such as infliximab, have been developed recently for the treatment of IBD, a number of clinical problems, such as side effects, are yet to be addressed.[57] Thus, IBD remains an uncured disease for which the development of new types of drugs is clinically important. As described above, some aspects of the ER stress response are positively and other aspects negatively involved in IBD development. As such, factors located downstream (such as CHOP) rather than upstream (such as ATF6 and IRE1) of the ER stress response may be better drug targets for IBD. Results in this study suggest that inhibitors of CHOP may be therapeutically beneficial for IBD.

In summary, results in this study show that CHOP is positively involved in the development of DSS-induced colitis. Furthermore, the results suggest that this effect involves various mechanisms, such as Mac-1-induced infiltration of macrophages, ERO-1α-induced ROS-production, Caspase-11-induced production of IL-1β, and stimulation of mucosal apoptosis.

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Figure Legends

Fig. 1. Development of DSS-induced colitis in CHOP-null mice and wild-type mice. CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT) were treated with or without 3% DSS for 7 days. Body weight (A) and DAI (B) were measured daily. After 7 days, length of colon (C), colonic MPO activity (D) and colonic TBARS (E) were determined as described in the materials and methods. After 7 days, sections of colonic tissues were prepared and subjected to histological examination (H & E staining) and the damage score and extent of lesion for 7 independent sections were determined (G, H). One of the sections is shown (F). Values are mean ± S.E.M. (n=5-13). $^{**}P<0.01$; $^{*}P<0.05$; n.s., not significant.

Fig. 2. Expression of CHOP and GRP78 in colonic tissues of CHOP-null mice and wild-type mice. DSS was administered to CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT), as described in the legend of Fig. 1. Colonic tissues were removed and total RNA was extracted. Samples were subjected to real-time RT-PCR, using a specific primer set

for *Chop or Grp78*. Values were normalized to *Gapdh*, expressed relative to the control sample (i.e. wild-type mice without DSS-treatment), and given as the mean ± S.E.M. (n=4). ** or $^{\#\#}P<0.01$; $*P<0.05$; n.s., not significant (A). Sections of colonic tissues were prepared and subjected to immunohistochemical analysis with an antibody against CHOP (B) or GRP78 (C). The right panel in each column is a 4-times magnified image of the boxed area defined in the left panel (B). Total protein was extracted from colonic tissues and analysed by immunoblotting with an antibody against CHOP, GRP78 or actin (D).

Fig. 3. Development of TNBS-induced colitis in CHOP-null mice and wild-type mice. CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT) were intrarectally administered with (TNBS) or without (EtOH) TNBS of 3 mg/mice (A, C - E) or 8 mg/mice (B) once and cultivated for indicated days. Body weight (A) and mice survival rate (B) were measured daily. After 1 day, sections of colonic tissues were prepared and subjected to histological examination (H & E staining) as described in the legend of Fig. 1 (C - E). Values are mean ± S.E.M. (n=6-12). $**P<0.01$; n.s., not significant.

Fig. 4. The mRNA expression of various genes in colonic tissues. DSS was administered to CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT), as described in the legend of Fig. 1. Relative mRNA expression of each gene in colonic tissues was monitored and expressed as described in the legend of Fig. 2A. Values are mean ± S.E.M. (n=4-5). ** or $^{\#\#}P<0.01$; * or $^{\#}P<0.05$; n.s., not significant.

Fig. 5. The mRNA expression of various genes in peritoneal macrophages. Peritoneal macrophages were prepared from CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT) and incubated with 10 ng/ml LPS for 12 h (3 h for the *Tnf-α*). Relative mRNA expression of each gene in colonic tissues was monitored and expressed as described in the legend of Fig. 2A. Values are mean ± S.E.M. (n=3). ** or $^{\#\#}P<0.01$; * or $^{\#}P<0.05$; n.s., not significant.

Fig. 6. Involvement of caspase-11-caspase-1-IL-1β pathway in CHOP-dependent excerebration of DSS-induced colitis. DSS was administered to CHOP-null mice

(*Chop*$^{-/-}$) and wild-type mice (WT), as described in the legend of Fig. 1 (A, B). Peritoneal macrophages were prepared from CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT) and incubated with 10 ng/ml LPS for 24 h (C, D). The amount of IL-1β was determined by ELISA (A, C). The caspase-1 activity was measured as described in materials and methods (B, D). Values are mean ± S.E.M. (n=3-4). $^{**}P<0.01$; $^{*}P<0.05$; n.s., not significant.

