SUPPLEMENTARY APPENDIX

Crucial role of hematopoietic *JAK2* V617F in the development of aortic aneurysms

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Crucial role of hematopoietic JAK2V617F in the development of aortic aneurysms

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Supplementary methods

Patients

We enrolled all 39 patients with *JAK2*V617F-positive MPNs whose data of a computed tomography were available in a series of our former studies.[1, 2] The diagnosis and classification of MPNs were based on World Health Organization 2016 criteria.[3] The *JAK2*V617F allele burden was evaluated as described previously.[2] Clinical data were obtained at the time of evaluation of the allele burden of *JAK2*V617F. Medical histories were assessed including thrombotic events including arterial thrombosis (myocardial infarction, cerebral infarction, and other arterial thrombosis) and venous thrombosis (deep vein thrombosis, pulmonary embolism, and other venous thrombosis). We evaluated the maximal diameters of the ascending aorta and abdominal aorta on a computed tomography (Aquilion 64, Toshiba Medical Systems Co., Ltd., Tokyo, Japan, or Somatom Definition Edge, Siemens Healthcare GmbH, Munich, Germany). According to the guidelines,[4, 5] TAA was defined as > 4.0 cm of the diameter of the ascending aorta, and AAA was determined as > 3.0 cm of the abdominal aortic diameter or when the maximum diameter is 50% greater than the suprarenal diameter. The protocol of the human studies was approved by the institutional ethics committee of Fukushima Medical University Hospital (approval ID, 29348 and 1242) and confirmed in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was given by all subjects.

Human blood sampling and reverse transcription-quantitative PCR

Peripheral blood samples were collected with a tube containing EDTA-2Na (VP-NA070K, TERUMO, Tokyo, Japan) from 13 patients with *JAK2*V617F-positive MPNs and 28 age- and gender-matched healthy volunteers who had never shown any cardiovascular diseases including AAs or hematological disorders. The blood samples were immediately hemolyzed with ammonium chloride solution, and then after centrifugation, the white blood cells were obtained and kept frozen at -80°C. Total RNA were isolated by using TRIzol Reagent (Thermo Fisher Scientific, MA, USA) and complementary DNA was synthesized using ReverTra Ace qPCR RT Kit (TOYOBO, Osaka, Japan). Quantitative PCR was performed to determine mRNA levels for *MMP2*, *MMP9*, *TGFB3, IL-8* and *ACTB* using THUNDERBIRD SYBR qPCR Mix (TOYOBO) in the CFX Connect Real-Time PCR System (Bio-Rad, CA, USA). A delta CT method was applied for the

quantification and all values were normalized to *ACTB* and expressed as a fold increase of the control group. Primer sequences were described in *Table S4*.

Animals

We used male *Jak2*V617F-expressing transgenic (JAK2^{V617F}) mice aged between 8 and 12 weeks (body weight range, 20–30 g) with a C57BL/6J background.[2, 6] Wild-type (WT) littermates were used as controls. Male apolipoprotein E-deficient (ApoE$^{-/-}$) mice on a C57BL/6 background were obtained from the Jackson Laboratory (JAX stock number, 002052, Bar Harbor, ME, USA).[7] CAG-EGFP reporter mice with a C57BL/J background were purchased from the Japan SLC (Shizuoka, Japan). JAK2^{V617F} mice were crossed with CAG-EGFP mice to generate JAK2^{V617F}/CAG-EGFP double transgenic mice (JAK2^{V617F}-GFP).[8] The WT littermates were used as controls (WT-GFP). WT mice (C57BL/6) were purchased from the Charles River Laboratories Japan, Inc. The mice were housed with standard chow and water ad libitum at room temperature under a 12 h light-dark cycle. Mice were euthanized by cervical dislocation. The investigations conform to the Guidelines for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication, 8th Edition, 2011). All efforts were addressed to minimize the suffering of the animals. All animal studies were approved by the Fukushima Medical University Animal Research Committee (approval ID, 2019078).

Bone marrow (BM) transplantation (BMT) and chimeric analysis

ApoE$^{-/-}$ mice aged between 8 and 12 weeks (body weight range, 20–30 g) or WT mice aged between 8 and 12 weeks (body weight range was from 20 to 30 g) were lethally irradiated (9.0 Gy) 24 h before BMT.[2] Whole BM cells were harvested from femurs and tibiae of the donor mice. The cells were washed with phosphate-buffered saline (PBS) and 5.0×10^6 of BM cells were injected in the recipient ApoE$^{-/-}$ mice or WT recipient mice via the tail vein. For the chimeric analysis of the ApoE$^{-/-}$ recipient mice, DNA was isolated using QuickGene-Mini80 (KURABO, Osaka, Japan) with QuickGene DNA whole blood kit (KURABO) from blood samples and quantitative polymerase chain reaction (PCR) was performed using THUNDERBIRD SYBR qPCR Mix (TOYOBO) using the following primers; apolipoprotein E wild-type for donor-derived DNA forward, 5'-GCCTAGCCGAGGGAGAGCCG-3'; apolipoprotein E wild-type for donor-derived DNA reverse, 5'-TGTGACTTGGGAGCTCTGCAGC-3'; ApoE$^{-/-}$ for recipient-

derived DNA forward, 5'-GCCTAGCCGAGGGAGAGCCG-3'; ApoE$^{-/-}$ for recipient-derived DNA reverse, 5'-GCCGCCCCCGACTGCATCT-3'. Chimerism was calculated as follows[9];

Chimerism = DNA_{donor}/ (DNA_{donor}+$DNA_{recipient}$) x 100

$(1+e_{wild})^{-CTwild}/((1+e_{wild})^{-CTwild}+(1+e_{KO})^{-CTKO})$ x 100

DNA_{donor}, DNA burden of donor; $DNA_{recipient}$, DNA burden of recipient;e_{wild}, PCR efficiency of apolipoprotein E wild-type; CTwild, threshold cycle of apolipoprotein E wild-type; e_{KO}, PCR efficiency of ApoE$^{-/-}$; CTKO, threshold cycle of ApoE$^{-/-}$.

