In Vivo Depletion of CD206+ M2 Macrophages Exaggerates Lung Injury in Endotoxemic Mice via Increased AP-1 Activation

（和訳；CD206陽性マクロファージの欠損はAP-1活性化を介してマウス敗血症性肺傷害モデルにおける肺傷害を増悪する）

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In Vivo Depletion of CD206^{+} M2 Macrophages Exaggerates Lung Injury in Endotoxemic Mice via Increased AP-1 Activation

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A short running head: M2 Macrophages in Acute Lung Injury

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Although phenotypically polarized macrophages are now generally classified into 2 major subtypes termed pro-inflammatory M1 and anti-inflammatory M2 macrophages, a contributory role of lung M2 macrophages in the pathophysiological features of acute lung injury is not fully understood. Here, we show in an endotoxemic murine model that M2 macrophages serve as key anti-inflammatory cells that play a regulatory role in the severity of lung injury. To study whether M2 macrophages can modify inflammation, we depleted M2 macrophages from lungs of CD206-diphtheria toxin receptor transgenic mice during challenge with lipopolysaccharide. Intrapenetreal administration of diphtheria toxin depleted CD206-positive macrophages in lungs and exaggerated lung inflammation, including up-regulation of pro-inflammatory cytokines and increased histologic lung damage. In CD206-diphtheria toxin receptor transgenic mice given diphtheria toxin, the endotoxemia-induced increase in NF-κB activity was significantly reduced, suggesting that M2 phenotype-dependent counteraction of inflammatory insult cannot be attributed to the inhibition of the NF-κB pathway. In contrast, AP-1 c-Jun and c-Fos activities were greatly enhanced in CD206-depleted mice with endotoxemia. These findings demonstrate that pulmonary M2 macrophages internally protect against lung inflammation by lessening AP-1 activation and may become part of a therapeutic strategy for pulmonary inflammatory disease.

Macrophages are critical regulators of many organ systems, including innate and adaptive immunity, systemic metabolism, hematopoiesis, vasculogenesis, malignancy, and reproduction.[1] Macrophages differentiate into functionally distinct immunological populations depending on the microenvironments. On the basis of Th1/Th2 polarization concepts,[2] phenotypically polarized macrophages are now generally termed pro-inflammatory M1 and anti-inflammatory M2.[3,4] Macrophages exposed to microbial products and IFN-γ become classically activated macrophages (M1), which produce copious amounts of pro-inflammatory cytokines and chemokines, keys among these are TNF-α, IL-1β, IL-6, IL-12, and CCL-2, as well as reactive oxygen intermediates, and function predominantly in inflammation, tissue damage, killing of intracellular microbes, and increased tumoricidal activity.[3] Recent studies also suggest that the phenotypic switching of adipose tissue macrophages from M2 to M1 is generated by obesity, leading to systemic inflammation and insulin resistance.[5-7] In contrast, alternative activation of macrophages is promoted by a variety of stimuli, such as IL-4, IL-10, IL-13, and glucocorticoids, that elicit different M2 forms. M2 macrophages up-regulate scavenger, mannose receptor, galactose receptor, and IL-1 receptor antagonist, and down-regulate IL-1β and other pro-inflammatory cytokines.[3,8,9] They are principally able to tune inflammatory responses and adaptive Th2 immunity, and to promote tissue remodeling, angiogenesis, and tumor development.[3,10] Furthermore, M2 macrophage activation can protect neuron and facilitate neuronal regeneration in Alzheimer's disease.[11] M2-polarized macrophages are further subdivided into the wound-healing M2a (elicited by IL-4 and IL-13), the regulatory M2b (following stimulation by immune complexes in the presence of Toll-like receptor ligands), and the M2c (when exposed to anti-inflammatory stimuli such as glucocorticoids, IL-10, and TGF-β),[4] but this subclassification may not fully represent

the complexity of the transitional states of macrophage activation, which is finely tuned in response to different microenvironments.[12]

Acute lung injury (ALI) is a common and highly morbid clinical disease marked by respiratory failure attributable to increased pulmonary endothelial and epithelial permeability, leading to alveolar flooding, and neutrophil accumulation in alveolar spaces and in adjacent capillaries.[13,14] Although neutrophil influx and activation within the lung play a crucial role in the pathogenesis of ALI,[15] growing evidence indicates that macrophages also contribute to the modulation of lung inflammation and the resultant lung injury.[16-18] Several studies have found that macrophage depletion with clodronate-lisosomes results in attenuation of lung injury following endotoxin administration, ischemia-reperfusion, and mechanical ventilation.[19-22] Conversely, other studies suggest that the recruitment of macrophages to the lung is associated with attenuated lung injury.[23,24] One possible explanation for such disparate roles of macrophages in ALI may be related to different depletion or recruitment of heterogeneous populations of pro-inflammatory and anti-inflammatory macrophages in the lung. However, how M2 macrophages have a protective anti-inflammatory role under the pathological condition of ALI are not fully understood.

In the present study, we decided to deplete M2 macrophages in mice during exposure to lipopolysaccharide (LPS). We generated a line of transgenic (Tg) mice expressing human diphtheria toxin (DT) receptor under the control of the *CD206* gene promoter. Murine cells are naturally resistant to DT as they lack functional cell surface receptors for the toxin.[25-27] Thus, the technique with human DT receptor transduction allows us to achieve efficient inducible ablation of a specific type of murine cells by DT administration *in vivo*. Because CD206 is a cell surface receptor as an M2 phenotypic marker, systemic administration of DT was able to lead to a sharp

decline in lung M2 macrophages in CD206-DT receptor TG mice. Here, we identify lung M2 macrophages as key anti-inflammatory cells that play a regulatory role in the severity of endotoxin-induced lung injury.

Materials and Methods

Generation and Screening of CD206-DT receptor Tg Mice

We obtained the mouse BAC clone RP 24-377 D19 carrying a 152 kbp insert containing the exon coding translational start Met and upstream 133 kbp sequence of CD206 gene from the BACPAC Resources Center CHORI (Oakland, CA). The plasmid pTRECK6 that includes noncoding exon and intron from rabbit β-globulin gene, human HB-EGF (DT receptor) cDNA, and rabbit β-globulin and simian virus (SV) 40 polyadenylation (pA) signals was kindly provided by Dr. Kenji Kohno (Nara Institute of Science and Technology).[28,29] Using a Counter-selection BAC modification kit (Gene Bridges, Dresden, Germany), we inserted the noncoding exon and intron from rabbit β-globulin gene, human DT receptor cDNA, and rabbit β-globulin and SV 40 pA signals at the initiation site of translation in the CD206 gene to yield the pTg-CD206-DT receptor (see Figure 1A).