Fig. 7. Involvement of infiltration of macrophages in CHOP-dependent exacerbation of DSS-induced colitis. DSS was administered to CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT), as described in the legend of Fig. 1. Sections of colonic tissues were prepared and subjected to immunohistochemical analysis with an antibody against CD68. The lowest panel in each column is a 4-times magnified image of the boxed area defined in the middle panel (A). RAW264 cells were transiently transfected with expression plasmid for CHOP and C/EBP-β and cultured for 12 h. The relative mRNA expression of each gene was monitored and expressed as described in the legend of Fig. 2A. Values are

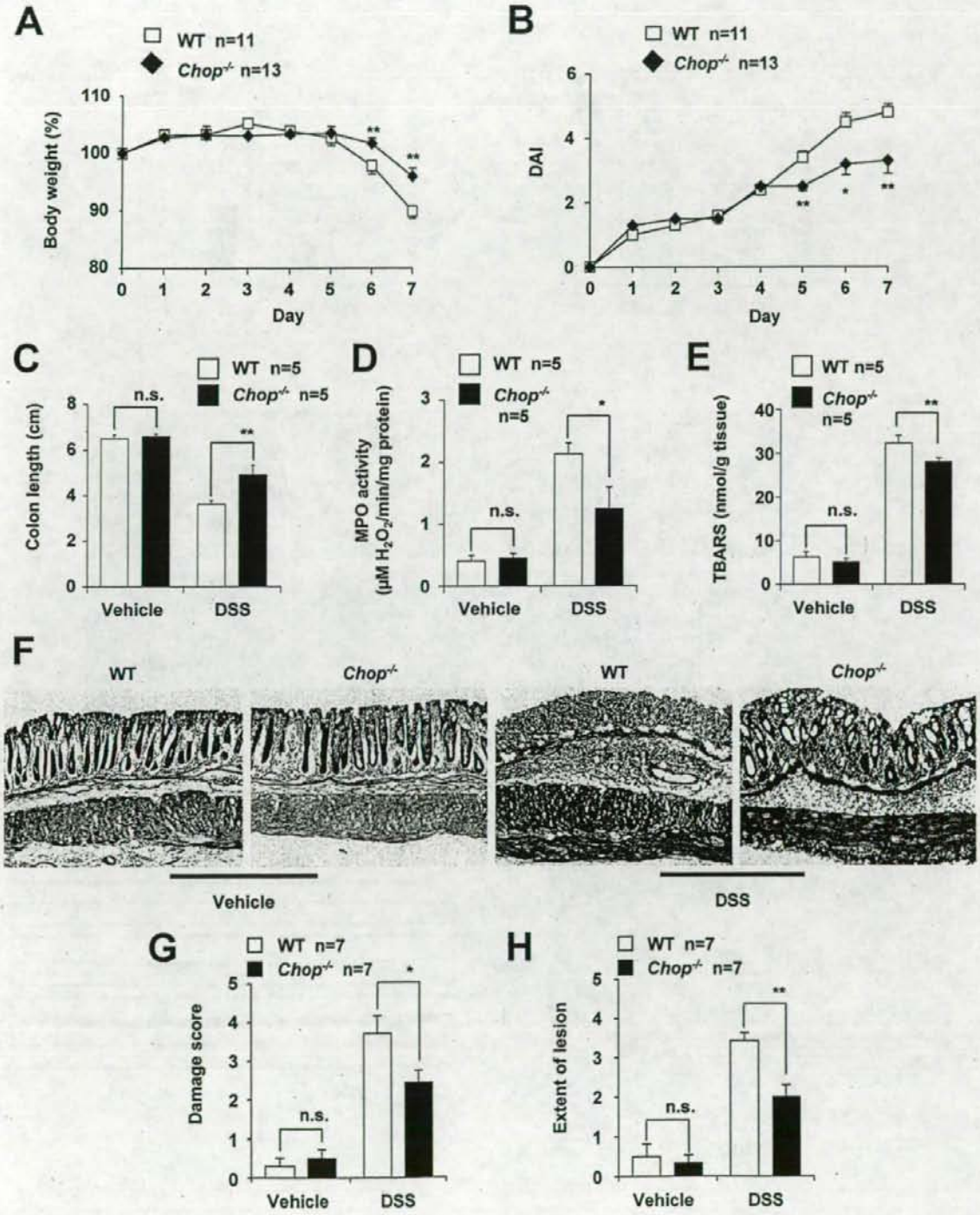
mean ± S.E.M. (n=3). $^{**}P<0.01$; $^{*}P<0.05$; n.s. not significant (B – D). The structure and sequences of the *CD11b* promoter are shown (E).

