Supplemental information

Mice and Mice maintenance

WT (C57BL/6J) and *Ldlr*$^{-/-}$ (B6.129S7-Ldlrtm1Her) 6-8 week old female mice were purchased from Jackson Laboratory. *Jak2*^{VF} MxCre mice were created and reported previously.[12] Bone Marrow (BM) from poly I:C treated female WT (C57BL/6J) or *Jak2*^{VF} MxCre mice were transplanted into irradiated (10.5 Gy for 8.4 minutes) WT or *Ldlr*$^{-/-}$ recipients. WT BM recipients were fed a chow diet, while *Ldlr*$^{-/-}$ recipients were fed a Western diet (WD) (Harlan Teklad, TD88137) for the indicated period of time. Mice were housed under a 12 hr light /dark cycle with ad libitum access to water and food. All protocols were approved by the Institution Animal Care and Use Committee of Columbia University.

Human subjects

The JAK2V617F positive MPN patients were newly identified, untreated, being treated with aspirin or phlebotomy but not hydroxyurea or ruxolitinib, male or female, White, non Hispanic from 40 to 80 years old. Sex, age and ethnic matched healthy subjects were used as the control.

Atherosclerosis Lesion Analysis and Metabolic profiling

The heart with the aortic root attached was embedded in paraffin and then serially sectioned. The sections were stained with hematoxylin and eosin or trichrome (sigma, HT15) for morphometric lesion analysis. Five sections per mouse was used for total lesional and necrotic core area quatification as previously described.[13] All these *in vivo* studies including the ones described below such as immunofluorescence and immunohistochemistry staining were performed with a blinding protocol. Each animal was assigned an arbitrary number and the data was collected based on the assigned number while the genotype of the mouse and experimental conditions were unknown to the data collector. Total plasma cholesterol and HDL-cholesterol were measured using kits from WAKO diagnostics, total plasma triglyceride was measured using kit from Thermo (TR22421).

Immunofluorescence Staining

Paraffin-embedded slides were deparaffinized with Histo-clear and then rehydrated in deceasing concentration of ethanol. Identification of macrophages, neutrophils in atherosclerotic lesions were performed by immunostaining using anti-mouse CD107b (Mac-3) (BD 553322, 1： 100), anti-mouse Ly6G (Biolegend 127601, 1:200), anti-myeloperoxidase (R&D BAF3667, 1:30) Lesional MerTK was incubated with biotin-labeled MerTK (R&D system, BAF591) and Mac-3. The sections were incubated with primary antibodies overnight at 4˚C overnight then incubated with secondary antibodies for 30 min. For antibody specificity in immunofluorescence staining, isotype matched normal IgG was used as the control for each assay. Iron staining and TUNEL staining were performed using a commercially available kit (Abcam ab83366) and (Roche 12156792910). Images were acquired by using Leica immunofluorescence microscope.

Immunohistochemistry

Paraformaldehyde-fixed and paraffin-embedded lesions were deparaffinized and rehydrated, then incubated with anti-Mac-3 antibody (BD 553322, 1： 100) or with anti-Ter119 antibody (eBioscience 14-5921-82,

1:200) at 4˚C overnight and with secondary anti-Rat IgG (VECTOR, MP-7405) for 30 min. The reaction was developed with diaminobenzidine (DAB) staining (VECTOR, SK-4100). For antibody specificity, isotype matched normal IgG was used as the control for each assay.

Flow Cytometry

Flow cytometry to quantify peripheral blood neutrophils, monocytes, platelet-neutrophil aggregates, platelet-monocyte aggregates or bone marrow hematopoietic stem and progenitor cells profiles were performed as previously described.[13] For analysis of erythrocytes from mice and human samples, the red blood cells were labeled with antibody against Ter119 (eBioscience 14-5921-82) or CD47 (BD 556045 for human and BD 563585 for mice) or Calreticulin (Abcam ab22683) in staining buffer (30 min, 4°C). For neutrophils activity assays, neutrophils were stained with antibodies against to CD45 (Biolegend 103133), CD11b (Biolegend 101211), Ly6G (BD 561104) in staining buffer (20 min, 4°C). Formyl-Nle-Leu-Phe-Nle-Tyr-Lys (FMLP) conjugated with Fluorescein (Thermofisher, 10 min, 4°C) was used to detect FPR1 expression. Flow cytometry was performed using the LSR Fortessa or LSRII (Beckton Dickinson) and data were analyzed using FlowJo software (Beckton Dickinson). Isotype matched normal IgG was used as the control in each flow cytometry assay.

Intravital Microscopy

Leukocyte-endothelial interactions along the carotid artery were analyzed by intravital epifluorescence microscopy. Mice were placed in supine position, and the right jugular vein was cannulated with a catheter for antibody injection. Intravital microscopy was performed after injection of antibodies to Ly6G (1µg; clone 1A8; eBioscience), Ly6C (1µg, HK1.4, Biolegend), and CD11b (1µg, M1/70, eBioscience) using an Olympus BX51 microscope equipped with a Hamamatsu 9100-02 EMCCD camera, and a 10× saline-immersion objective. Movies of 30s were acquired and analyzed offline. Rolling flux was assessed as cell moving across a line perpendicular to the carotid artery. Cells were considered adherent if not moving during 30 seconds.

Flow Adhesion Assays

Flow adhesion assays were performed in vitro using IBIDI-Slide IV 0.1 flow chambers (Ibidi). Flow chambers were coated with 12 µg/mL of intercellular adhesion molecule-1 (ICAM1), vascular cell adhesion molecule-1 (VCAM1) and P-selectin (all from R&D systems). Neutrophils were stained with Ly6G (eBioscience 17-9668-82，1:100) for 15 min on ice. After adding 1mM of Ca2+/Mg2+, $5x10^5$ neutrophils were placed into flow chambers and flow was created during 3 minutes at 5µL/min at 37°C using a high-precision syringe pump. After perfusion with PFA 4%, cells were washed with PBS. Pictures were acquired using a climate chamber fluorescence microscope (Leica, DMi8) and adhered neutrophils were quantified using ImageJ software.

Erythrophagocytosis Assays.

Bone marrow derived macrophages (BMDM) were prepared as described.[14] For erythrophagocytosis assays, macrophages were plated in CutureSlides (BD Falcon 354102) or 24-well tissue culture plates at the density of $2x10^5$ per well. PKH26-labeled (Sigma mini26) 2 million erythrocytes were added to macrophages. Following 20 hour incubation, the macrophages were thoroughly washed with phosphate buffered saline

(PBS) four times to remove the free erythrocytes. The slides were fixed by 1% paraformaldehyde and then counterstained with DAPI. Images were acquired by Leica immunofluorescence microscope. The erythrophagocytosis rate was assessed by flow cytometry. To assess the impact of erythrophagocytosis on efferocytosis, Calein-AM (Molecular Probe C3100MP) labeled, UV-induced (254nm UV lamp for 15 mins) apoptotic Jurkat cells were incubated with bone marrow derived macrophages with or without 5 million erythrocytes for 20 hours. Then uptake of the apoptotic Jurkat cells by macrophages was assessed by Leica immunofluorescence microscope.