The purified pTg-CD206-DT receptor BAC DNA was microinjected into pronuclei of fertilized one-cell embryos from C57BL/6 mice. Founder mice were crossed with C57BL/6 mice to produce +/CD206$^{\text{DT receptor}}$ mice. The wild type (+/+) and heterozygous CD206-DT receptor(+/CD206DTR) littermates were used for analysis. All mice were housed in a specific pathogen-free facility. Experiments were performed according to institutional guidelines.

For the genotyping of the Tg mouse lines by Southern blot analysis, genomic DNA prepared from tail biopsy was digested with BamHI, separated by electrophoresis on a 0.6 % agarose gel, and transferred to a nylone membrane (Hybond-N^{+}). Hybridization was conducted with a 900-bp ^{32}P-labeled DNA fragment derived from the exon1 and intron1 (see Figure 1B). The detected band sizes of endogenous and transgenic genes were 8.3 kb and 3.8 kb, respectively.

Experimental Procedures

In a first series of experiments to examine the effects of M2 macrophage depletion under normal non-inflammatory conditions, CD206-DT receptor Tg mice and WT littermates received an intraperitoneal injection of 15 μg/kg DT. Mice were administered DT for three consecutive days and were killed 24 h after the last injection (see Figure 2A). Blood samples were collected and various tissues and organs were removed. Some mice were given PBS instead of DT.

In another series of experiments to address the role of M2 macrophages in endotoxin-induced ALI, mice received an intraperitoneal injection of 15 μg/kg DT for three consecutive days and subsequently challenged with intravenous LPS (0.1 mg/kg; List Biological Laboratories, Campbell, CA) 24 h after the last injection (see Figure 3A). Mice were killed 2 h later, and blood collection and lung tissue isolation were performed.

RNA Extraction and Quantitative Real-Time PCR

Total RNA was isolated from tissues with an RNeasy Mini Kit (Qiagen, Tokyo, Japan). RNA was reverse-transcribed to cDNA, and real-time PCR analyses were performed as described previously[30] using Takara RNA PCR kit (Takara Bio). The sequences of specific primer pairs for target genes are available upon request from a website of Takara Bio Inc. (http://www.takara-bio.co.jp/). Thermal cycler parameters were as follows: 1 cycle of 50°C for 2 min, 50 cycles of denaturation (95°C, 15 s), and combined annealing/extension (60°C, 30 s). Gene expression changes were calculated by the comparative Ct method, and *18S* ribosomal RNA was used as reference genes for *normalization*.

Enzyme Immunoassay for Cytokines

Blood levels of IL-1β, TNF-α, MCP-1, and IL-6 were measured by the use of commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN) according to the manufacture's instructions. The plate was read on a microplate reader (Nippon-InterMed, Tokyo, Japan). Assays were performed in duplicate.

Histologic Examination

For routine histology, inflation-fixed lungs were harvested, fixed, dehydrated, paraffin-embedded, and sliced into 4-μm-thick sections.[30,31] After deparaffinization, slides were stained with hematoxylin and esosin using standard methods. A semiquantitative morphometric analysis of lung injury was performed by scoring from 0 to 4 (none, light, moderate, severe, very severe) for the following categories: neutrophil infiltration, pulmonary edema, and disorganization of lung parenchyma and hemorrhage.[30] A total lung injury score was calculated by adding the individual scores in every animal and averaging the total values in each group. All the histological studies were performed in a blinded fashion.

Immunohistochemistry

Five-mm-thick frozen lung sections were fixed in acetone for 10 min at -20^{o}C and air-dried. Bronchoalveolar lavage (BAL) was performed with five aliquots of 5 ml of PBS instilled into the lungs and gently withdrawn. Cells obtained from the total BAL fluid (BALF) were spun at 113 *g* for 10 min onto glass slides by use of a Cytospin 4 (Thermo Fisher Scientific, Yokohama Japan). Then, they were fixed with 4% buffered

formalin solution for 10 min at room temperature. Endogenous peroxidase activity was quenched by incubation in 0.3% hydrogen peroxide for 15 min. After incubation overnight at 4^{o}C with anti-CD206 antibody (AbD Serotec, Raleigh, NC, USA), the samples were exposed to anti-rat igG conjugated with horseradish peroxidase. Bound antibody was visualized by a light microscopy with diaminobenzidine. Omission of primary antibody and staining with non-immune IgG served as negative control.

NF-κB and AP-1 Binding Assays

TransAM NF-κB and TransAM AP-1 kits (Active Motif, Carlsbad, CA) were used to quantify the binding of p65 to the NF-κB site and c-Jun and c-Fos to the AP-1 site, according to the manufacture's instructions.

Statistical Analysis

Data were analyzed by the use of Prism software (ver. 6; GraphPad Software, San Diego, CA). Statistical analysis was performed by one-way analysis of variance followed by Tukey's multiple comparison test. Differences were considered significant at $P \leq 0.05$. Data are presented as means ± SEM.

Results

CD206-Positive Cell Expression and Distribution in Mice

CD206 mRNAs were transcribed in a variety of tissues, including lung, liver, spleen, and kidney, of normal C57BL/6 mice, but they were most abundant in lung tissues (Figure 1C). Immunohistochemical studies showed that CD206-positive cells were present in the alveoli (Figure 1D) and constituted a great portion of the whole cells in BALF (Figure 1E).

DT Treatment of CD206-DT Receptor Tg Mice

DT treatment resulted in a sharp reduction in CD206 mRNA levels in lungs of CD206-DT receptor Tg mice without a significant change in those of WT mice (Figure 2B). In line with the effect on CD206 mRNA in lung tissues, DT treatment caused a marked decrease in the number of CD206-positive cells in BALF from the CD206-DT receptor Tg animal as compared with WT (Figure 2C and D). DT treatment did not substantially affect mRNA levels of pro-inflammatory cytokines, IL-1β, TNF-α, MCP-1, and IL-6 in lungs of WT mice. However, CD206-DT receptor Tg mice exhibited up-regulation of these pro-inflammatory cytokine mRNAs in response to DT treatment (Figure 2E). When blood levels of pro-inflammatory cytokines were measured via an enzyme-linked immunosorbent assay, the WT animals had very low levels of the cytokines examined here regardless of whether DT was given. In contrast, IL-1β, MCP-1, and IL-6 showed an evident increase in CD206-DT receptor Tg mice when treated with DT, except that TNF-α was undetectable even though DT was administered (Figure 2F).

