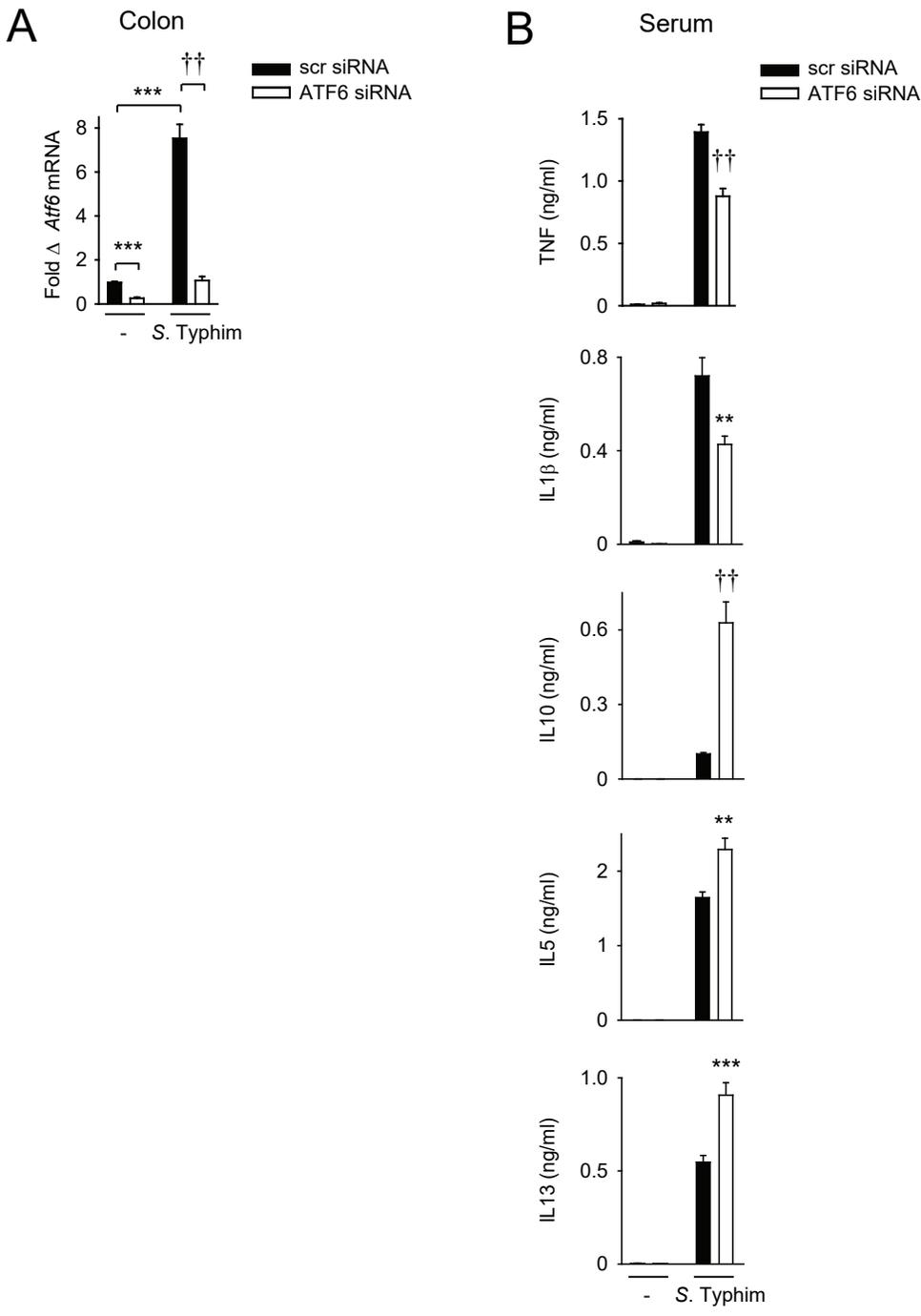
**Figure S22. ATF6 promotes the UPR, cytokines, bacterial uptake and bacterial clearance in colonic lamina propria macrophages.** Mice (n=5) were administered scrambled or ATF6 siRNA i.p. **(A)** Colon *Atf6* mRNA expression. **(B-E)** Colonic lamina propria macrophages were isolated and co-cultured with *S. Typhimurium*. **(B)** *Atf6* mRNA at 4h. **(C)** Fold mRNA expression at 4h. **(D)** Cytokines at 24h. **(E)** Bacterial uptake at 20min and gentamicin was then added and intracellular bacterial clearance was assessed 4h after initial bacterial co-culture. Mean + SEM. LP, lamina propria; M $\Phi$ , macrophage, scr, scrambled. \*, p<0.05; \*\*, p<0.01; \*\*\*, p<0.001; †, p<1 $\times$ 10<sup>-4</sup>; ††, p<1 $\times$ 10<sup>-5</sup>.