Fig. 8. Involvement of ROS-production in CHOP-dependent exacerbation of DSS-induced colitis. DSS was administered to CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT), as described in the legend of Fig. 1. After administration of POBN, the colons were dissected and subjected to radical adduct ESR spectrum analysis (A). The intensity of the ESR signal of radical adduct (shown by bars) was determined, expressed relative to the control sample (i.e. wild-type mice without DSS-treatment), and given as the mean ± S.E.M. (n=3). $^{*}P<0.05$; n.s., not significant (B). Peritoneal macrophages were prepared from CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT) and incubated with 10 ng/ml LPS for 24 h (C). RAW264 cells were transiently transfected with siRNA for CHOP (D-F) or ERO-1α (G, H) or non-silencing (ns) siRNA (D-H) and incubated with 50 ng/ml LPS for 24 h (D-H). The production of ROS was monitored by FACS analysis with H_2DCF, as described in materials and methods (C, D, G). Relative mRNA expression of

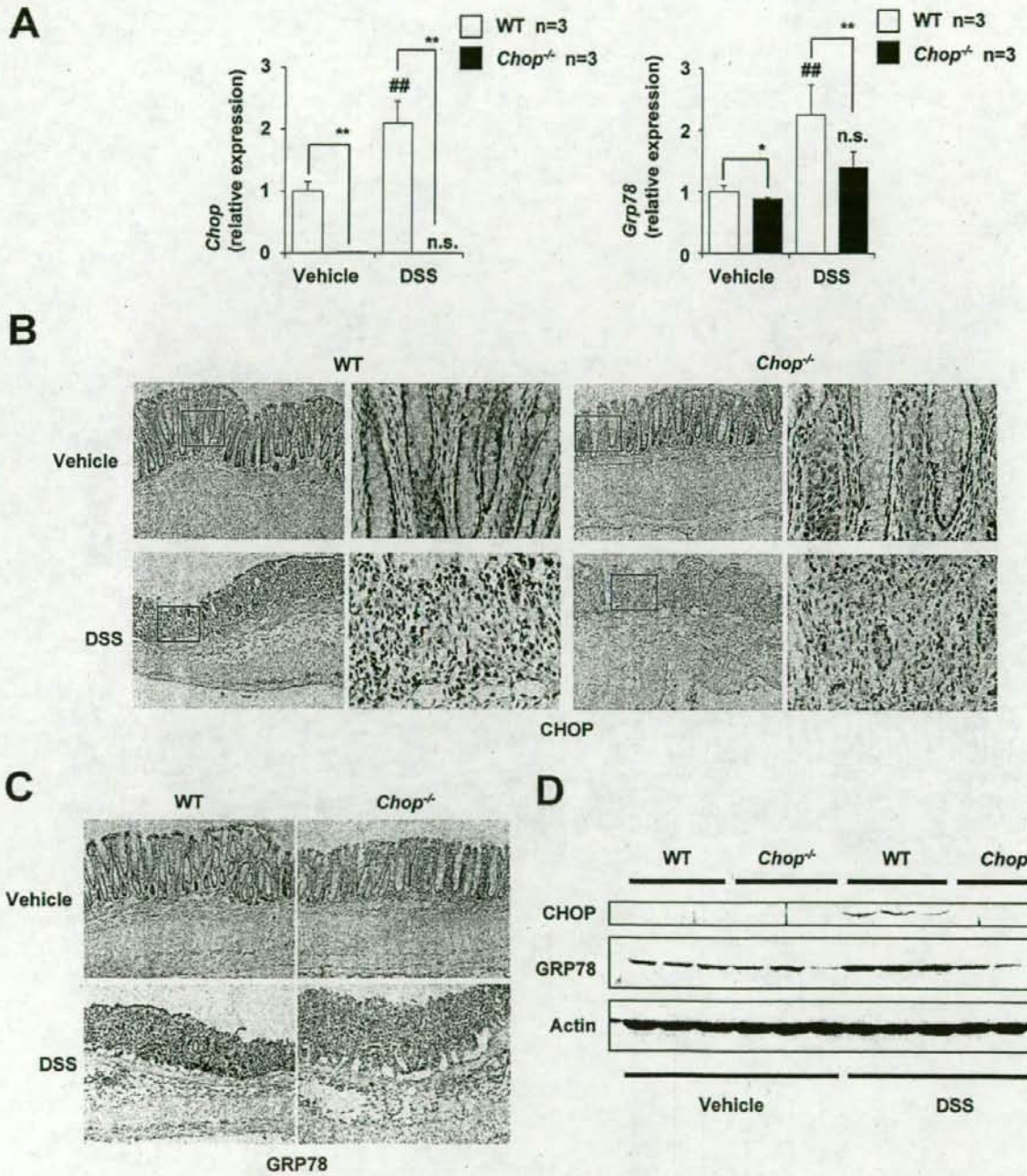
the *CHOP* (E) or *Ero-1α* (F, H) was monitored and expressed as described in the legend of Fig. 2A. Values are mean ± S.E.M. (n=3). ** or $^{\#\#}P<0.01$.