Effect of LPS in DT-Treated CD206-DT Receptor Tg Mice

When a low dose (0.1 mg/kg) of LPS was intravenously injected, lung CD206 mRNA levels were significantly decreased in DT-treated WT mice. The reduction in CD206 mRNA in lungs was more marked in DT-treated CD206-DT receptor Tg mice (Figure 3B).

LPS challenge led to up-regulation of IL-1β, TNF-α, MCP-1, and IL-6 mRNA levels in lungs of WT mice. This up-regulation was strikingly enhanced in DT-treated CD206-DT receptor Tg mice (Figure 3C). The ability of LPS at 0.1 mg/kg to up-regulate blood levels of TNF-α, MCP-1, and IL-6 was less pronounced in WT mice regardless of treatment with DT, although IL-1β responded relatively well to LPS. However, LPS caused a significant and substantial impact on all pro-inflammatory cytokines examined here in DT-treated CD206-DT receptor Tg mice (Figure 3D).

Histologic examination of hematoxylin and eosin-stained sections of the lungs revealed that WT mice had alveolar septae that were normal in appearance, with no intra-alveolar inflammation (Figure 4A). Lung histopathology of WT mice given LPS showed modest inflammatory cell infiltrate (Figure 4B). DT-treated CD206-DT receptor Tg mice that received LPS had massive cell infiltration, hemorrhage resulting from ruptured capillary vessels, and diffuse septal edema (Figure 4C). Semiquantitative assessment using lung injury score demonstrated that the score was dramatically increased when DT-treated CD206-DT receptor Tg mice was challenged with LPS (Figure 4D).

To examine the activation of the pro-inflammatory transcription factor NF-κB in lungs after LPS challenge, NF-κB DNA binding activity was measured in nuclear protein extracts from lungs. The DNA binding activity of NF-κB was greatly increased in WT mice following LPS injection. This increase was significantly attenuated in DT-treated CD206-DT receptor Tg mice (Figure 5A). Another

pro-inflammatory transcription factor AP-1 consists of Jun family and Fos family proteins. Thus, c-Jun and c-Fos proto-oncogenes encode proteins that form a complex which regulates transcription from promoters containing AP-1 activation elements.[32] Both c-Jun and c-Fos binding activities were significantly increased when WT mice were challenged with LPS. Further rises in these binding activities in response to LPS were observed in DT-treated CD206-DT receptor Tg mice (Figure 5B and C).

Discussion

The concept of macrophages existing in functionally distinct phenotypes from pro- to anti-inflammatory states has been fully established by detailed studies on M1 and M2 macrophages.[3,4,8,9] A growing body of evidence is accumulating that macrophages are key regulators of the induction and resolution phases of ALI, suggesting distinct roles of macrophage subpopulations.[33] Understanding this heterogeneity of pulmonary macrophages may be important in developing strategies to modulate ALI. However, there is no direct evidence that pulmonary M2 macrophages make a significant contribution to the pathophysiological features of ALI. In this work, we addressed this issue by studying how depletion of M2 macrophages can modify lung inflammation in endotoxemic mice.

CD206 encodes the mannose receptor C-type lectin, a cell surface protein that belongs to a family of C-type lectin receptors.[34] This endocytic receptor is known to be expressed on macrophages and probably other types of cells, including airway smooth muscle cells.[35-38] The expression of the receptor is highly regulated and correlates with the functional state of macrophages. Thus, fully mature activated M2 macrophages express CD206 as a phenotypic hallmark.[39] Therefore, CD206 can be widely used to identify the M2 phenotype.[3] In the present study, DT administration on CD206-DT receptor Tg mice led to conditional CD206-positive cell ablation, which implies that M2 macrophages were effectively depleted *in vivo*.

A significant number of M2 macrophages were found in the BALF of C57BL/6 mice under normal conditions. Lung sections from normal C57BL/6 mice showed that M2 macrophages were also detected in the alveoli. DT-mediated M2 macrophage depletion in CD206-DT receptor Tg mice demonstrated that mRNA levels of pro-inflammatory cytokines, IL-1β, TNF-α, MCP-1, and IL-6, were up-regulated in

lungs. We interpret these observations to indicate that M2 macrophages lead to a tonic inhibition of pulmonary production of pro-inflammatory cytokines. Blood levels of IL-1β, MCP-1, and IL-6 were apparently increased when abolition of M2 macrophages was made by administration of DT to CD206-DT receptor Tg mice. This suggests that M2 macrophage-mediated tonic inhibition of the genesis of pro-inflammatory cytokines under basal sub-inflammatory conditions may be found systemically.

Administration of LPS at a low dose resulted in a significant reduction in expression of CD206 mRNA in mouse lungs, suggesting that M2 macrophages may be down-regulated by a low dose of LPS. In agreement with this finding, a recent report has shown that low doses of LPS can effectively suppress expression of the M2 marker arginase-1 when bone marrow-derived macrophages were treated with M2 skewing mediators.[40] Alternatively, M2 macrophages may be transformed into M1 macrophages following LPS stimulation to promote inflammation. Despite low levels of M2 macrophages under endotoxemic conditions, the presence of M2 macrophages appears to be important in the regulation of the inflammatory state during endotoxemia. DT-mediated depletion of M2 macrophages in CD206-DT receptor Tg mice led to a discernible enhancement of the LPS-induced increases in pulmonary mRNA levels of pro-inflammatory cytokines. Furthermore, this manipulation significantly aggravated LPS-induced lung injury. These results suggest that M2 macrophages serve as a protective phenotype and can lessen lung inflammation during endotoxemia. DT treatment accelerated the deleterious effect of LPS on blood pro-inflammatory cytokine levels in CD206-DT receptor Tg mice, implying that M2 macrophages appear essential for appropriate moderation of systemic endotoxemic inflammation.