# Supplementary Figure 23



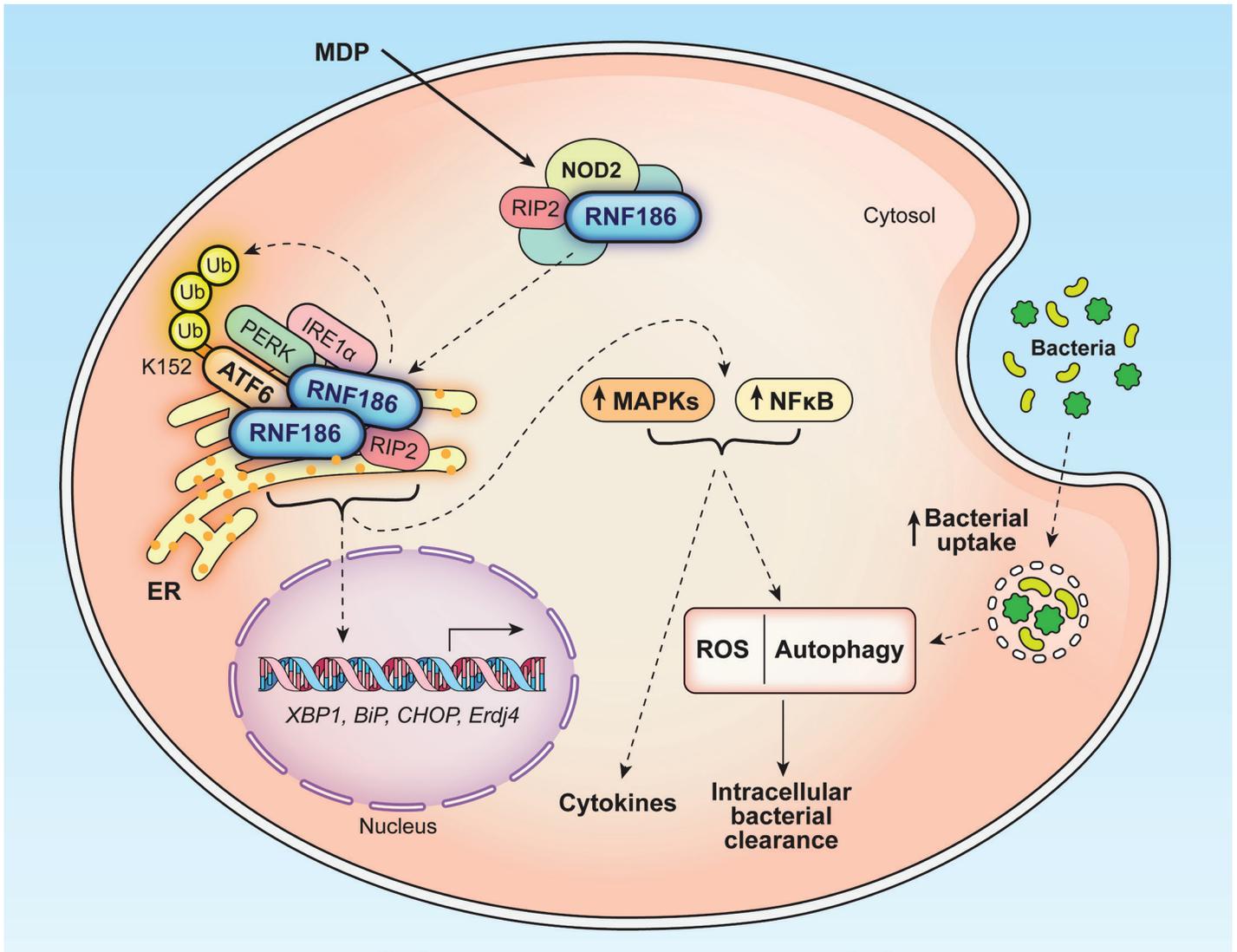
**Figure S23. ATF6 regulates serum cytokines in mice administered DSS.** Mice in **Figure 13** were assessed for: **(A)** Colon *Atf6* mRNA expression. **(B)** Serum cytokines. Mean + SEM. Scr, scrambled. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; †,  $p < 1 \times 10^{-4}$ ; ††,  $p < 1 \times 10^{-5}$ .