For the chimeric analysis of the WT recipient mice, quantitative PCR was performed using the following primers; recipients and donors-derived DNA forward, 5'-CTTTCTTCGAAGCAGCAAGCATGA-3', reverse primer for recipients ; 5'-CTGGCTTTACTTACTCTCCTCTCCACAGAC-3' reverse primer for donor ; 5'-AACCAGAATGTTCTCCTCTCCACAGAA-3'. Delta Ct (Ct_{donor}–Ct_{total}) was calculated to estimate the chimerism of *Jak2*V617F in the WT recipient mice transplanted with bone marrow cells from JAK2^{V617F} mice. For BMT using the JAK2^{V617F}-GFP transgenic mice, the lethally irradiated male ApoE$^{-/-}$ mice were used as the recipient mice. BM cells from male JAK2^{V617F}-GFP mice or WT-GFP littermates were injected into the recipient mice via the tail vein.

Peripheral blood analysis in mice

Peripheral blood parameters were analyzed at 5 weeks after BMT and at the time of termination of the experiments. Blood test was performed using pocH-100i (Sysmex, Kobe, Japan).

Angiotensin II (Ang II)-induced aortic aneurysm model

Five weeks after BMT, the recipient ApoE$^{-/-}$ mice were anesthetized with an intraperitoneal injection of a mixed anesthetic agent consisting of medetomidine (0.75 mg/kg), midazolam (4 mg/kg) and butorphanol (5 mg/kg) in a sterile saline. After an adequate depth of anesthesia was confirmed, an osmotic pump (ALZET micro-osmotic pump MODEL 1004, DURECT Co., Cupertino, CA, USA) was placed into the subcutaneous space through a small incision in the back of the neck that we closed by suturing.[10] Ang II (1900 ng/kg per min) or saline was continuously infused for 4 weeks.[11,12]

Ultrasound imaging and determination of aortic aneurysms

The mice were lightly anesthetized with 1% isoflurane and placed on a heating

pad to maintain body temperature. The mice were monitored with electrocardiogram and heart rate was verified between 400 and 500 beats per minute. Thoracic and abdominal aortic diameters were evaluated by ultrasound images using Vevo 2100 Imaging System (FUJIFILM Visualsonics, Toronto, Canada) at the baseline prior to implantation of osmotic pumps, and at 2 weeks and 4 weeks after Ang II or saline infusion. The images of the thoracic aorta were acquired from right parasternal long axis view, and then the maximal diameter of the ascending aorta was measured between the aortic root and aortic arch regions. The maximal diameter of the abdominal aorta was analyzed around the renal arteries from short axis view. All measurements were performed at end-diastole of cardiac cycles according to R wave of electrocardiogram, and the data were averaged over 3 separate heart beats. Presence of TAA and AAA was defined as an increase of ≥50% compared with the same area of the aortas at the baseline as previously described.[13] Ruptures of TAA and AAA were defined by blood in the cavity and large thrombi in the aorta when the mice were sacrificed.[12]

Measurements of blood pressure and heart rate

The systolic, mean, and diastolic blood pressures, as well as heart rate, were measured by the tail-cuff method using a programmable sphygmomanometer (BP-98A-L, Softron, Tokyo, Japan) in the conscious mice at the baseline prior to implantation of osmotic pumps, and at 2 weeks and 4 weeks after starting Ang II or saline infusion.[14] The data were averaged over 3 consecutive measurements.

Histological analysis

After continuous Ang II or saline infusion for 4 weeks, the mice were euthanized by cervical dislocation, and aortas were perfused by cold PBS from the apex of the left ventricle for histological analysis. The samples of aorta were fixed with 4% paraformaldehyde, embedded in paraffin, and then sectioned to 3 μm thickness. The paraffin sections were stained with hematoxylin-eosin (HE), Elastica-Masson (EM), or used for immunostaining. To analyze the degradation of medial elastic lamina, EM-stained sections were blindly classified into four elastin degradation-grading groups; grade 1, no degradation; grade 2, mild degradation; grade 3, severe degradation; grade 4, aortic rupture as previously described.[15] Paraffin sections were stained with CD45 (sc-53665, Santa Cruz Biotechnology, CA, USA), CD68 (MCA1957GA, Bio-Rad), Ly6B.2 (MCA771GA,

Bio-Rad) or TER119 (116201, Biolegend, CA, USA) antibody followed by 0.02% 3, 3-diaminobenzidine-4HCl (DAB; 347-00904, Dojindo, Kumamoto, Japan) and counterstained with hematoxylin. For preparation of the frozen sections, the abdominal aortas were embedded in the O.C.T. compound (4583, Sakura Finetek Japan, Tokyo, Japan) and were sectioned to 12 μm thickness using a cryostat (CryoStar NX70, Thermo Fisher Scientific). The O.C.T.-embedded sections were used for immunohistochemical staining with the following primary antibodies; CD45, CD68, Ly6B.2, and GFP (ab6556, abcam, Cambridge, United Kingdom), followed by the appropriate secondary antibodies including goat anti-rat IgG H&L (Alexa Fluor 647, ab150159, abcam), anti-rabbit IgG H&L, (Alexa Fluor 647, 4414, Cell Signaling Technology, MA, USA), and then mounted with DAPI containing mounting media (Fluoro-Gel II, Electron Microscopy Sciences, PA, USA). All images were acquired by a microscope (BZ-X700, KEYENCE, Osaka, Japan).

Measurement of total cholesterol and triglyceride

After the mice were euthanized by cervical dislocation, whole blood was taken from the heart, and then immediately centrifuged at 10000 g for 5 min at 4°C. The plasma samples were kept frozen at -80°C. Total cholesterol and triglyceride concentrations were measured by an enzymatic method in Oriental Yeast Co., Ltd (Tokyo, Japan) with their standard protocols.