In quiescent cells, NF-κB is maintained in inactive form by IκB.[41] LPS stimulates IκB-kinase (IKK) that specifically phosphorylates IκB, resulting in IκB

polyubiquitination and subsequent degradation, followed by liberation of NF-κB.[42] Many M1 genes have κB sites in their promoter region, including iNOS and COX-2.[43] Thus, in M1 macrophages, NF-κB orchestrates the expression of many pro-inflammatory genes in response to LPS. In this study, we showed that M2 macrophage depletion resulted in a significant reduction in LPS-induced NF-κB activity, suggesting that M2 phenotype-dependent counteraction of inflammatory insult cannot be attributed to the inhibition of the NF-κB pathway. At the present time, however, we do not have a clear understanding of whether the reduced NF-κB activity was the result of the critical regulation of the transcription factor by M2 macrophages. Another major pro-inflammatory transcription pathway within macrophages involves the AP-1 pathway whose pro-inflammatory targets overlap those of the NF-κB pathway.[44] AP-1 is a group of a basic leucine zipper transcription factors, including the Fos and Jun families of transcription factors.[44] We found that AP-1 c-Jun and c-Fos activities were greatly enhanced when DT was given to CD206-DT receptor Tg mice with endotoxemia, which implies that M2 macrophages negatively regulate the AP-1 pathway. We thus suggest that AP-1 signaling is a key transcriptional regulator involved in the inhibitory modulation of lung inflammation by M2 macrophages. There were slight differences in the extents of the enhancing effect of DT treatment on an array of pro-inflammatory cytokine levels in CD206-DT receptor Tg mice. This may be associated with some differences in the dependence of the transcription of cytokines on the NF-κB and AP-1 pathways.

We have previously demonstrated that short-term treatment with high doses of statin can increase the number of alveolar macrophages in mouse lungs.[45] These alveolar macrophages display an unusual phenotype compared with typical tissue macrophages. Thus, we have found that alveolar macrophages express high levels of

CD11c,[45] a molecule that is not expressed by their counterparts in other body sites and is generally expressed by dendritic cells.[46] However, contrary to dendritic cells, alveolar macrophages are very unlikely to emigrate from the tissue and seem to have distinct roles in the initiation and maintenance of immune response.[47] These alveolar macrophages may be now identified as M2 macrophages. It is noteworthy that our statin treatment has been able to mitigate acute lung injury and improve the survival of mice with cecal ligation and puncture-induced sepsis.[45]

In conclusion, our findings identify lung CD206-positive M2 macrophages as key anti-inflammatory cells during endotoxemic lung injury. We demonstrate that pulmonary M2 macrophages internally protect against lung inflammation by lessening AP-1 activation and may become part of a therapeutic strategy for pulmonary inflammatory disease.

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Figure Legends

Figure 1 CD206 expression in C57BL/6 mice. **A:** Schematic representation of wild-type (WT) and DT receptor-inserted transgene in BAC DNA(pTg-CD206-DT receptor). Exons 1 through 3 of *CD206* gene are indicated by open box. Met is the initiation site of translation in *CD206*. In the pTg-CD206-DT receptor BAC DNA, rabbit β-globin intron, human DT receptor cDNA and rabbit β-globin/SV40 poly A signals (pA) are indicated by line and gray box. The probe used for Southern blot analysis is indicated as a solid line together with the predicted hybridizing fragments. After insertion of DT receptor, the CD206 of the transgene is not expressed. **B:** Southern blot analysis of *Bam*HI-digested genomic DNA from CD206-DT receptor Tg mice. Genomic DNAs were hybridized with the CD206 probe, as shown in **A**. Location of WT (8.6 kbp), and CD206-transgene (Tg, 3.8 kbp)-derived bands are shown. The positions of DNA size markers (kbp) are indicated on the *left*. **C:** CD206 mRNA expression in different tissues of normal mice. The data are expressed as a fold increase above kidney value normalized 18 S. Values are means ± SEM (n = 3 mice). *P<0.05, **P<0.01, ***P<0.001. **D** and **E:** Immunohistochemical detection of CD206-positive cells in lung sections (**D**) and in BALF (**E**) from normal mice. Lung sections and BALF were stained with hematoxylin. Shown are representative micrographs from two independent experiments. In **D**, *arrows* indicate CD206-positive cells.

Figure 2 DT treatment on CD206 expression and pro-inflammatory cytokine production in CD206-DT receptor Tg mice. **A:** Experimental protocol. Mice were administered DT (or PBS) once daily for 3 days. The animals were then sacrificed 24 h after the last DT injection. **B:** CD206 mRNA expression in lung tissues. The data are expressed

as a fold increase above WT PBS normalized to 18 S. **C** and **D:** Immunohistochemical detection of CD206-positive cells in BALF from WT mice (**C**) and from DT-treated CD206-DT receptor Tg mice (**D**). Shown are representative micrographs from two independent experiments. **E:** mRNA levels of cytokines in lungs. The data are expressed as a fold increase above WT normalized 18 S. **F:** Blood levels of cytokines. **B**, **E**, and **F**: Values are means ± SEM (n = 7-8 mice). *P<0.05, **P<0.01, ***P<0.001

Figure 3 DT treatment on CD206 expression and pro-inflammatory cytokine production in CD206-DT receptor Tg mice exposed to LPS. **A:** Experimental protocol. Mice were administered DT once daily for 3 days. The animals then received an intravenous injection of LPS 24 after the last DT treatment and were sacrificed 2 h later. **B:** CD206 mRNA expression in lung tissues of mice given LPS. **C:** Effect of LPS challenge on mRNA levels of cytokines in lungs. **B** and **C:** The data are expressed as a fold increase above control WT normalized 18 S. **D:** Effect of LPS challenge on blood levels of cytokines. **B-D:** Values are means ± SEM (n =5-10 mice). *P<0.05, **P<0.01, ***P<0.001.