For erythrophagocytosis assay with human erythrocytes, blood samples were obtained from JAK2VF positive myeloproliferative neoplasm patients or matched healthy controls. Erythrocyte rich fractions were obtained with High Efficiency Leukocyte Reduction Filter (Haemonetics Corporation, NEO1). Human peripheral monocyte derived macrophages generated with a protocol as reported[15] from samples of healthy donors were used for the assays and plated in 24-well no tissue culture plates. Human erythrocytes were labeled with PKH26 and 2 million of them were add to incubate for overnight. After washing for three times with PBS to remove the free erythrocytes, the erythrophagocytosis rate was assessed by flow cytometry.

MerTK Cleavage Assays

Spleens from 5-wk WD-fed WT and *Jak2*^{VF} BM recipient mice were harvested and gently homogenized with PBS. Spleen cells were separated from supernatant by centrifuge. Pelleted cells were co-stained with anti-F4/80 (Biolegend 123124) and MerTK antibodies. Surface MerTK on macrophages was detected by flow cytometry. Supernatant was assayed for sol-Mer by immunoblot. For plasma soluble Mer level which was measured by ELISA kit (DY591 and DY008) as instructions.

Analysis of Pro-inflammatory Response of Macrophages

Macrophages were derived from bone marrow cells or obtained as peritoneal macrophage following peritoneal injection of concanavalin A (ConA) as reported.[16] After stimulation by specified concentration of lipopolysaccharides (LPS) for indicated period of time and as indicated, p38 or c-Jun N-terminal kinase (JNK) inhibitor (Selleckchem, SB203580 for p38 and SP600125 for JNK) or RUX or rapamycin were added. After treatment, the cells were washed with phosphate buffered saline. RNA was extracted from samples using RNeasy Mini kit (QIAGEN, 74106). The RNA concentration was assessed by Termo Scientific Nanodrop spectrophotometer. cDNA was synthesized and quantitative RT-PCR was performed using Real time PCR system (Applied Biosystem) and SYBR Green Master Mix. Primers were purchased from Integrated DNA Technologies. Sequences of primers for qPCR are listed below：

Gene	Forward Primer	Reverse Primer
Mouse IL-1β	TGTGAATGCCACCTTTTGACA	GGTCAAAGGTTTGGAAGCAG
Mouse IL-6	ACAACCACGGCCTTCCCTACTT	CACGATTTCCCAGAGAACATGTG
Mouse iNOS	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Mouse Tnf-α	CCAGACCCTCACACTCAGATC	CACTTGGTGGTTTGCTACGAC
Mouse Mcp-1	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCCTTCTTG

Immunoblotting

Macrophage Cells were lysed in 4X Laemmli sample buffer containing 50 mM DTT and heated at 95°C for 5 minutes. protein was separated by 4-20% gradient SDS-PAGE and transferred onto PVDF membranes, which were immunostained with corresponding primary antibodies at 4°C overnight and detected using HRP-conjugated secondary antibodies.

Erythrocyte Ghost Membrane Isolation

Collected blood samples were lysed by RBC lysis buffer (5mM Tris, 1mM EDTA, PH=8) and incubated on ice for 5 min. Pelleted the ghost erythrocytes by centrifugation at 13,000 rpm for 10 minutes. Washed the pellet again using lysis buffer until it was white and clean. Then used RIPA buffer to lyse and resuspend the pellet. After measuring protein concentration, we used approximately 100μg protein/sample, mixed with 2-mercaptoethanol in SDS sample buffer and then heated the samples at 95°C for 5 minutes. Running on 10% SDS-PAGE reducing gels and then stained gel with coomassie brilliant blue solution.

Primary $CD11b^+$ Cells Isolation

$CD11b^+$ cells were isolated from spleen using CD11b coated magnetic beads (MACS，130-049-601) according to the manufacturer's instructions. Isolated cells were then lysed by RIPA buffer for western blot or cultured in 96 well plates in DMEM supplemented with 10% FBS for following treatment.

Respective animal and human ethics

All animal and human studies were performed according to the approved respective IRB or IACUC protocols.

Statistics

The number of mice used for each study was estimated by power analysis based on the data from our previous studies. Normality assumption of the data distribution was assessed using Kolmogorov-Smirnov test. Data were analyzed by unpaired *t* test if data were normally distributed and two groups were involved. Otherwise, Mann-Whitney *U* test was used. One or two-way ANOVA was used for more than two groups. Bonferroni post-hoc test was further performed to compare specific two groups. Pearson correlation was used to detect the linear correlation between two parameters that were normally distributed. Otherwise, Spearman correlation was used. Chi-squared test was used to compare iron or Ter119 lesional staining. Two-tailed analysis was performed in all statistical analyses. p-value less than 0.05 was considered as a significant difference.

Supplementary Figure legends

Online Figure I: Plasma cholesterol levels and hematopoietic cell profile in peripheral blood of WT and *Jak2^{VF}* mice.
The mice were fed chow or WD respectively following brief recovery from bone marrow transplantation. **(A)** Plasma total cholesterol levels of WT and *Jak2^{VF}* mice after 4, 5, 6 and 7 weeks of WD. Unpaired *t* test. **(B)** Plasma HDL level of WT and *Jak2^{VF}* mice after 6 weeks of WD. Mann-Whitney *U* test. **(C)** Plasma total cholesterol levels of WT and *Jak2^{VF}* mice on chow diet. Unpaired *t* test. **(D)** Plasma triglyceride after 7 weeks of WD. Unpaired *t* test. **(E)** Hematopoietic cell profile in bone marrow. Progenitor cells were defined as LSK (Lin^- $Sca1^+$ $c\text{-}Kit^+$), CMP (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^{int}Fc\gamma RII/III^{int}$), GMP ($Lin^-$ $Sca1^-$ $c\text{-}Kit^+$ $CD34^{int}Fc\gamma RII/III^{hi}$), MEP ($Lin^-$ $Sca1^-$ $c\text{-}Kit^+$ $CD34^{int}Fc\gamma RII/III^{low}$), ERP($Lin^-$ $Sca1^-$ $cKit^+$ $CD34^{int}Fc\gamma RII/III^{int}CD71^+$ $CD41^-$) and MKP (Lin^- $Sca1^-$ $c\text{-}Kit^+$ $CD34^{int}Fc\gamma RII/III^{int}CD71^-$ $CD41^+$) by flow cytometry. 2-way ANOVA. **(F)** Red blood cells and **(G)** Hemoglobin in whole blood. 2-way ANOVA. **(H)** Monocyte, **(I)** Platelet and **(J)** neutrophils cell count in whole blood of WT and *Jak2^{VF}* mice. 2-way ANOVA .*p<0.05,**p<0.01,***p<0.001, N.S., not significant.

Online Figure II: WT or *Jak2^{VF}* platelet responses.
The mice were on chow or WD for 7 weeks. **(A)** Average red blood cell size (MCV) and **(B)** red blood cell distribution width (RDW). 2-way ANOVA. **(C)** Platelet/monocyte aggregate or **(D)** platelet/neutrophil aggregate content in blood. 2-way ANOVA. **(E)** P selectin in whole blood at basal state. 2-way ANOVA. **(F)** P selectin in whole blood stimulated by AYPGKF at 100μM for 30 min at room temperature. 2-way ANOVA. *p<0.05,**p<0.01,***p<0.001.