Gelatin zymography

The matrix metalloproteinase 2 (MMP-2) activity and pro metalloproteinase 9 (MMP-9) expression levels were evaluated in the abdominal aorta. The frozen tissue of abdominal aorta (20 g) was initially homogenized in 200 μl of a lysis buffer containing Tris-HCl (pH 7.4, 50 mM), NaCl (150 mM), $CaCl_2$ (10 mM) and 0.25% Triton X-100 using TissueLyser II (QIAGEN, Hilden, Germany).[16] After centrifugation, the supernatant was collected, and the protein concentration was determined using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific). Ten μg of protein was used for gelatin zymography. Gelatin zymography analysis was performed using a Gelatin zymography kit (AK45, Cosmo Bio, Tokyo, Japan) according to the manufacturer's instructions. The gelatin electrophoresis gel was visualized using an Amersham imager 600 RGB (GE Healthcare Life Sciences, MA, USA). The results were evaluated by densiometric analysis of the lytic areas obtained from the gelatin electrophoresis using ImageJ software (U. S. National

Institutes of Health, MD, USA).[17] The sum of MMP-2 and pro MMP-2 bands was evaluated as MMP-2 activity.[18]

Preparation for bone marrow-derived macrophages

BM cells were obtained by isolating and flushing tibias and femurs from WT or $JAK2^{V617F}$ mice after euthanasia by cervical dislocation. The BM-derived mononuclear cells were collected by density gradient centrifugation at 500 g for 40 min at room temperature using Lymphocyte Separation Media (Promo Cell, Heidelberg, Germany). On a 24-well cell culture plate (662160, CELLSTAR, Greiner Bio-One, Kremsmünster, Austria), 7.0×10^5 of the separated mononuclear cells were cultured in DMEM (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) containing 10% fetal bovine serum, 100 IU/mL of penicillin, 100 mg/mL of streptomycin and 10 ng/mL of granulocyte-macrophage colony stimulating factor (AFL415, R&D Systems, MN, USA) for 6 days at 37°C in the presence of 5% CO_2 as previously described.[19] To determine the purity of the macrophages, the adherent cells were seeded in 4 well chamber slide (Thermo Fisher Scientific) and fixed by 4% paraformaldehyde and stained with a CD68 antibody followed by Alexa Fluor 647-conjugated goat anti-rat secondary antibody and then mounted with DAPI containing mounting media.

Western blot analysis

Frozen mouse aorta samples were initially homogenized in lysis buffer. The protein concentration was determined using a Piece BCA Protein Assay Kit (Thermo Fisher Scientific). Aliquots of proteins were subjected to SDS-polyacrylamide gel electrophoresis, transferred onto polyvinylidene difluoride membranes (Merck Millipore, MA, USA) and probed with the following primary antibodies; phosphorylated-STAT3 (9145S, Cell Signaling Technology), total-STAT3 (4904S, Cell Signaling Technology), and Beta actin (sc-47778, Santa Cruz Biotechnology) followed by appropriate goat anti-rabbit (4050-05, SouthernBiotech, AL, USA) or mouse (sc-516102, Santa Cruz Biotechnology) horseradish peroxidase conjugated secondary antibodies. The immunoreactive bands were visualized by an Amersham ECL system and the signals were detected with an Amersham imager 600 RGB (GE Healthcare Life Sciences, MA, USA). The results were evaluated by densiometric analysis of the lytic areas obtained from the gelatin electrophoresis using ImageJ software (U. S. National Institutes of Health, MD, USA).[17]

Reverse transcription-quantitative PCR

Total RNA was extracted from the cultured cells using Trizol reagent according to the manufacturer's protocol (Thermo Fisher Scientific). Total RNA from the aorta samples were extracted using RNeasy Fibrous Tissue Mini Kit (QIAGEN, Hilden, Germany). Complementary DNA was synthesized using ReverTra Ace qPCR RT Kit (TOYOBO). Quantitative PCR was performed to determine the levels of mRNA expression for *Mmp2*, *Mmp9, Mmp13, Ccl6*, *Tgfb1*, and *Actb* using THUNDERBIRD SYBR qPCR Mix (TOYOBO) in the CFX Connect Real-Time PCR System (Bio-Rad). A standard curve method was applied for the aorta samples and a delta CT method was applied for the cultured cell samples. All values were normalized to *Actb* and expressed as a fold increase of the control group. Primer sequences were described in *Table S4*.

Administration of a JAK1/2 inhibitor

For in vitro experiments, ruxolitinib (Novartis Pharmaceuticals, Basel, Switzerland) was used at concentration of 250 nM for 24 hours prior to the RNA extraction. Dimethylsulfoxide was used as a control. For in vivo study, ruxolitinib dissolved in 0.5% methylcellulose was administered to the mice orally at 60 mg/kg twice daily for 4 weeks.[20, 21] We used 0.5% methylcellulose as a vehicle control.

Statistical analysis

Data are expressed as mean ± standard error (SEM). The statistical significance of differences was analyzed using the unpaired Student's t-test for parametric continuous variables. The Kaplan-Meier method and log-rank test were used to compare survival from the incidence of AAA or TAA. Categorical variables were compared using the Chi-square test or Fisher's exact test. The parameters of more than 2 groups were evaluated by one-way analysis of variance (ANOVA) followed by multiple comparisons with the Tukey post-hoc test. A value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed using the Statistical Package for Social Sciences version 26 software (SPSS Inc., Chicago, IL, USA).

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Table S1. Patient characteristics by classification of myeloproliferative neoplasms.