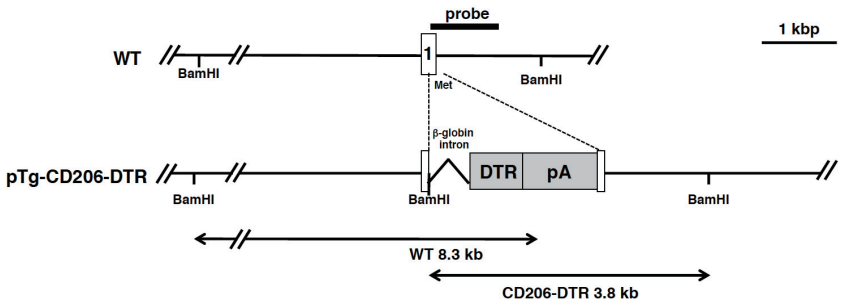
Figure 4 Lung sections stained with hematoxylin and eosin. **A:** Control WT mice. **B:** DT-treated WT mice who received LPS. **C:** DT-treated CD206-DT receptor Tg mice who received LPS. **D:** Semiquantitative analysis of lung tissues by lung injury score. Values are means ± SEM (n = 5-7). ***P<0.001.

Figure 5 Activation of pro-inflammatory transcriptional factors in lungs of DT-treated WT and CD206-DT receptor Tg mice who were challenged with LPS. **A:** NF-κB p65

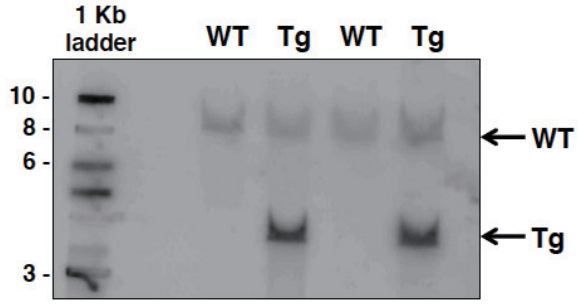
DNA binding activity. **B:** AP-1 c-Jun DNA binding activity. **C:** AP-1 c-Fos DNA binding activity. Values are means ± SEM (n = 5-7 mice). *P<0.05, **P<0.01, ***P<0.001.

Figure 1

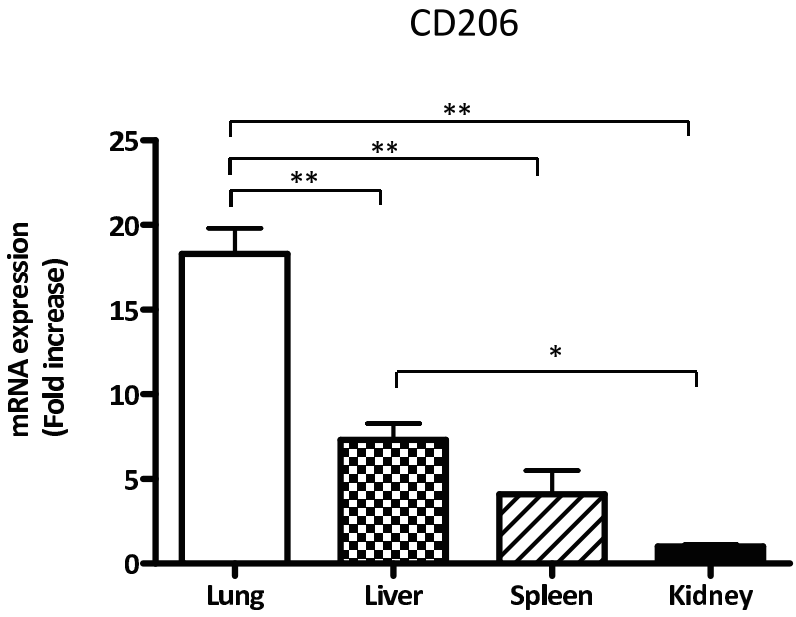
A



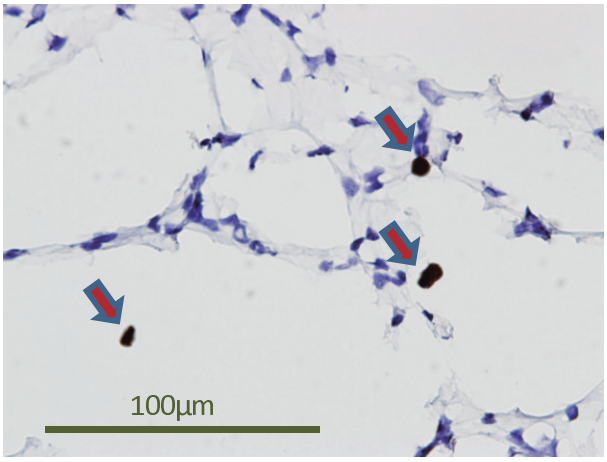
B



C



D



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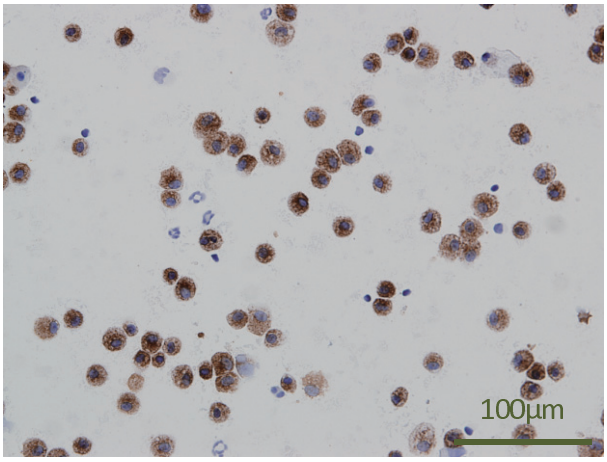