Online Figure III: Macrophage infiltration and monocyte rolling of WT and *Jak2^{VF}* mice.
(A) Immunofluorescence isotype negative control of myeloperoxidase (MPO) and Ly6G staining of aortic root lesions from mice fed WD for 7 weeks. **(B)** Mac-3+ macrophage in early lesions (7 weeks). Mann-Whitney *U* test. Scale bar, 200μm. **(C)** Ly6C rolling flux and **(D)** Ly6C monocyte adhesion were assessed by intravital fluorescence microscopy in mice fed WD for 5 weeks. Unpaired *t* test. *p<0.05. N.S., not significant.

Online Figure IV: Lesional collagen content and iron or erythrocyte marker accumulation.
(A) Plasma total cholesterol levels of WT and *Jak2^{VF}* mice after 12 weeks of WD. n=5 to 10 per group. Unpaired *t* test. **(B)** Plasma triglyceride after 12 weeks of WD. Unpaired *t* test. **(C)** Quantification of Ly6G positive cells in lesions of 12 weeks WD-fed mice. Mann-Whitney *U* test. **(D)** Correlation between MPO+ neutrophils and total lesion size after 12 weeks of WD. Pearson correlation test. **(E)** Quantification of Trichrome positve area as a percentage of total lesion area and Fribrosis cap thickness of *Ldlr$^{-/-}$* recipients after 12 weeks WD. Mann-Whitney *U* test. Number of mice that showed positive or negative lesional iron **(F)** or Ter119 **(G)** staining (12 weeks of WD). Chi-Squared test. **(H)** IHC isotype negative control of Mac-3 and Ter119 stainig. Scale bar, 100μm. *p<0.05,**p<0.01,***p<0.001. N.S., not significant.

Online Figure V :*Jak2^{VF}* mice showed increased complex of erythrocytes with monocytes or neutrophils in blood after 7 weeks WD feeding.
(A) Representative images of iron staining, immunohistochemistry staining of red blood cell marker Ter119 and macrophage marker Mac-3 in lesions of *Ldlr$^{-/-}$* recipients after 7 weeks WD. Scale bar, 100μm. **(B)**

Flow cytometry analysis of RBC (Ter119+)/monocyte (CD45+CD115+) or RBC/neutrophil (CD45+Ly6G+) complex in the blood of 7 weeks WD-fed mice. Unpaired *t* test. **p*<0.05,***p*<0.01.

Online Figure VI: Increased erythrophagocytosis of *Jak2*^{VF} mice.
Bone marrow derived macrophages were challenged with 2 million PKH26-labeled (Red) red blood cells overnight. **(A)** Erythrophagocytosis of WT and *Jak2*^{VF} macrophage (Figure 4B) merged with bright field. Scale bar, 50μm. **(B)** 3D image of erythrophagocytosis of red blood cells (Red) by macrophage (DAPI), obtained by confocal fluorescence microscopy. **(C)** Representative image of human control and *JAK2*^{VF} erythrophagocytosis. Scale bar, 100μm. **(D)** SDS-PAGE/Coomassie Blue staining analysis of mouse erythrocyte ghost proteins. **(E)** Quantification of band 4.2. Unpaired *t* test. **(F)** ELISA assay of soluble MerTK in mice plasma. 2-way ANOVA. **(G,H)** Immunofluorescence isotype negative control of Mac-3 and MerTK. Scale bar, 200μm. ****p*<0.001. N.S., not significant.

Online Figure VII: Response of ConA and bone marrow derived macrophages to LPS.
(A) ELISA of IL-1β and **(B) I**L-6 in cultured media of ConA macrophages which were challenged by LPS (10ng/ml) for indicated period of time. 1-way ANOVA. **(C)** ConA macrophages were treated with or without 10ng/ml LPS for the indicated period of time. qPCR was used to determine mRNA level of iNOS, Tnf-α and Mcp-1. 1-way ANOVA. **(D)** Bone marrow derived macrophages were challenged with or without 10 ng/ml LPS for the indicated period of time. qPCR was used to determine mRNA level of IL-1β, iNOS, IL-6, Tnf-α and Mcp-1. 1-way ANOVA. **p*<0.05, ***p*<0.01,****p*<0.001, N.S., not significant.

Online Figure VIII: Inflammatory signaling pathways in ConA macrophages.
(A) The level of various signaling molecules was determined by immunoblot in ConA macrophages following 6 hour culture. Unpaired *t* test. **(B)** The level of various signaling molecules was determined by immunoblot in ConA macrophages following 48 hours culture in vitro. Unpaired *t* test. **p*<0.05, ***p*<0.01, ****p*<0.001, N.S., not significant.

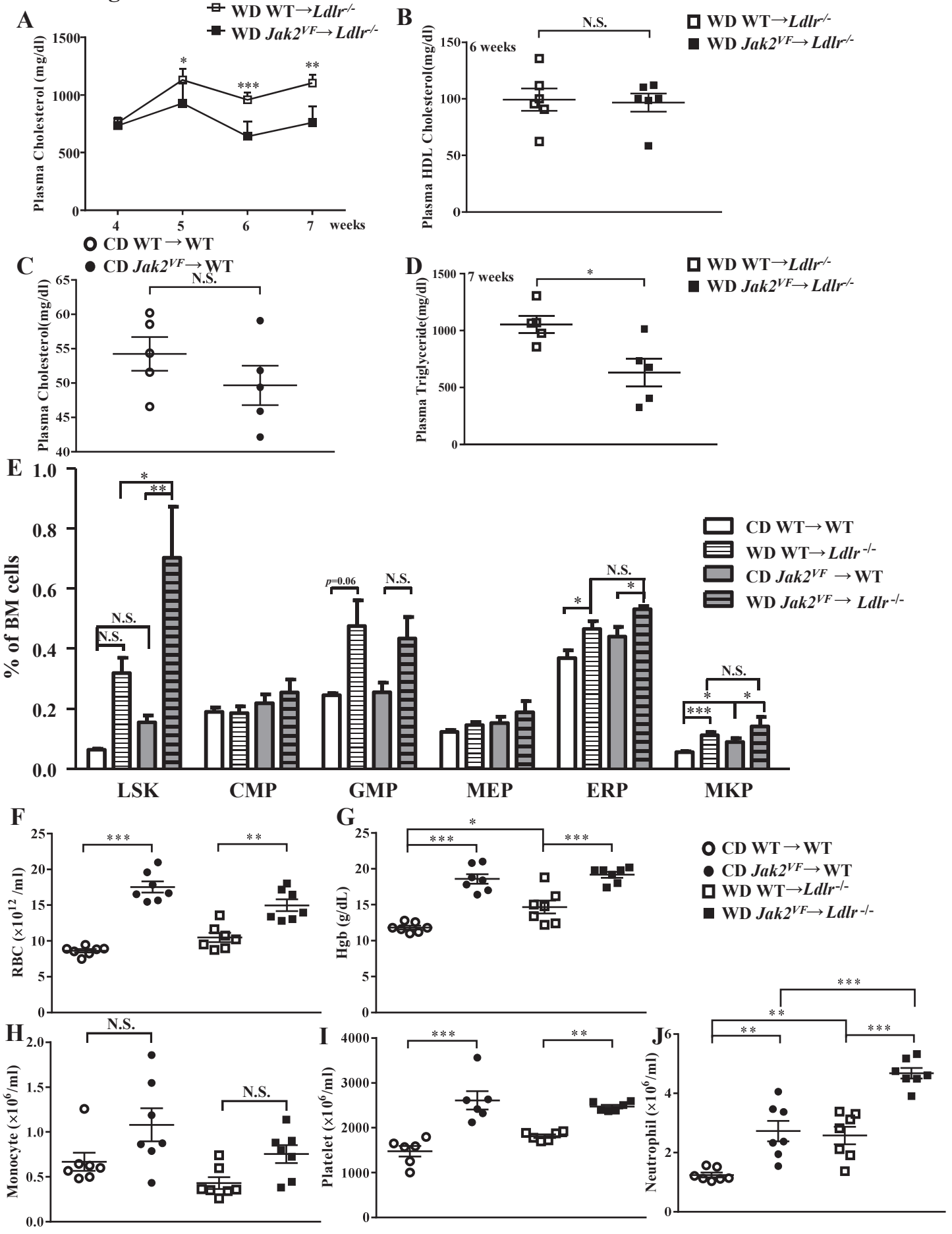
Online Figure IX: Impact of p38 or JNK inhibition on macrophage response.
ConA macrophages were treated with or without p38 inhibitor SB203580 or JNK inhibitor SP600125 at 10μM for 1 hour followed by 4-hour treatment with LPS (10ng/ml). Gene expression was quantified by qPCR. 1-way ANOVA. **p*<0.05, ***p*<0.01,****p*<0.001.