	PV (n = 16)	ET (n = 12)	PMF (n = 11)
Age, years	68 ± 11	69 ± 9	69 ± 16
Female, n (%)	8 (50)	5 (42)	5 (46)
Laboratory data			
White blood cell count, $\times 10^9$/L	12.7 ± 5.2	10.0 ± 5.5	18.7 ± 14.8
Red blood cell count, $\times 10^{12}$/L	5.7 ± 1.5	4.0 ± 0.9	3.7 ± 1.1
Hemoglobin concentration, g/dL	16.9 ± 3.4	13.0 ± 1.7	10.8 ± 3.4
Platelet count, $\times 10^9$/L	479 ± 304	472 ± 158	580 ± 721
*JAK2*V617F allele burden, %	63 ± 26	34 ± 31	49 ± 31
Comorbidities			
Hypertension, n (%)	8 (50)	8 (67)	1 (9)
Diabetes mellitus, n (%)	3 (19)	3 (25)	0 (0)
Dyslipidemia, n (%)	1 (6)	6 (50)	0 (0)
Smoking history, n (%)	6 (38)	5 (42)	2(18)
Thrombosis history, n (%)	2 (13)	5 (42)	3 (27)
Arterial thrombosis, n (%)	1 (6)	4 (33)	2 (18)
Venous thrombosis, n (%)	1 (6)	1 (8)	1 (9)
Cytoreductive treatment			
Hydroxyurea, n (%)	8 (50)	6 (50)	3 (27)
Ruxolitinib, n (%)	0 (0)	0 (0)	1 (9)
Presence of aortic aneurysms	6 (38)	2 (17)	1 (9)
Thoracic aortic aneurysm, n (%)	5 (31)	1 (8)	1 (9)
Abdominal aortic aneurysm, n (%)	1 (6)	1 (8)	0 (0)

All data are expressed as mean ± SD or number (%).

PV, polycythemia vera; ET, essential thrombocythemia; PMF, primary myelofibrosis.

Table S2. Patient characteristics used for reverse transcription quantitative polymerase chain reaction in peripheral leukocytes.

	Control subjects (n = 28)	Patients with MPNs (n = 13)	P value
Age, years	61 ± 15	68 ± 13	0.138
Female, n (%)	13 (46)	7 (54)	0.658

Values are mean ± SD or number (%). Comparisons of means between 2 groups were performed by the unpaired Student's t-test. Categorical variables were compared using Fisher's exact test. MPNs, myeloproliferative neoplasms.

Table S3. Plasma total cholesterol (TCHO) and triglyceride (TG) in response to angiotensin II (Ang II) infusion.

	Saline		Ang II	
	WT	$JAK2^{V617F}$	WT	$JAK2^{V617F}$
TCHO, mg/dL	328 ± 57	101 ± 17*	340 ± 33	170 ± 33*
TG, mg/dL	29 ± 10	20 ±7	76 ± 18	21 ± 5*

Plasma levels of TCHO and TG in WT-BMT mice and $JAK2^{V617F}$-BMT mice 4 weeks after saline or Ang II infusion (n = 4 each). All the data are presented as mean ± SEM. *P < 0.05 versus the corresponding WT group by one-way ANOVA with Tukey post-hoc analysis. WT, $ApoE^{-/-}$ mice transplanted with bone marrow cells from wild-type mice; $JAK2^{V617F}$, $ApoE^{-/-}$ mice transplanted with bone marrow cells from $JAK2^{V617F}$ mice.

Table S4. Primers used for reverse transcription quantitative polymerase chain reaction.

Species	Gene		Sequences		
Human	*MMP2*	Forward	5'-	GCTACGATGGAGGCGCTAAT	-3'
		Reverse	5'-	TCAGGTATTGCACTGCCAACT	-3'
	MMP9	Forward	5'-	AGCGAGAGACTCTACACCCA	-3'
		Reverse	5'-	AGAAGCCGAAGAGCTTGTCC	-3'
	IL-8	Forward	5'-	AAGGAAAACTGGGTGCAGAG	-3'
		Reverse	5'-	ATTGCATCTGGCAACCCTAC	-3'
	TGFB3	Forward	5'-	GCTGCACTTTGGAGTGATCG	-3'
		Reverse	5'-	GCTTGAGGGTTTGCTACAACA	-3'
	ACTB	Forward	5'-	TGGCACCCAGCACAATGAA	-3'
		Reverse	5'-	CTAAGTCATAGTCCGCCTAGAAGCA	-3'
Mouse	*Ccl6*	Forward	5'-	TATCCTTGTGGCTGTCCTTGG	-3'
		Reverse	5'-	GAAGAAGTGTCTTGAAAGCCTTGAT	-3'
	Tgfb1	Forward	5'-	AGCTGCGCTTGCAGAGATTA	-3'
		Reverse	5'-	AGCCCTGTATTCCGTCTCCT	-3'
	Mmp2	Forward	5'-	ACCTTGACCAGAACACCATCGAG	-3'
		Reverse	5'-	CAGGGTCCAGGTCAGGTGTGTA	-3'
	Mmp9	Forward	5'-	GCCCTGGAACTCACACGACA	-3'
		Reverse	5'-	TTGGAAACTCACACGCCAGAAG	-3'
	Mmp13	Forward	5'-	TCGCCCTTTTGAGACCACTC	-3'
		Reverse	5'-	AGCACCAAGTGTTACTCGCT	-3'
	Actb	Forward	5'-	CATCCGTAAAGACCTCTATGCCAAC	-3'
		Reverse	5'-	ATGGAGCCACCGATCCACA	-3'

Table S5. Plasma total cholesterol (TCHO) and triglyceride (TG) after vehicle or ruxolitinib treatment in the angiotensin II-infused JAK2^{V617F}-BMT mice.

	Vehicle	Ruxolitinib
TCHO, mg/dL	180 ± 38	380 ± 19*
TG, mg/dL	17 ± 4	115 ± 44

Plasma levels of TCHO and TG in the vehicle- or ruxolitinib-treated JAK2^{V617F}-BMT mice 4 weeks after angiotensin II infusion (n = 3–4). All data are presented as mean ± SEM. *P < 0.05 versus the vehicle group by the unpaired Student's t-test.