Figure 2

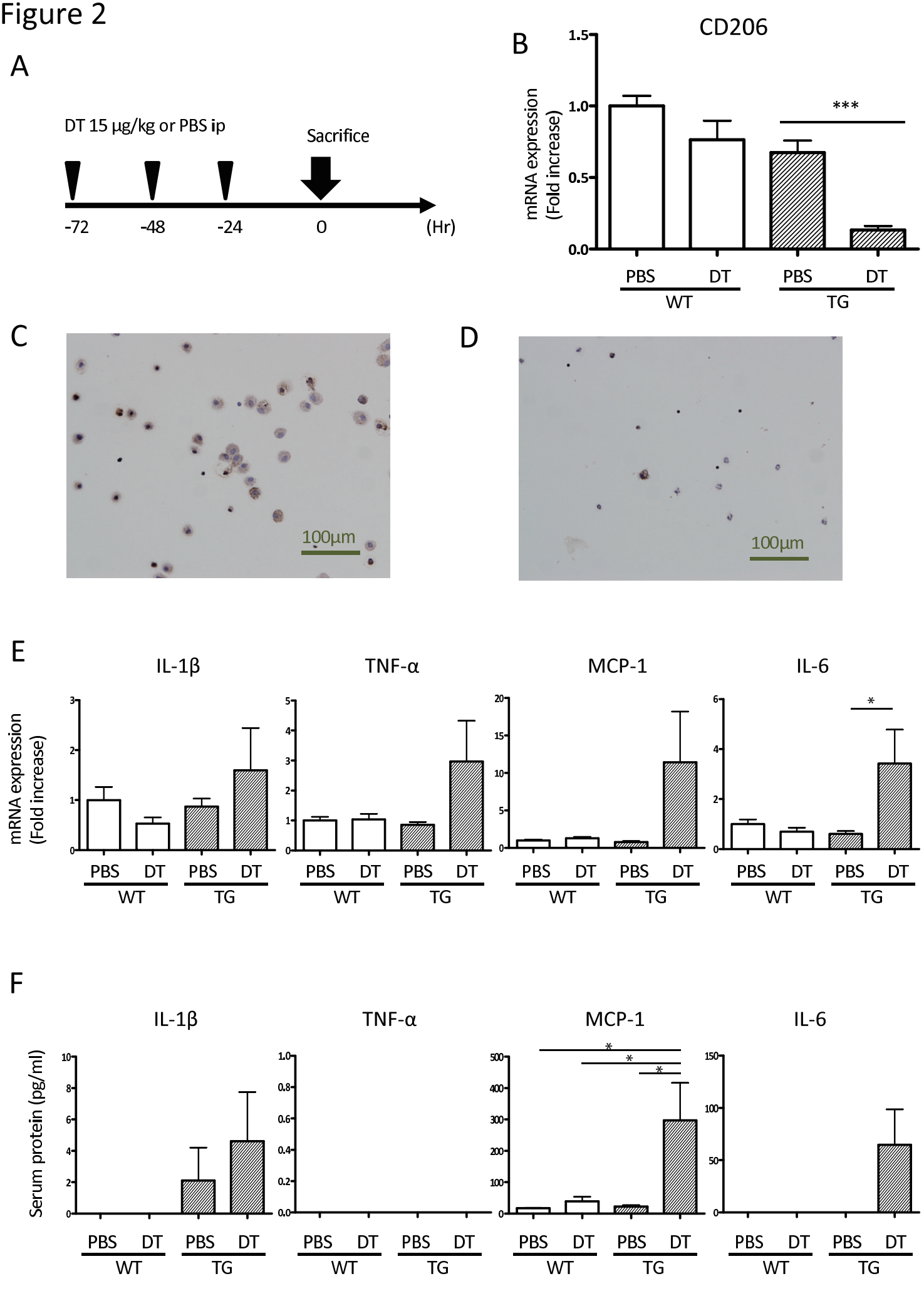
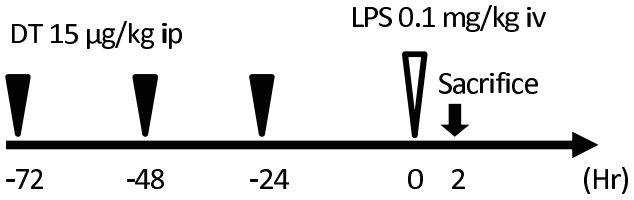
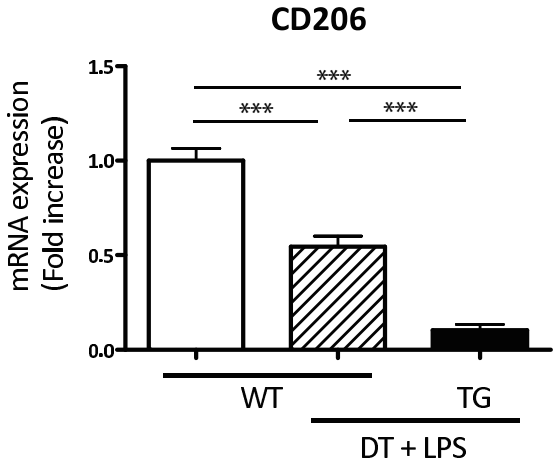

A
DT 15 μg/kg or PBS ip
Sacrifice
-72
-48
-24
0
(Hr)
B
CD206
mRNA expression (Fold increase)
1.5
1.0
0.5
0.0
***
PBS
DT
PBS
DT
WT
TG
C
100μm
D
100μm
E
IL-1β
TNF-α
MCP-1
IL-6
mRNA expression (Fold increase)
PBS
DT
PBS
DT
WT
TG
F
IL-1β
TNF-α
MCP-1
IL-6
Serum protein (pg/ml)
PBS
DT
PBS
DT
WT
TG