Online Figure X: Autophagy and ER stress in WT and *Jak2*^{VF} macrophages.
(A) ConA macrophages were treated with tunicamycin 10μg/ml for 8 hours. Cellular protein levels of CHOP were assessed by immunoblot. Unpaired *t* test. **(B)** ConA macrophages were pretreated with 100nM rapamycin for 2 hours and then challenged with LPS 10ng/ml for 8 hours. Gene expression was quantified by qPCR. 1-way ANOVA. **(C)** Bone marrow derived macrophages were pretreated with JAK1/2 inhibitor ruxolitinib 0.5μM for 1 hour and then challenged by 2 million PKH26-labed red blood cells. Erythrophagocytosis was assessed by determining mean fluorescence intensity of PKH26 in macrophages with flow cytometry. 2-way ANOVA. **p*<0.05, ***p*<0.01,****p*<0.001, N.S., not significant.

Online Figure XI: Impact of JAK1/2 inhibitor ruxolitinib on macrophage response.
ConA macrophages were pretreated with JAK1/2 inhibitor ruxolitinib 0.5μM for 1 hour and then challenged with LPS 10ng/ml for indicated times. Gene expression was quantified by qPCR. 1-way ANOVA.

*p<0.05,**p<0.01,***p<0.001. N.S., not significant.

Online Figure I



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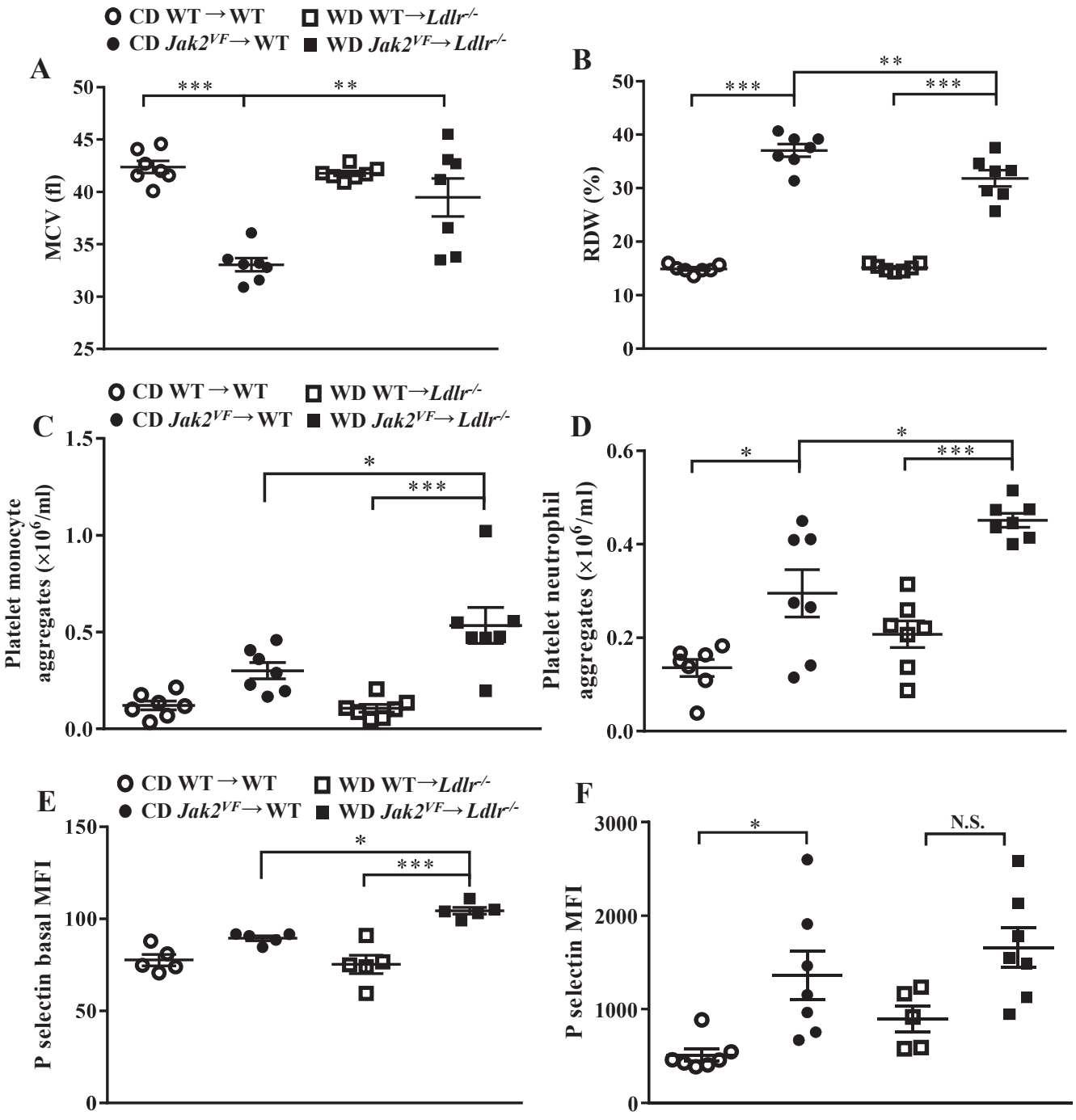