# Supplementary Figure 24



**Figure S24. ATF6 regulates serum cytokines in mice orally infected with *S. Typhimurium*.** Mice in **Figure 14** were assessed for: **(A)** Colon *Atf6* mRNA expression. **(B)** Serum cytokines. Mean + SEM. Scr, scrambled. \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ ; ††,  $p < 1 \times 10^{-5}$ .

## Supplementary Figure 25



**Figure S25. Model of RNF186-mediated regulation of PRR-initiated UPR-dependent outcomes.** Upon PRR stimulation in human MDMs, RNF186 localization to the ER transiently increases and forms a complex with the UPR sensors IRE1 $\alpha$ , PERK and ATF6. RNF186 localization to the ER is critical for PRR-induced, UPR-dependent MAPK and NF $\kappa$ B signaling, cytokine secretion, and the induction of multiple antimicrobial pathways, including bacterial uptake, ROS and autophagy. Importantly, RNF186 ubiquitinates ATF6 at K152, and this ubiquitination is required for PRR-induced, UPR-dependent outcomes. Both the rare RNF186 A64T IBD risk variant and the common rs64268331 A IBD risk variant in the *RNF186* region demonstrate an impaired PRR-induced UPR, and in turn, reduced downstream functions; these functions are restored with complementation of the UPR. Further, RNF186-deficient mice demonstrate impaired clearance of resident intestinal bacteria during acute colitis and of *S. Typhimurium* upon oral infection; ATF6-deficient mice demonstrate similar outcomes consistent with the role of ATF6 downstream of RNF186.

Table S1

GENE	PRIMER SEQUENCE
<b>Human</b>	
<i>RNF186</i>	FWD: 5' GAGGATGGACAGGATGAAGTAAG REV: 5' AGACACCCGGGTAGATGAA
<i>BiP</i>	FWD: 5' GAACGTCTGATTGGCGATGC REV: 5' TCAACCACCTTGAACGGCAA
<i>CHOP</i>	FWD: 5' TTAAGATGAGCGGGTGGCA REV: 5' GTTGGATCAGTCTGCTTTCAGG
<i>ERDJ4</i>	FWD: 5' TAGTCGGAGGGTGCAGGATA REV: 5' CGCTCTGATGCCGATTTTGG
<i>sXBP1</i>	FWD: 5' AGACAGCGCTTGGGGATGGAT REV: 5' CCTGCACCTGCTGCGGACTC
<i>GAPDH</i>	FWD: 5' GGCATGGACTGTGGTCATGAG REV: 5' TGCACCACCAACTGCTTAGC
<b>Mouse</b>	
<i>Rnf186</i>	FWD: 5' CCTGGAATGCTTGGTGTGCCG REV: 5' GGATGGACCAGGTGTCTTCCT
<i>Atf6</i>	FWD: 5' GCGGATGATAAAGAACCGAGAG REV: 5' ACAGACAGCTCTTCGCTTTG
<i>Bip</i>	FWD: 5' TCATCGGACGCACTTGGAA REV: 5' CAACCACCTTGAATGGCAAGA
<i>Chop</i>	FWD: 5' GTCCTAGCTTGGCTGACAGA REV: 5' TGGAGAGCGAGGGCTTTG
<i>Erdj4</i>	FWD: 5' GCGCACAGGTTATTAGAAATG REV: 5' TCGCTCTGAGGCAGACTTTG
<i>sXbp1</i>	FWD: 5' AAGAACACGCTTGGGAATGG REV: 5' CTGCACCTGCTGCGGAC
<i>Gapdh</i>	FWD: 5' CCACTCACGGCAAATTC AAC REV: 5' CTCCACGACATACTCAGCAC

Table S1. Primer Table.