Figure3

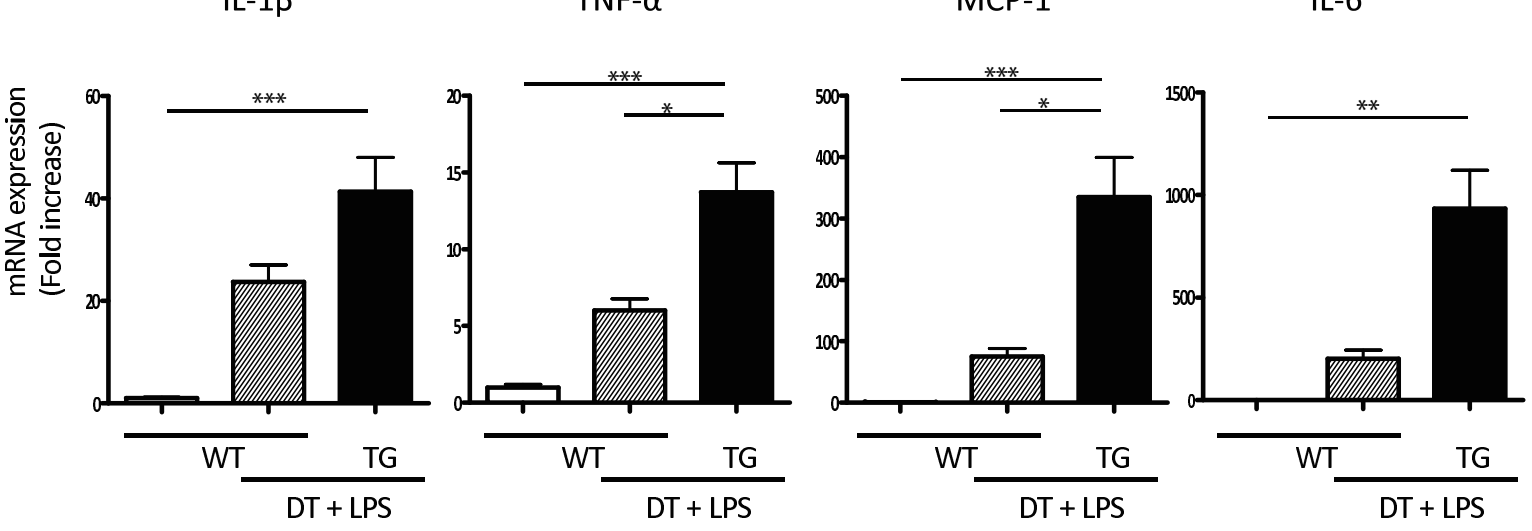
A



B



C



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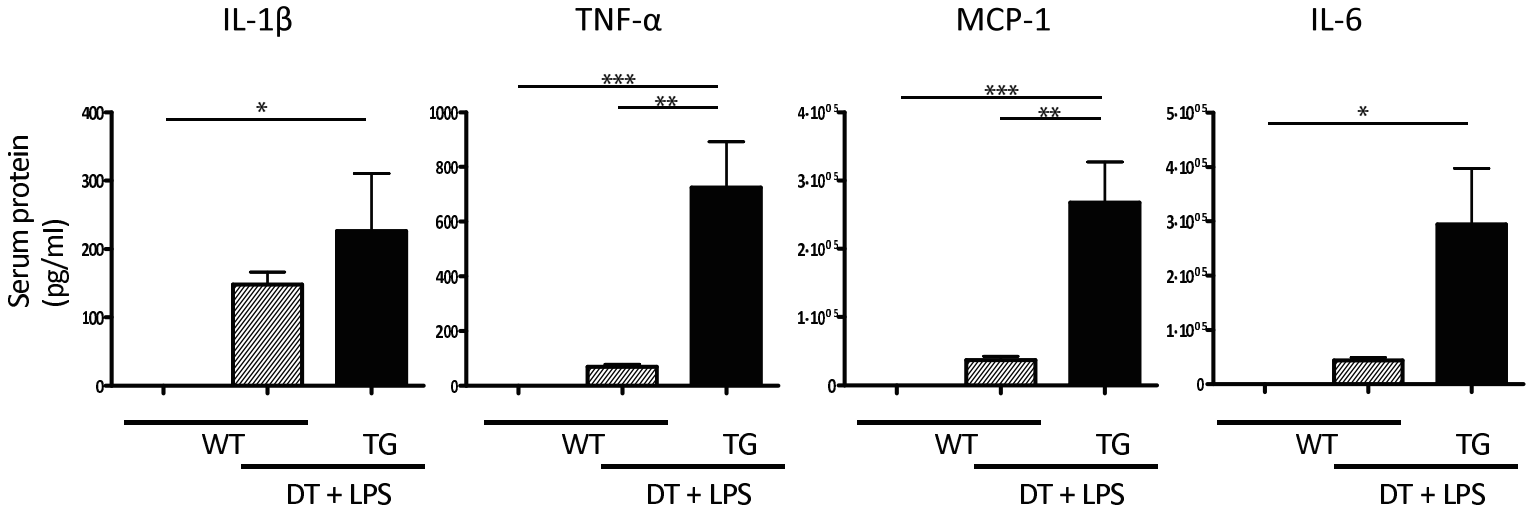
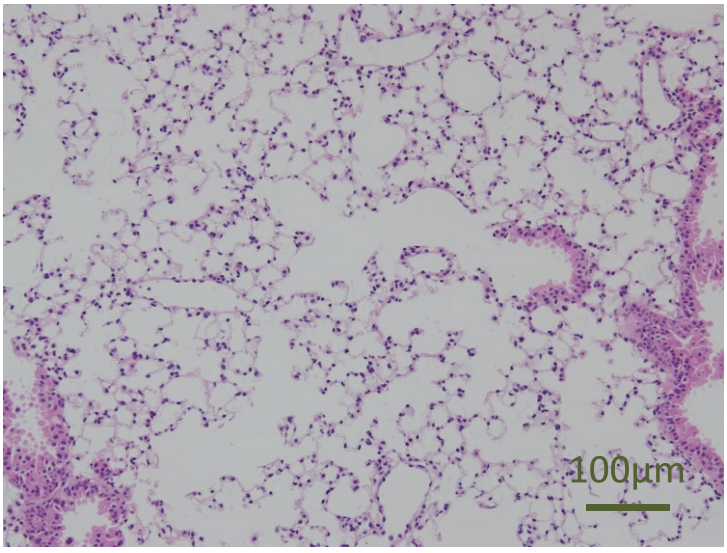
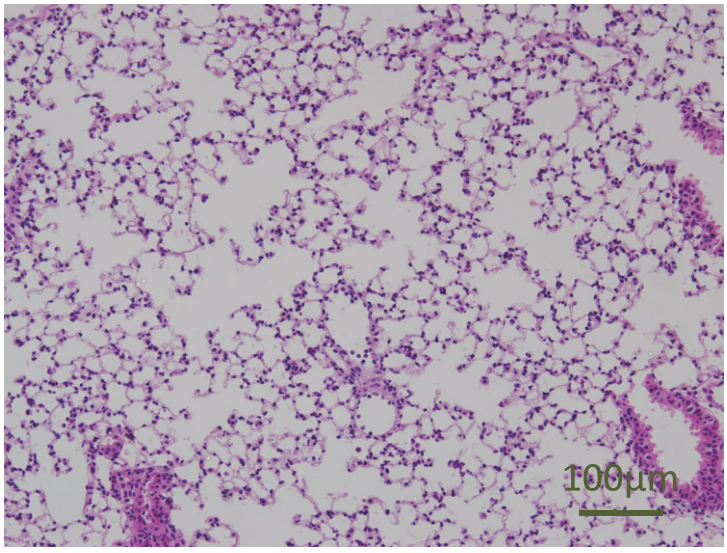