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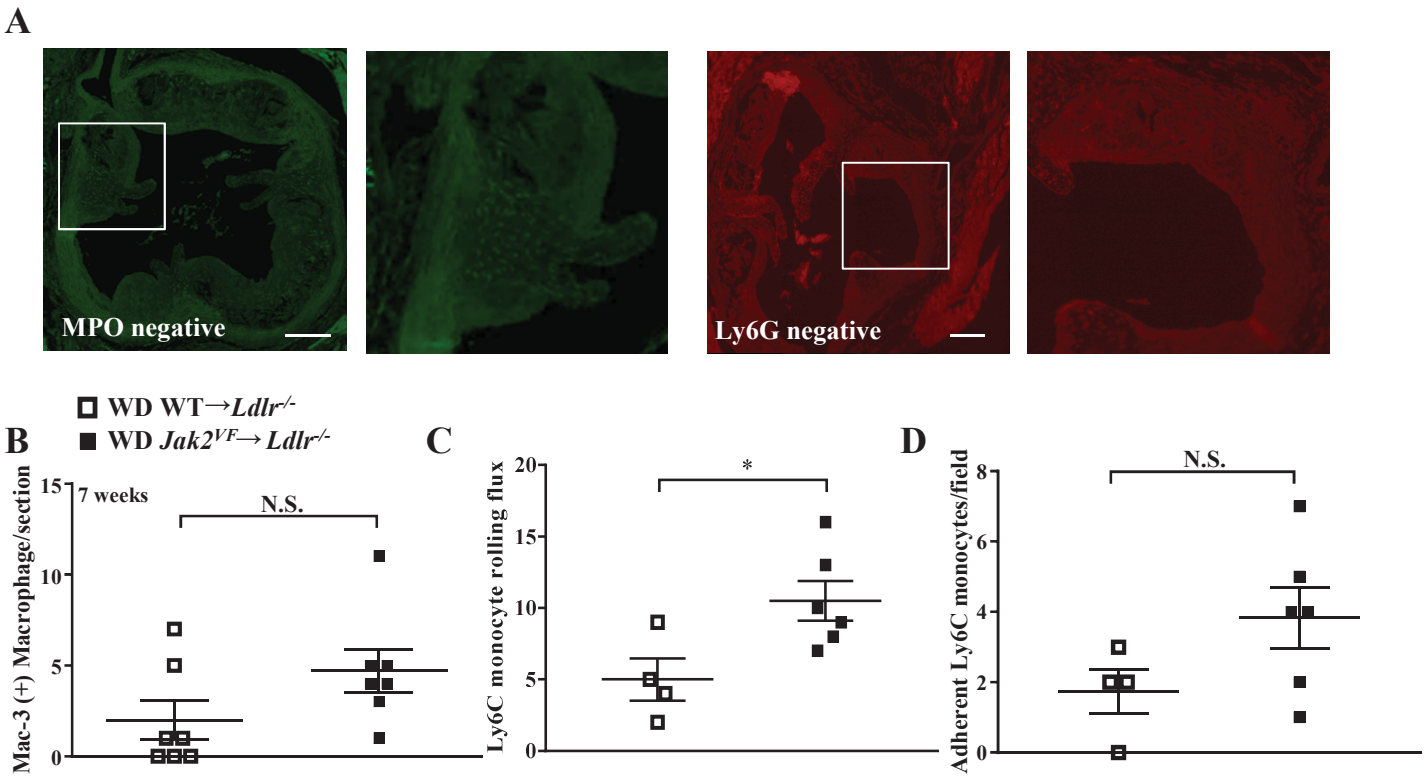

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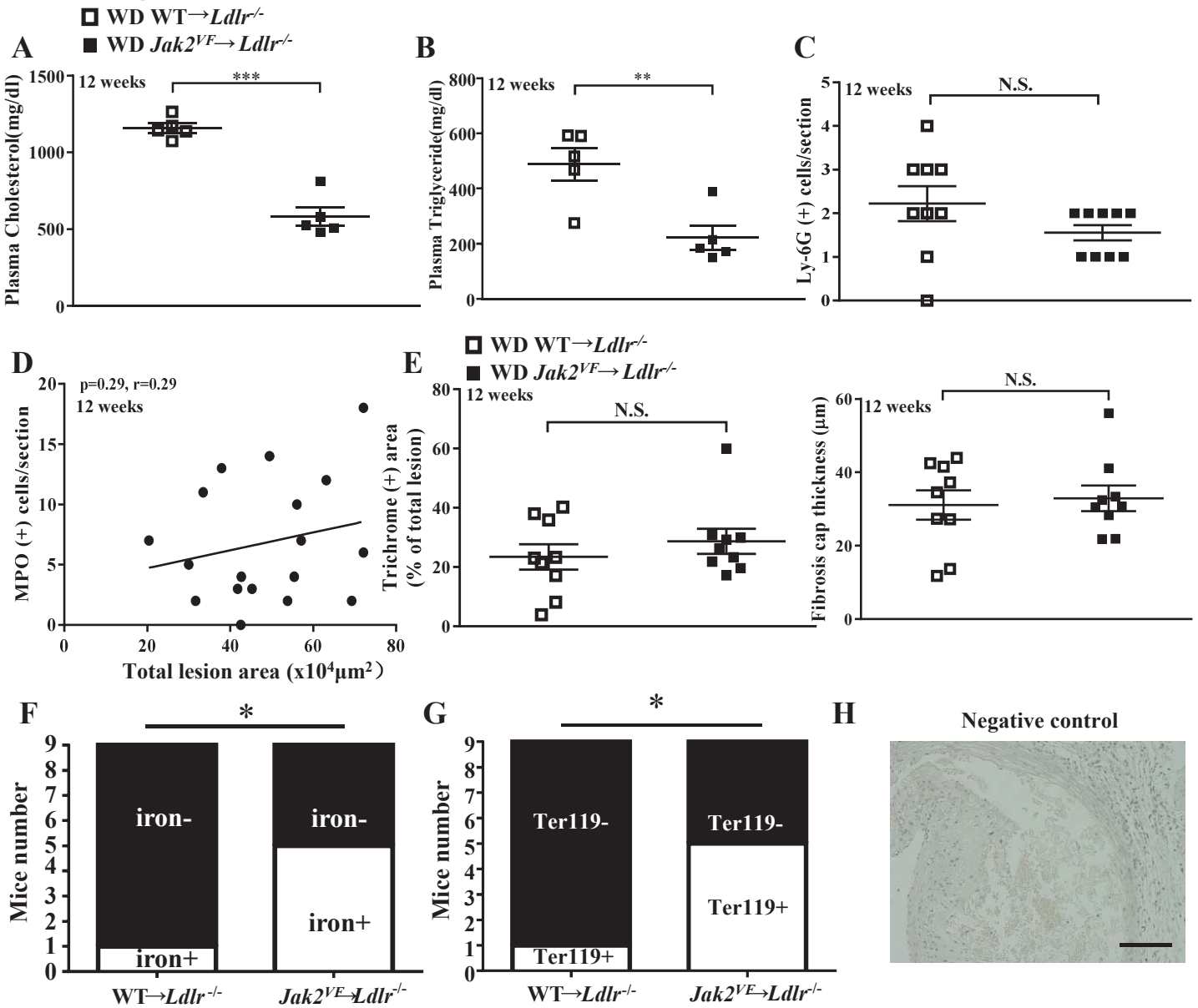