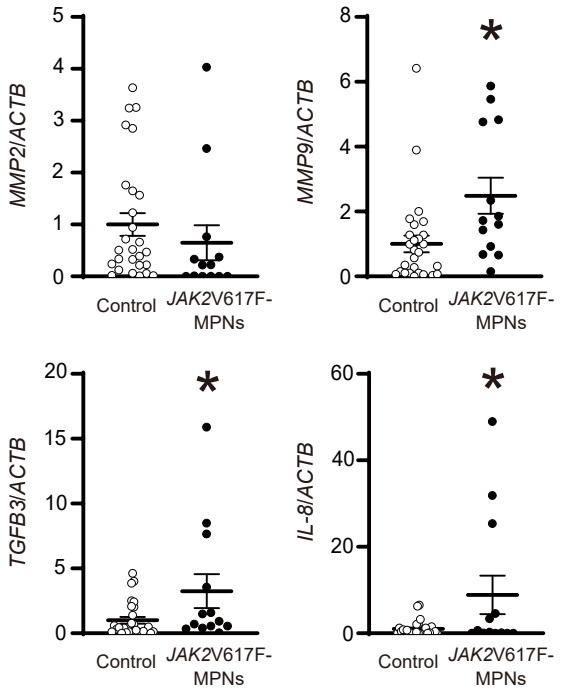

Figure S1. Matrix metalloproteinases and cytokine mediators were increased in the circulating leukocytes in patients with *JAK2*V617F-positive myeloproliferative neoplasms.
Relative mRNA expression levels of *MMP2*, *MMP9*, *TGFB3*, and *IL-8* in the peripheral leukocytes of myeloproliferative neoplasm patients (n = 13) in comparison with the age- and gender-matched control subjects (n = 28). *ACTB* was used for normalization. The average value of the control subjects was set to 1. All data are presented as mean ± SEM. *P < 0.05 versus the control group by the unpaired Student's t-test. MPNs, patients with myeloproliferative neoplasms.

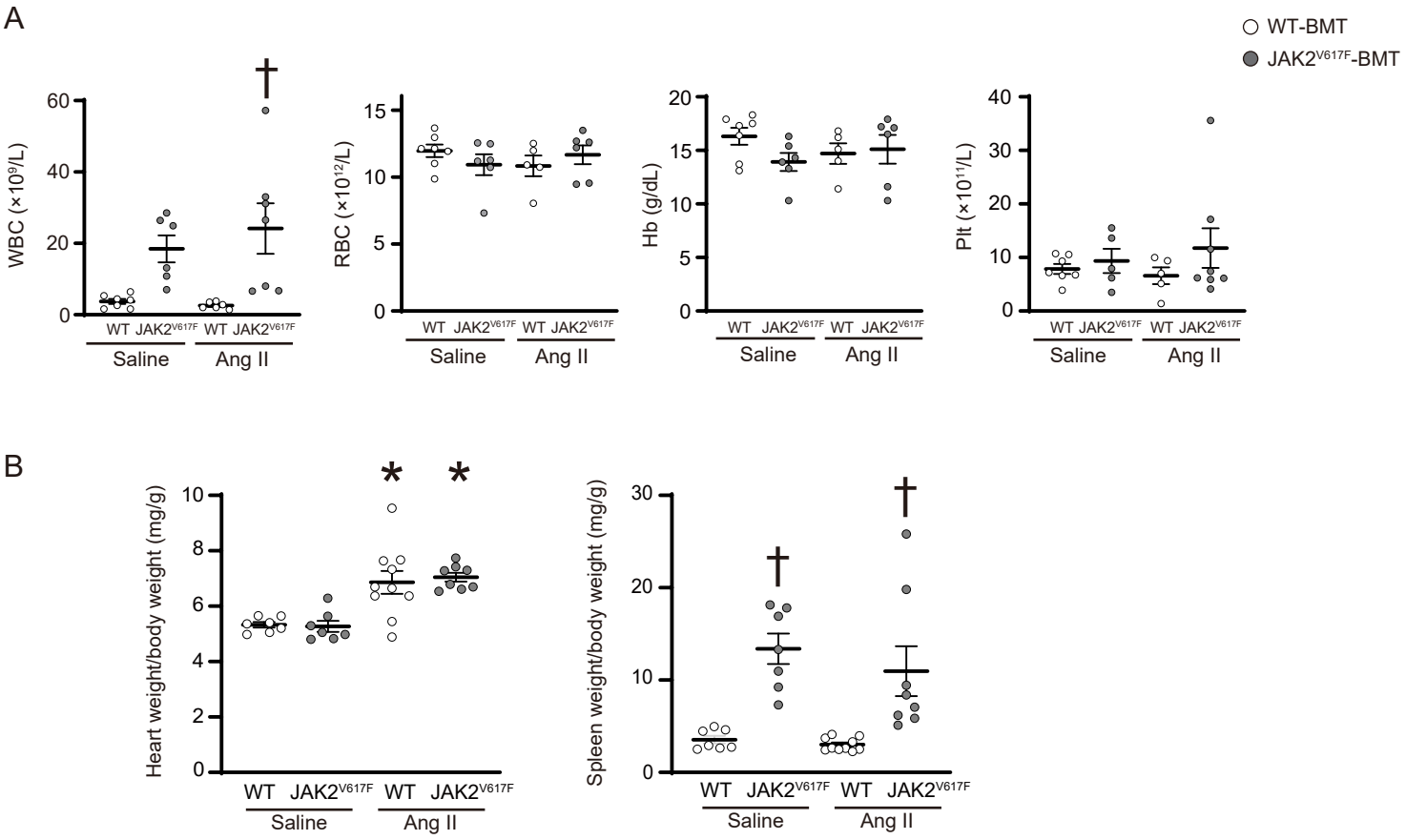

Figure S2. Blood cell counts and gravimetric data in response to angiotensin II (Ang II) infusion. (A) Peripheral blood cells counts (n = 5–7) and (B) gravimetric data (n = 7–10) in the WT-BMT mice and JAK2^{V617F}-BMT mice under saline or Ang II for 4 weeks. All data are presented as mean ± SEM. $^{*}P < 0.05$ versus the corresponding saline-infused mice and $^{\dagger}P < 0.05$ versus the corresponding WT-BMT mice by one-way ANOVA with Tukey post-hoc analysis. WT, ApoE$^{-/-}$ mice transplanted with bone marrow cells from wild-type mice; JAK2^{V617F}, ApoE$^{-/-}$ mice transplanted with bone marrow cells from JAK2^{V617F} mice; BMT, bone-marrow transplantation; WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin concentration; Plt, platelet count.