Fig. 9. Involvement of ROS-induced apoptosis in CHOP-dependent exacerbation of DSS-induced colitis. DSS was administered to CHOP-null mice (*Chop*$^{-/-}$) and wild-type mice (WT), as described in the legend of Fig. 1. Sections of colonic tissues were prepared and subjected to TUNEL assay and DAPI staining. The right panel in each column is a 3-times magnified image of the boxed area defined in the left panel (A). HCT-15 cells were transfected with siRNA for CHOP (siCHOP) or non-silencing (ns) siRNA and were incubated with indicated concentrations of menadione for 12 h. Relative mRNA expression of the *CHOP* was monitored as described in the legend of Fig. 2A (B). Cell viability was determined using the MTT method (C). The intracellular antioxidant activity was measured as described in materials and methods. Values shown are mean ± S.E.M. (n=3). ** or $^{\#\#}P<0.01$; n.s., not significant.

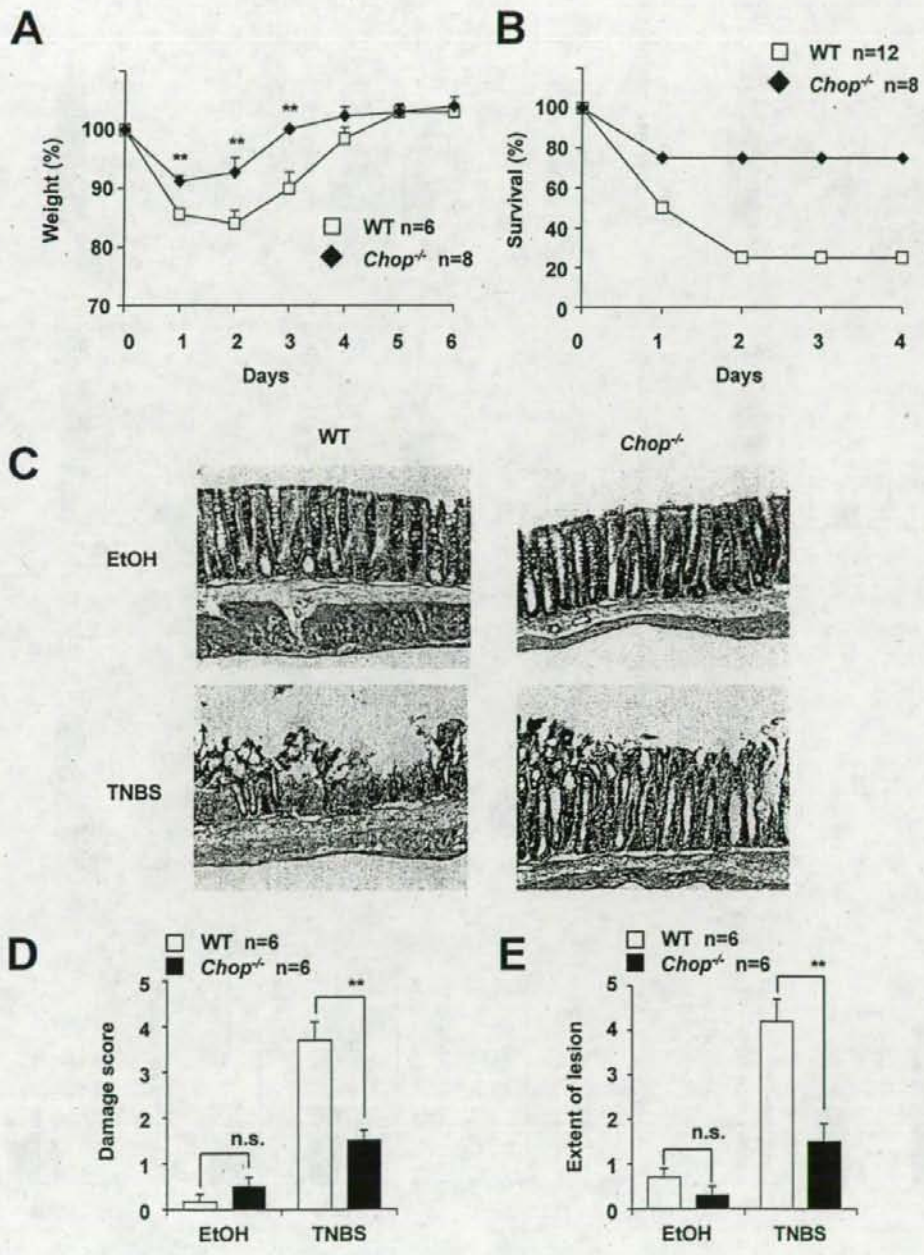
Namba *et al.* Fig.1



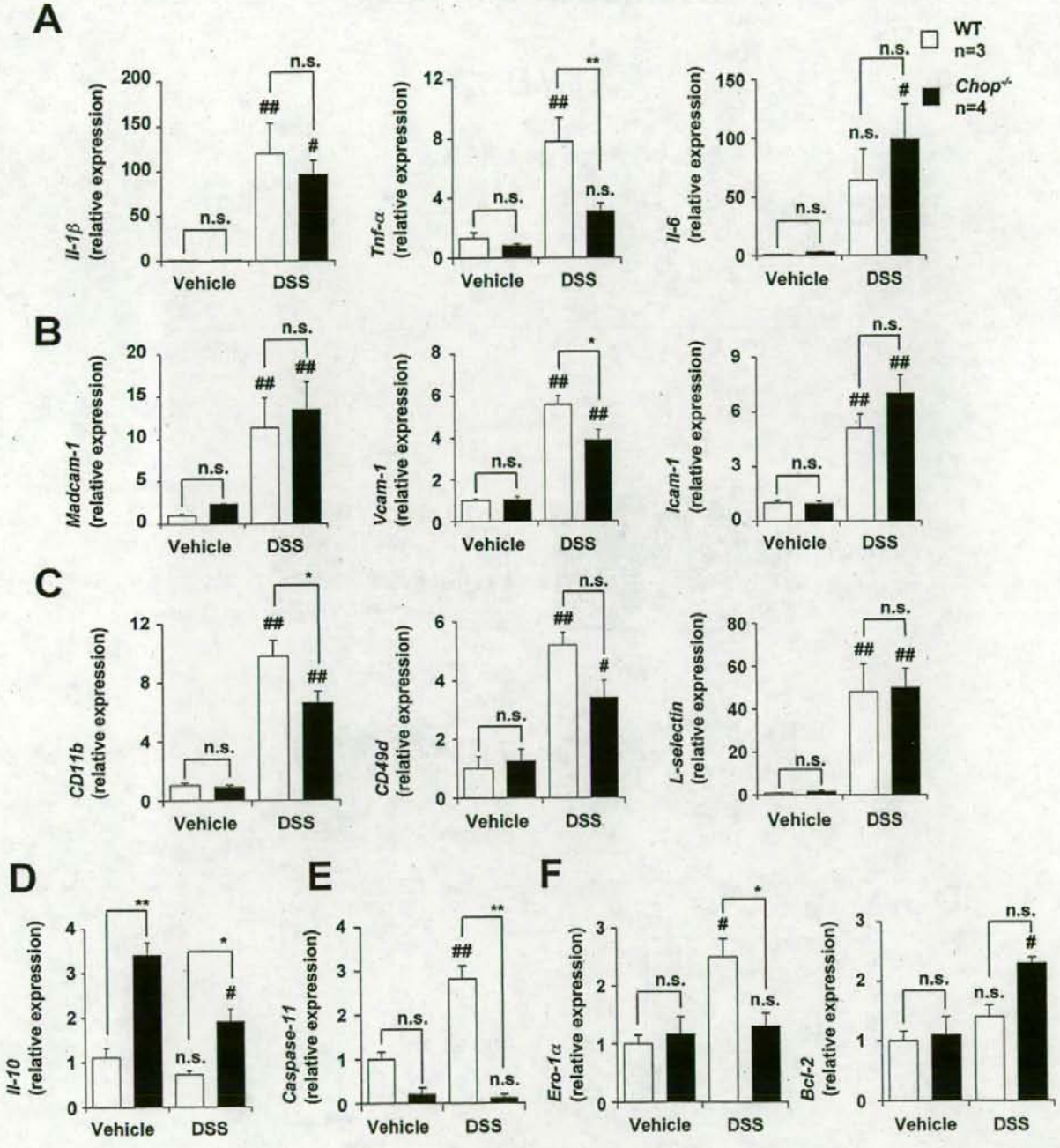
Namba *et al.* Fig.2



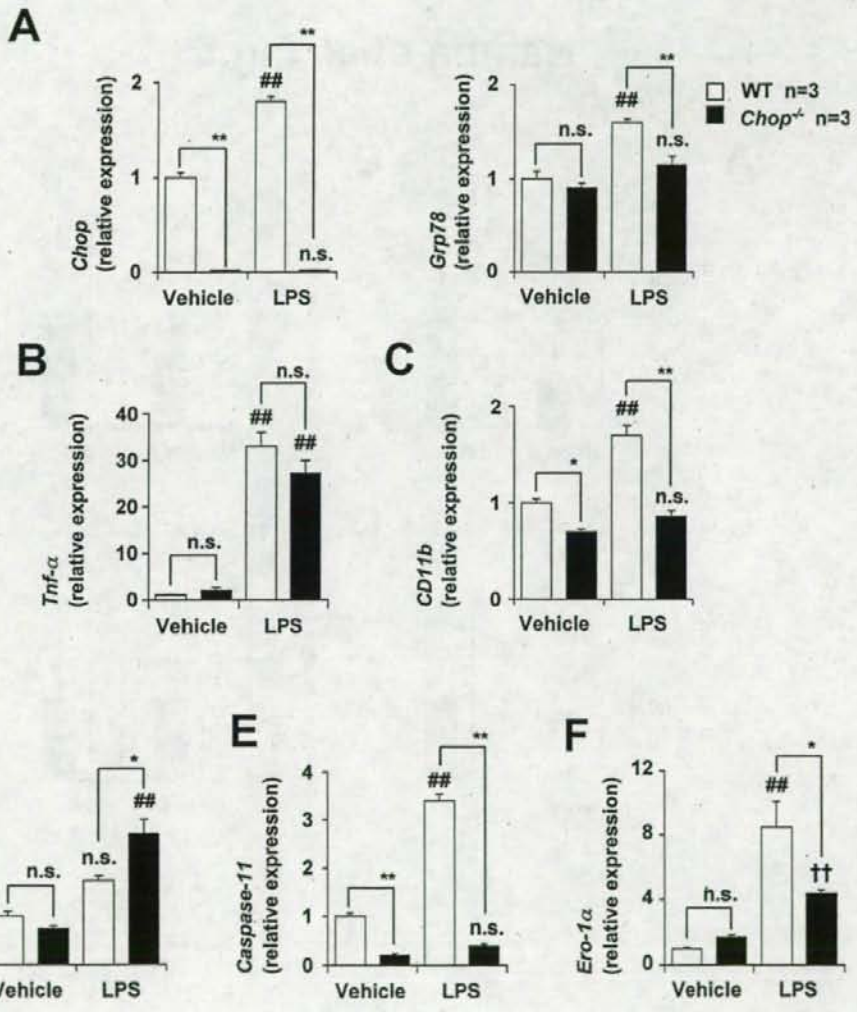
Namba *et al.* Fig.3



Namba *et al.* Fig.4



Namba *et al.* Fig.5



Namba *et al.* Fig.6