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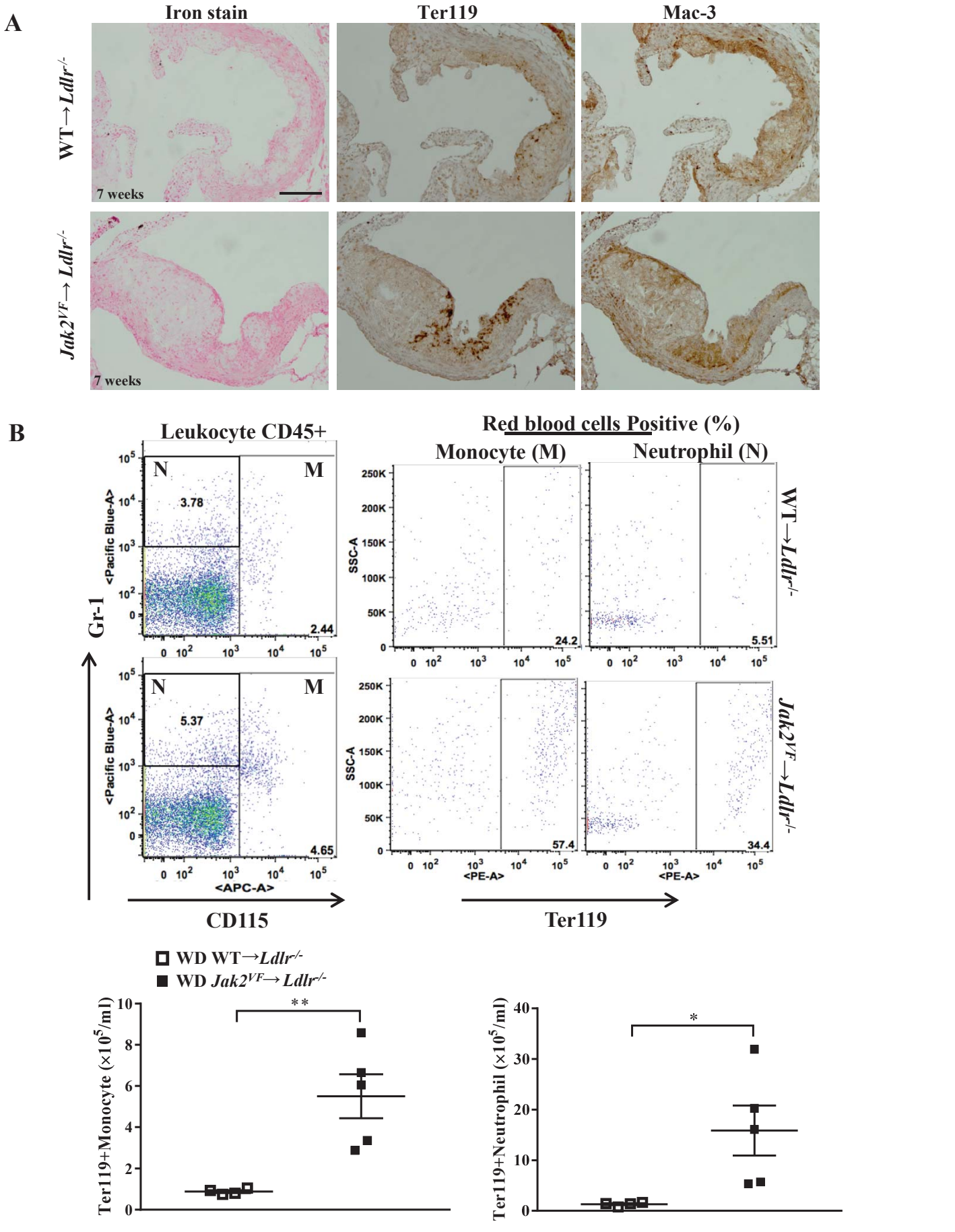

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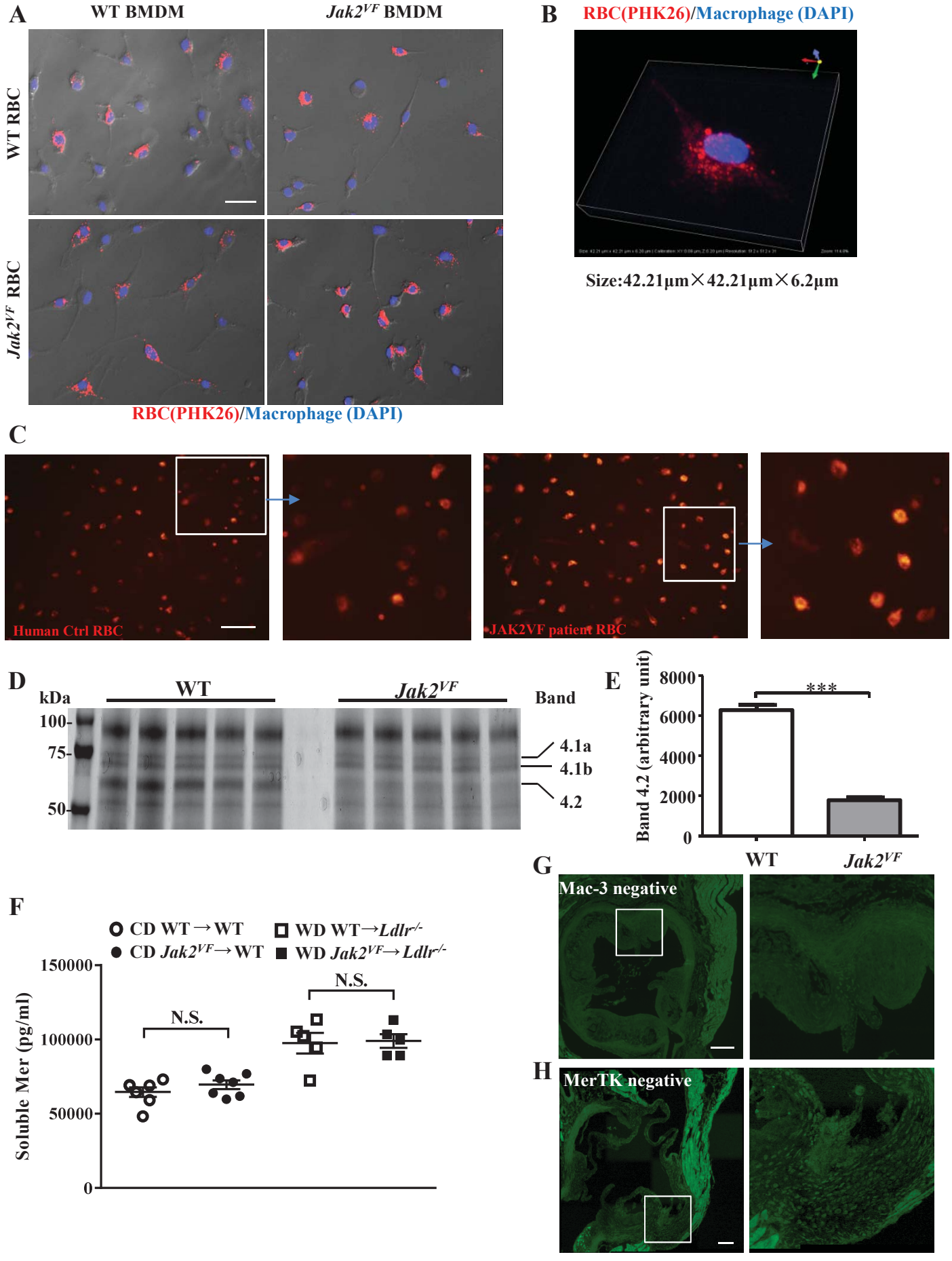