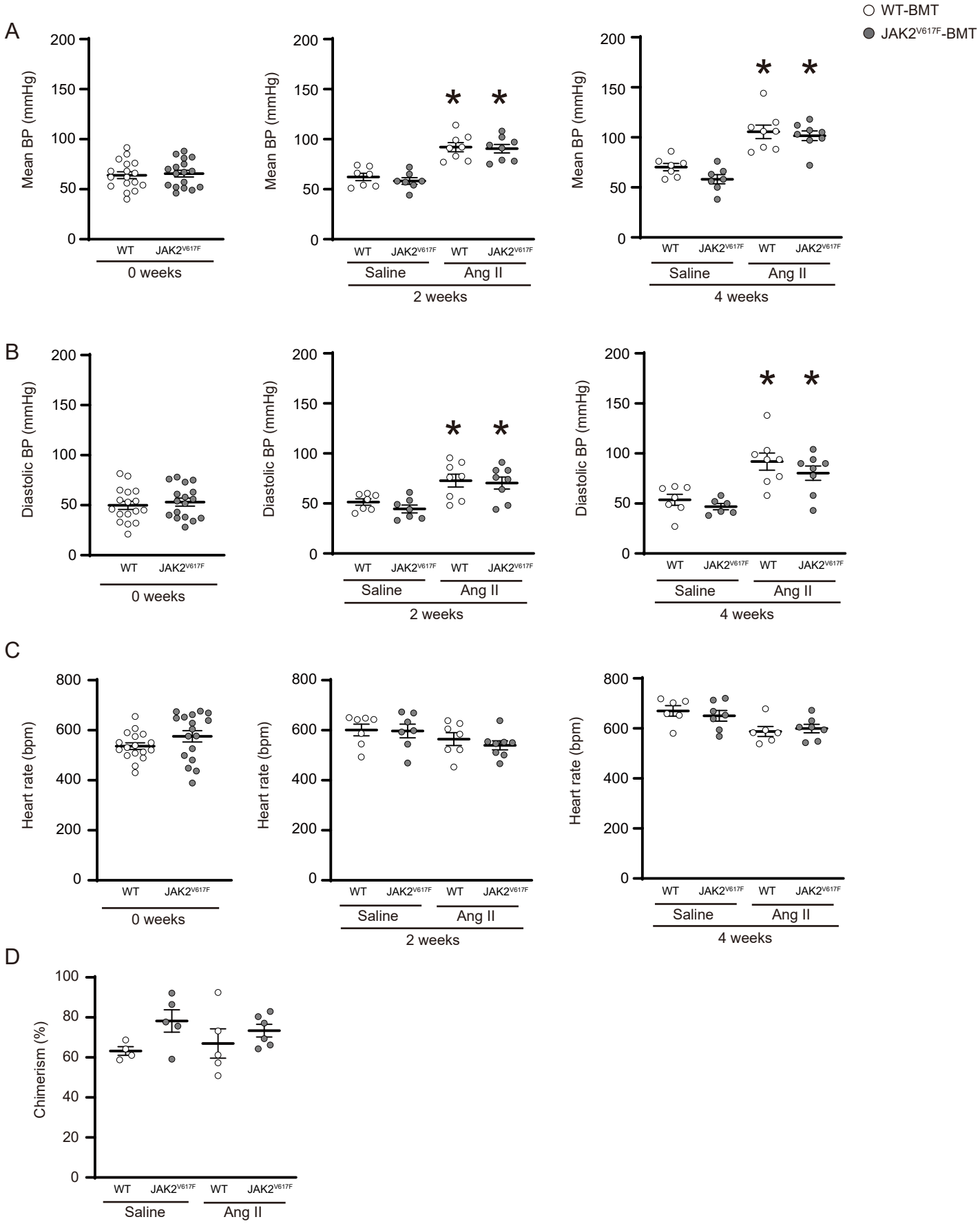

Figure S3. Blood pressures, heart rate, and chimerism in response to angiotensin II (Ang II) infusion.

(A) Mean blood pressure (BP), (B) diastolic BP, and (C) heart rate in the WT-BMT mice and JAK2^{V617F}-BMT mice at the baseline (n = 17 in each) and 2 weeks (n = 6–10) and 4 weeks (n = 6–8) under saline or Ang II infusion. (D) Chimerism (n = 4–6) in the WT-BMT mice and JAK2^{V617F}-BMT mice 4 weeks under Ang II infusion. All data are presented as mean ± SEM. *P < 0.05 versus the corresponding saline-infused mice by one-way ANOVA with Tukey post-hoc analysis. WT, ApoE$^{-/-}$ mice transplanted with bone marrow cells from wild-type mice; JAK2^{V617F}, ApoE$^{-/-}$ mice transplanted with bone marrow cells from JAK2^{V617F} mice; BMT, bone-marrow transplantation.