Figure4

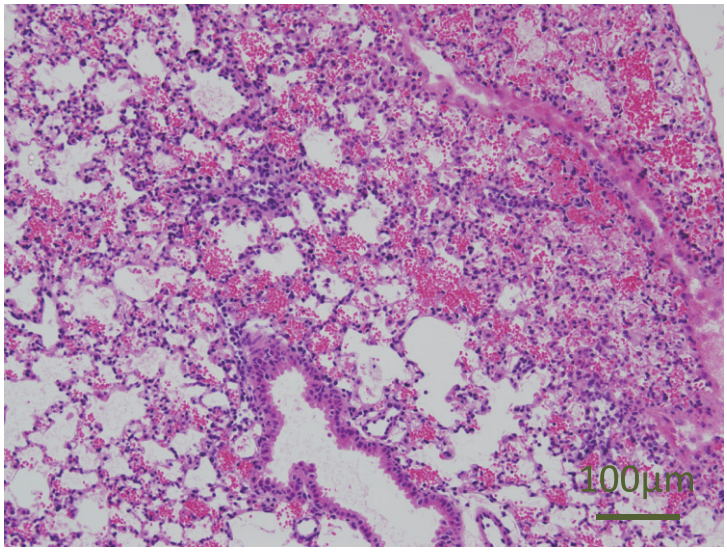
A WT



B WT + DT/LPS



C TG + DT/LPS



D

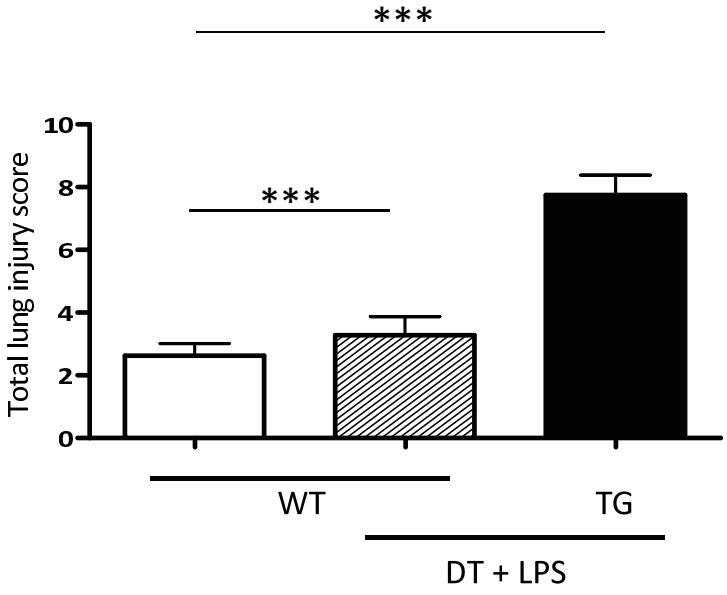
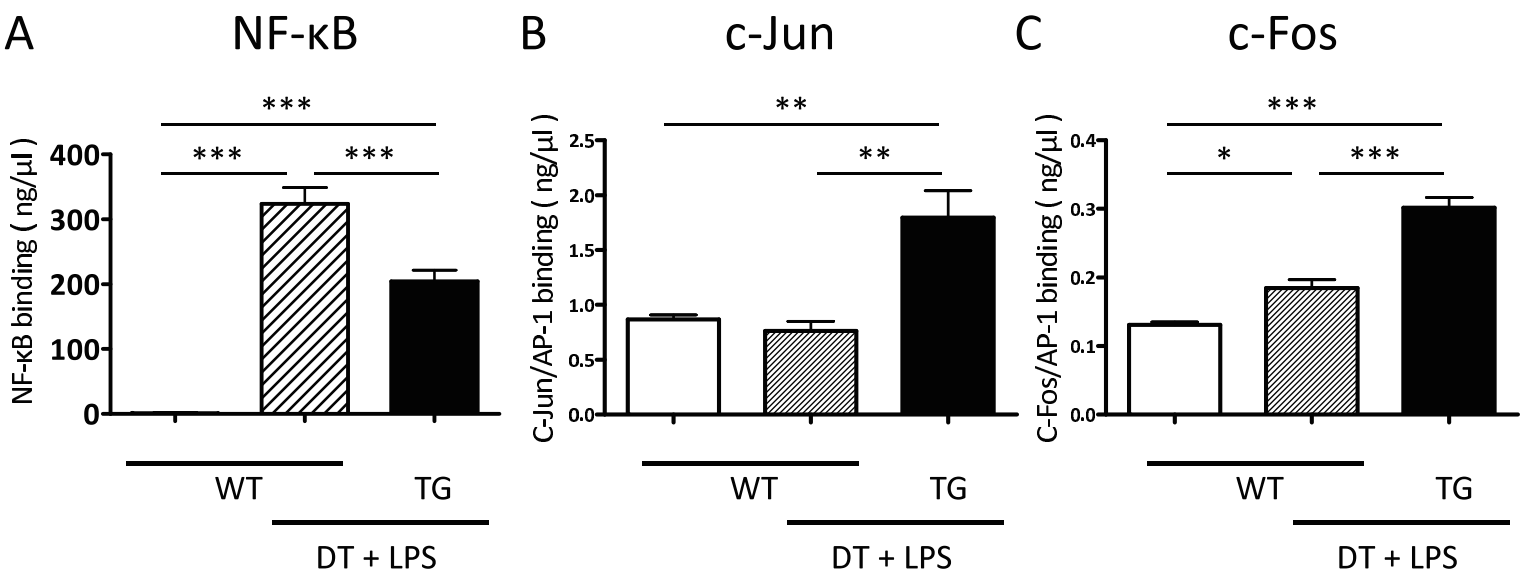


Figure5


A
NF-κB
B
c-Jun
C
c-Fos
NF-κB binding (ng/μl)
C-Jun/AP-1 binding (ng/μl)
C-Fos/AP-1 binding (ng/μl)
***
***
***
**
**
*
***
400
300
200
100
0
2.5
2.0
1.5
1.0
0.5
0.0
0.4
0.3
0.2
0.1
0.0
WT
TG
DT + LPS