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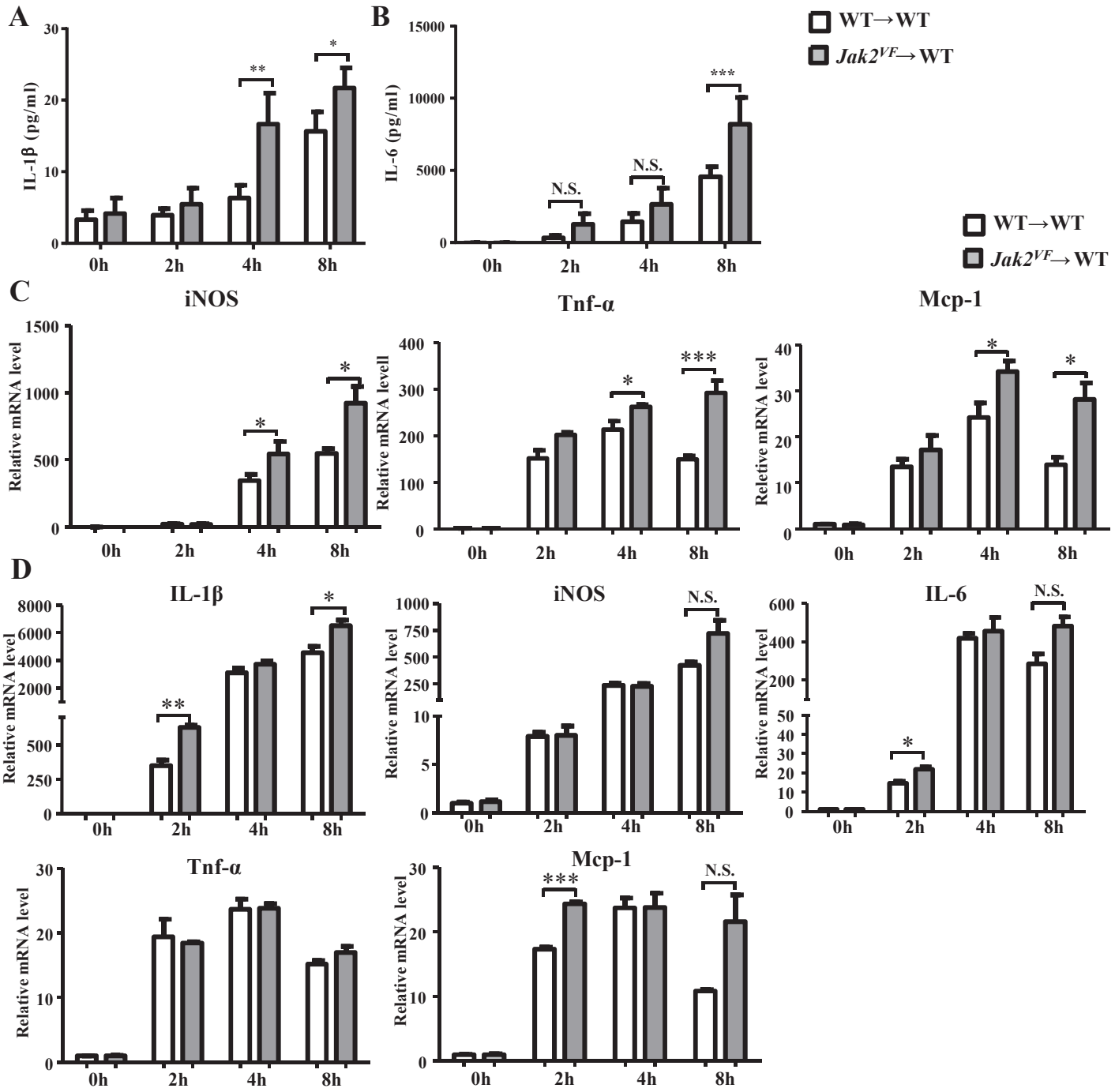

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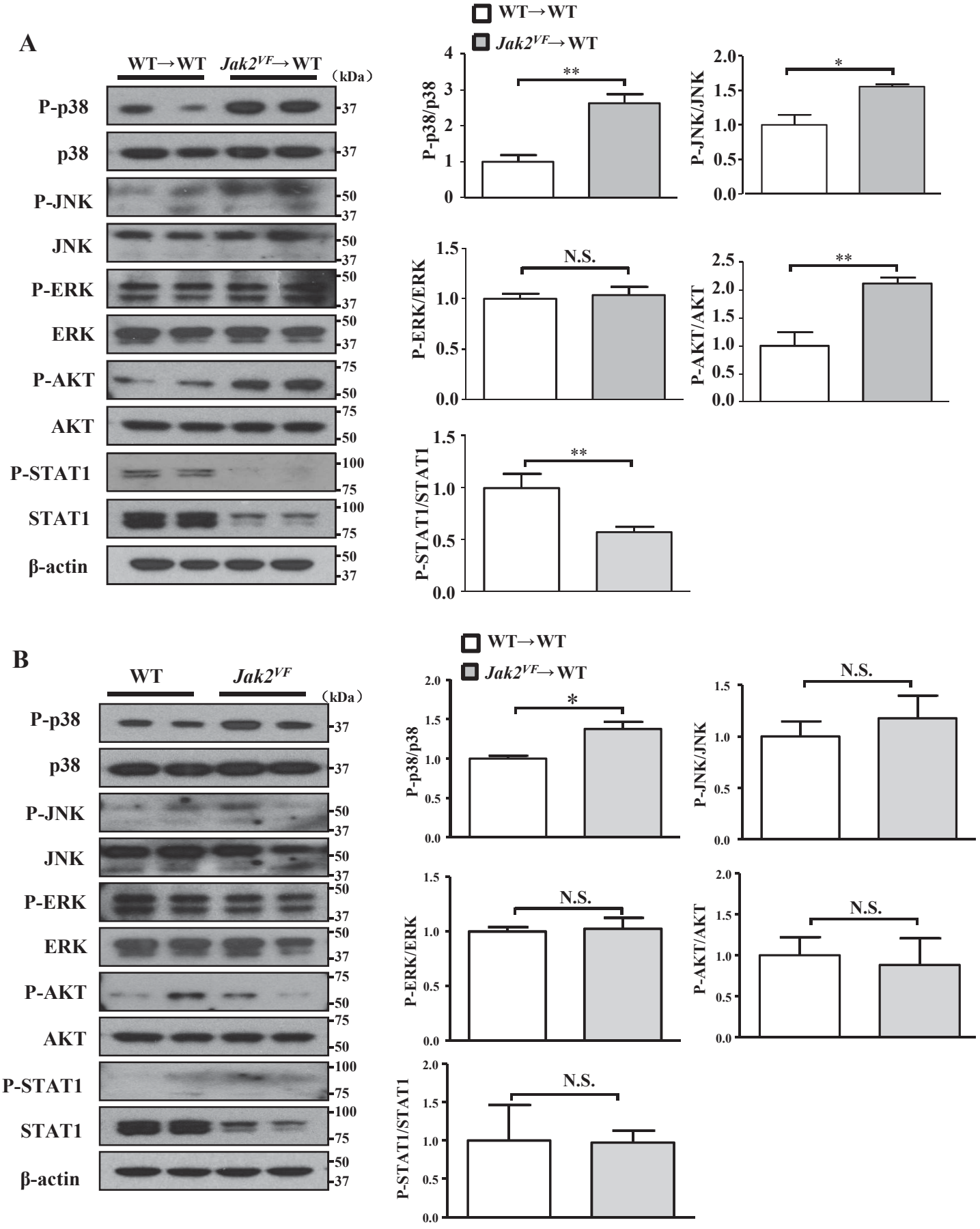