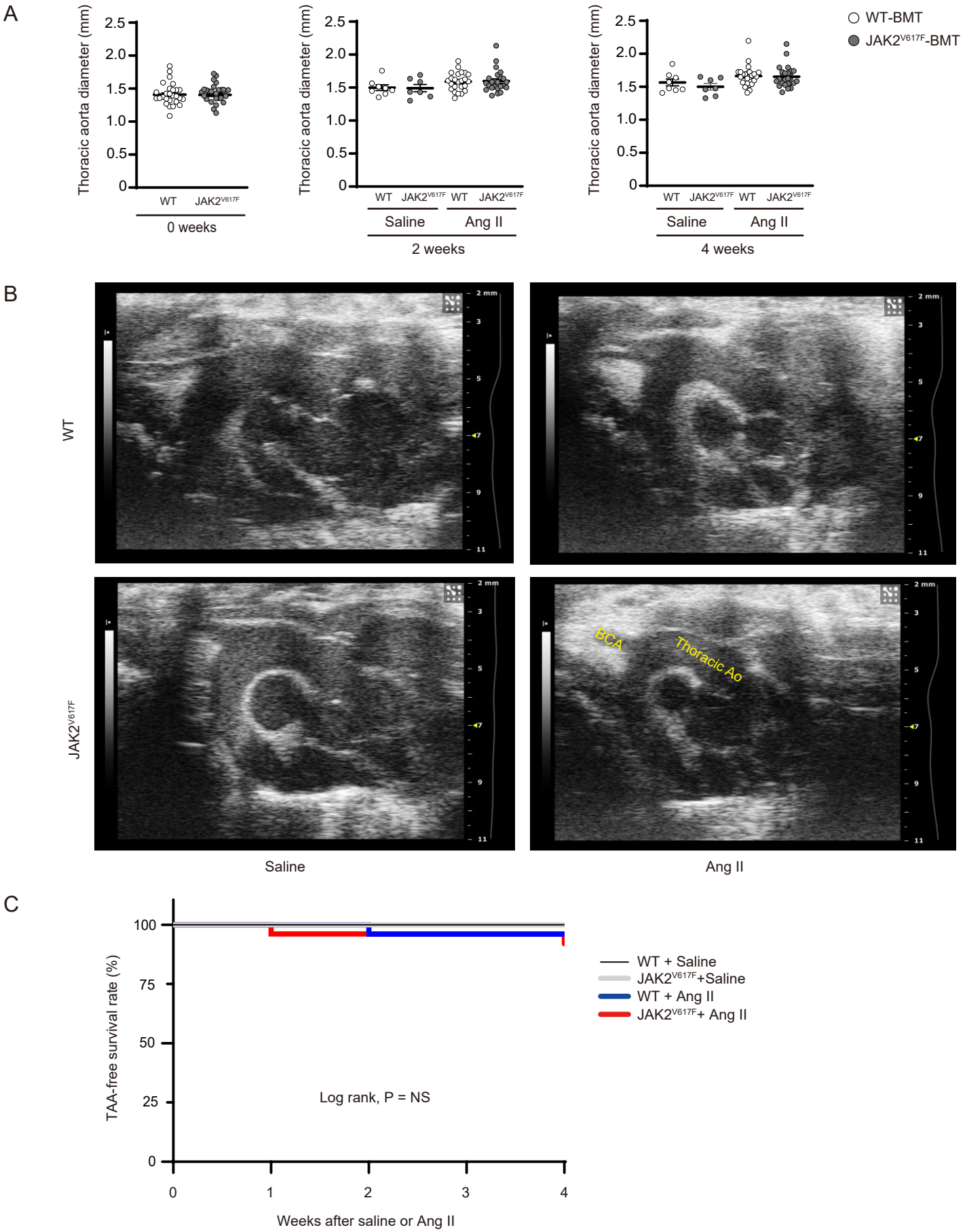

Figure S4. Incidence of thoracic aorta aneurysm (TAA) under continuous angiotensin II (Ang II) infusion.

(A) Time course of thoracic aorta diameters in the WT-BMT mice and JAK2^{V617F}-BMT mice at the baseline (n = 33–34), and 2 weeks (n = 7–24) and 4 weeks (n = 7–24) under saline or Ang II infusion. (B) Representative ultrasound images of asceinding aorta in the WT-BMT mice and JAK2^{V617F}-BMT mice under saline or Ang II for 4 weeks. (C) TAA-free survival rate in the WT-BMT mice and JAK2^{V617F}-BMT mice under saline or Ang II infusion (n = 7–27). The Kaplan-Meier analysis was used with a log-rank test. All data are presented as mean ± SEM. WT, ApoE$^{-/-}$ mice transplanted with bone marrow cells from wild-type mice; JAK2^{V617F}, ApoE$^{-/-}$ mice transplanted with bone marrow cells from JAK2^{V617F} mice; BMT, bone-marrow transplantation; BCA, brachio cephalic artery; Ao, Aorta.