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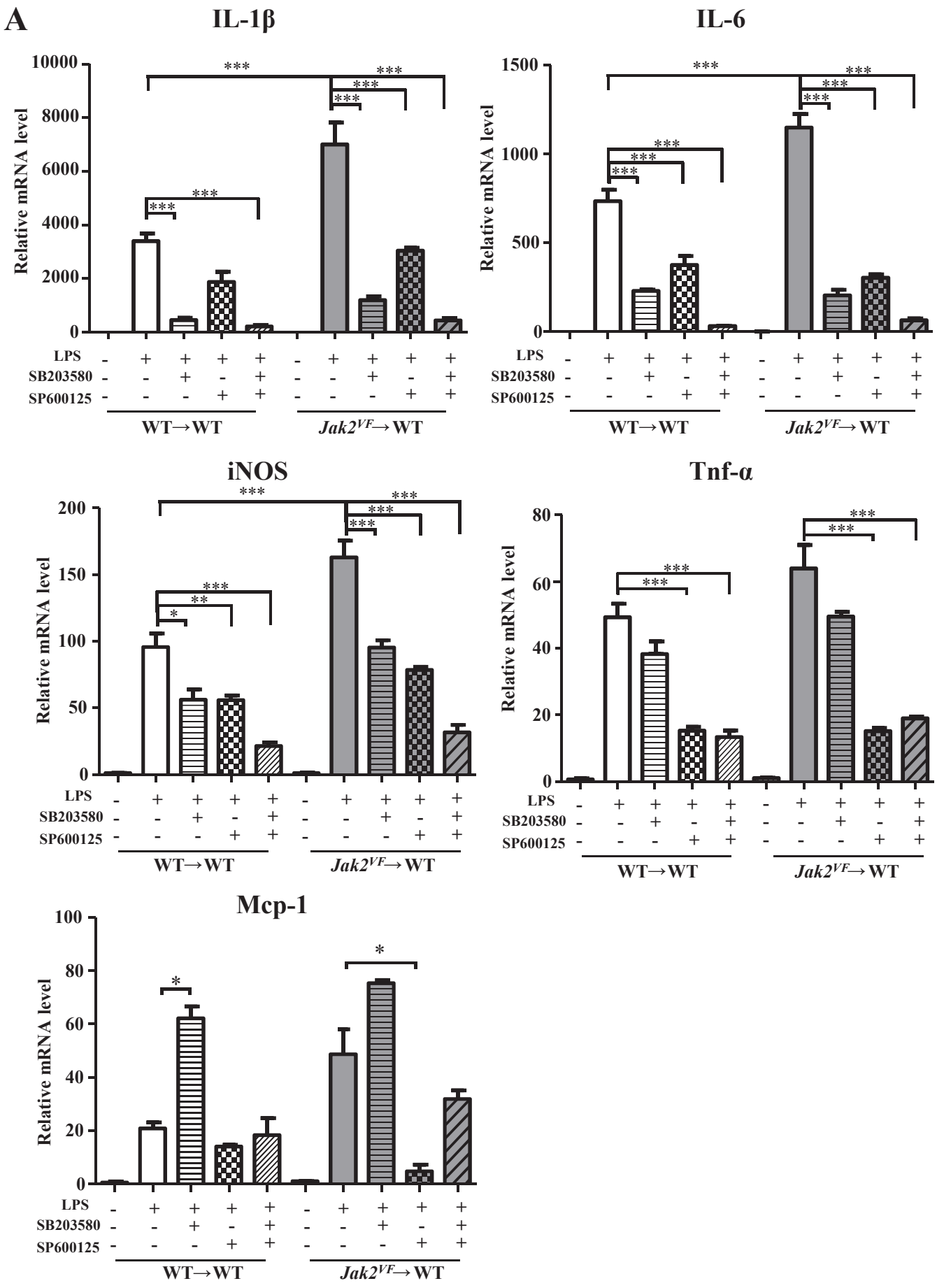

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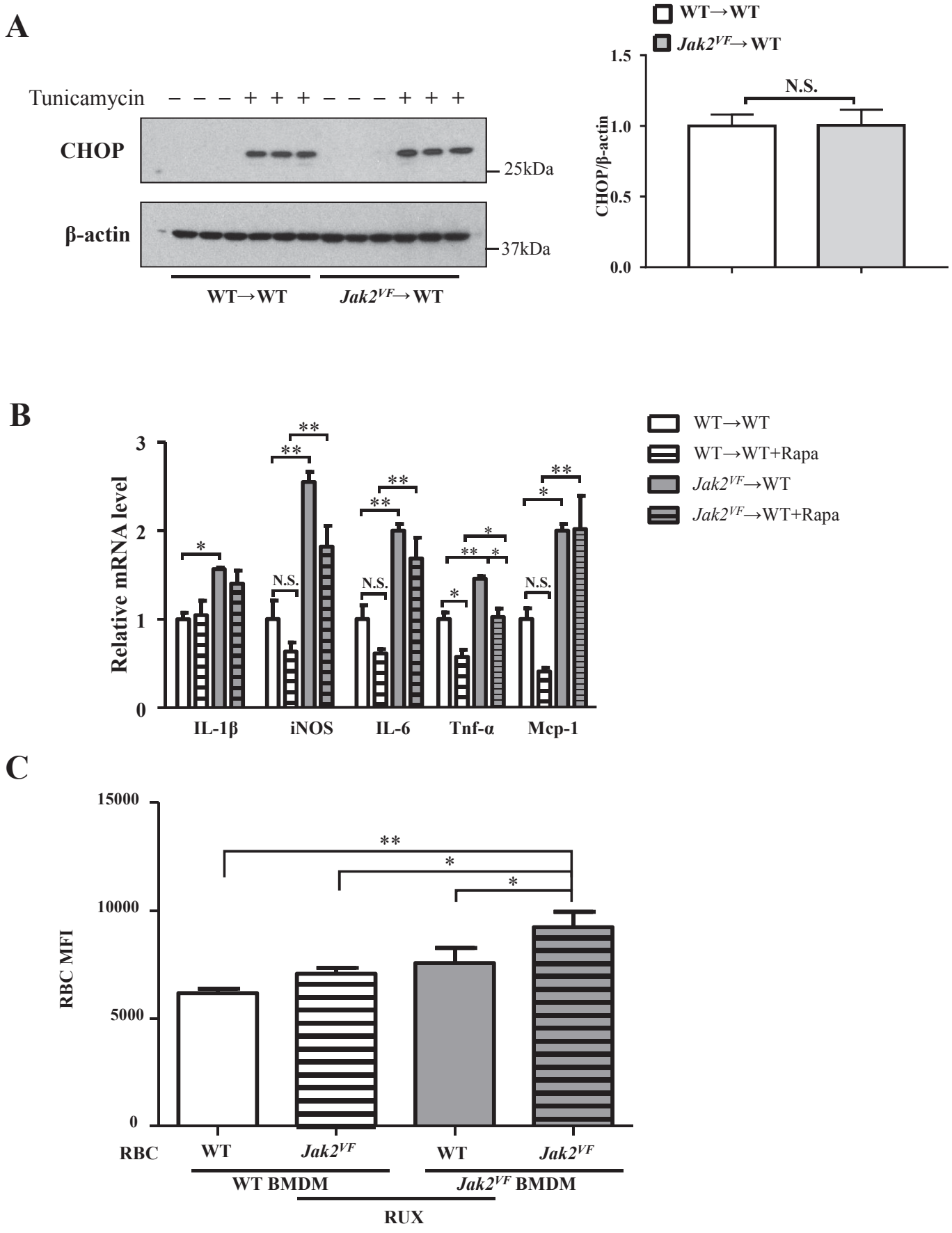

Online Figure XI