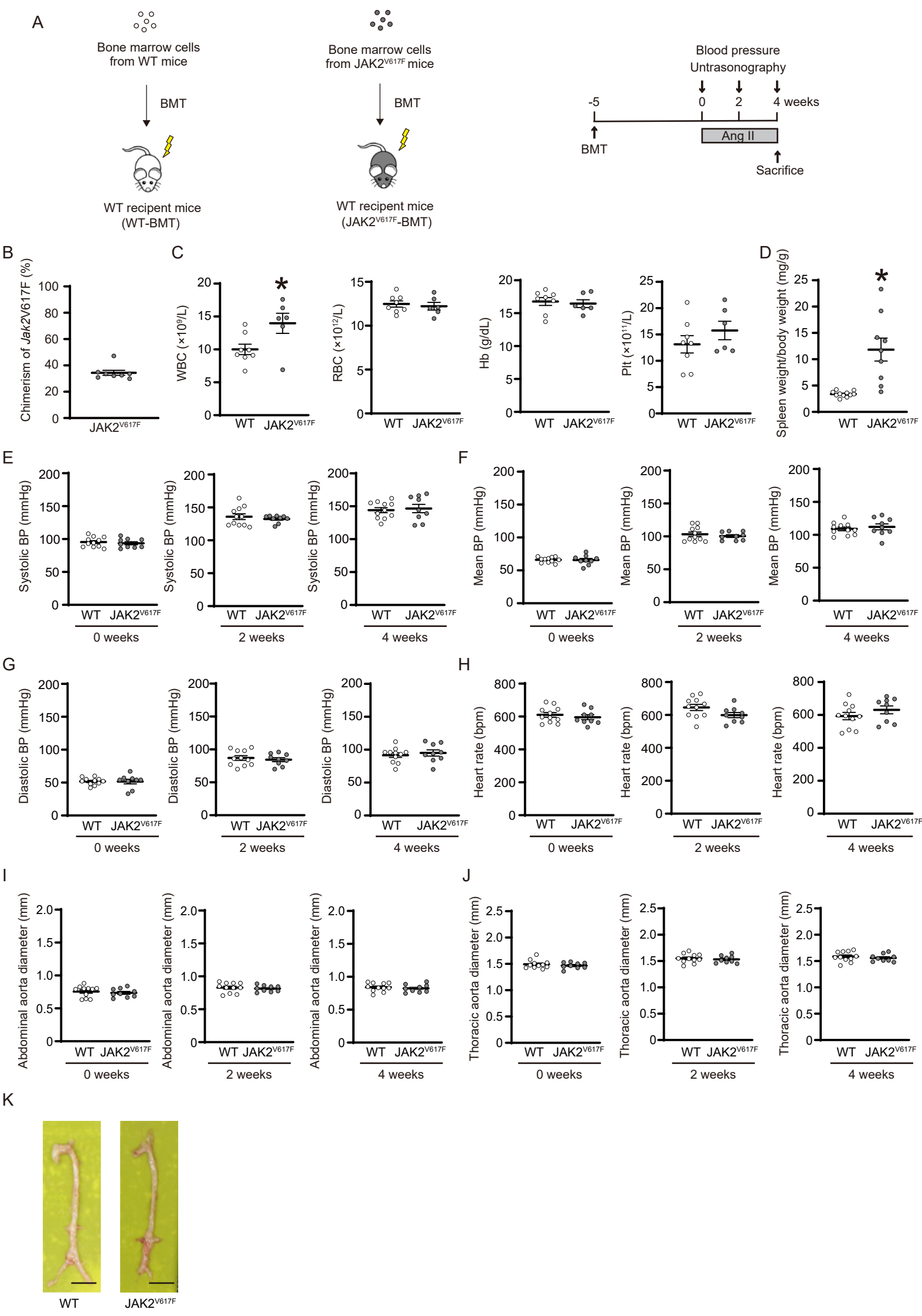
A
Bone marrow cells from WT mice
BMT
WT recipent mice (WT-BMT)
Bone marrow cells from JAK2V617F mice
BMT
WT recipent mice (JAK2V617F-BMT)
Blood pressure
Untrasonography
-5
0
2
4 weeks
BMT
Ang II
Sacrifice
B
Chimerism of Jak2V617F (%)
JAK2V617F
C
WBC (×10⁹/L)
RBC (×10¹²/L)
Hb (g/dL)
Plt (×10¹¹/L)
WT
JAK2V617F
D
Spleen weight/body weight (mg/g)
E
Systolic BP (mmHg)
0 weeks
2 weeks
4 weeks
F
Mean BP (mmHg)
G
Diastolic BP (mmHg)
H
Heart rate (bpm)
I
Abdominal aorta diameter (mm)
J
Thoracic aorta diameter (mm)
K
WT
JAK2V617F

Figure S5. Hematopoietic JAK2V617F does not induce the abdominal aortic aneurysm in the recipient mice with a wild-type (WT) background after angiotensin II (Ang II) infusion.
(A) Schematic diagram of the experimental design using the mice with a WT background (C57BL/6J) as recipients. Bone marrow cells from the WT mice or $JAK2^{V617F}$ mice were injected into the lethally irradiated WT recipient mice. Five weeks after bone marrow transplantation (BMT), Ang II (1900 ng/kg/min) infusion was initiated for 4 weeks. (B) Chimerism of *Jak2*V617F at 5 weeks after BMT in the $JAK2^{V617F}$-BMT mice (n = 8). (C) Blood cell counts 4 weeks after Ang II infusion in the WT-BMT mice and $JAK2^{V617F}$-BMT mice(n = 6–8). (D) Speen weight/body weight after Ang II infusion (n = 9–11). (E) Systolic blood pressure (BP), (F) mean BP, (G) diastolic BP, and (H) heart rate at baseline (n = 9–11), 2 weeks (n = 9–11), and 4 weeks (n = 9–11) in the Ang II-infused WT-BMT mice and $JAK2^{V617F}$-BMT mice. (I) Abdominal aorta diameters and (J) thoracic aorta diameters at baseline (n = 9–11), 2 weeks (n = 9–11), and 4 weeks (n = 9–11) after Ang II infusion. (K) Representative images of the aorta after Ang II infusion. Scale bars, 5 mm. All data are presented as mean ± SEM. *P < 0.05 versus the WT-BMT mice by the unpaired Student' s t-test. WT, WT recipients transplanted with bone marrow cells from WT mice. $JAK2^{V617F}$, WT recipients transplanted with bone marrow cells from $JAK2^{V617F}$ mice.

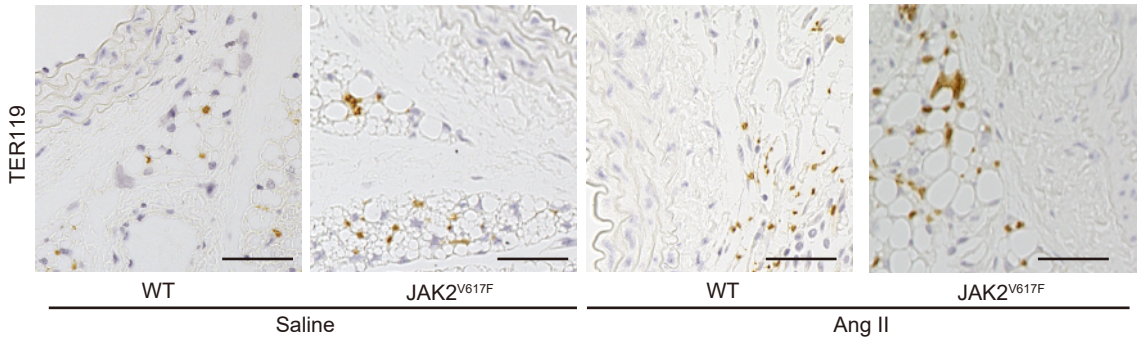

Figure S6. Immunohistochemical images of the abdominal aorta in ApoE$^{-/-}$ recipients after saline or angiotensin II (Ang II) infusion.

Representative immunohistochemical images of the abdominal aorta stained by an anti-TER119 antibody. Scale bars, 50 μm. WT, ApoE$^{-/-}$ mice transplanted with bone marrow cells from wild-type mice. JAK2^{V617F}, ApoE$^{-/-}$ mice transplanted with bone marrow cells from JAK2^{V617F} mice.

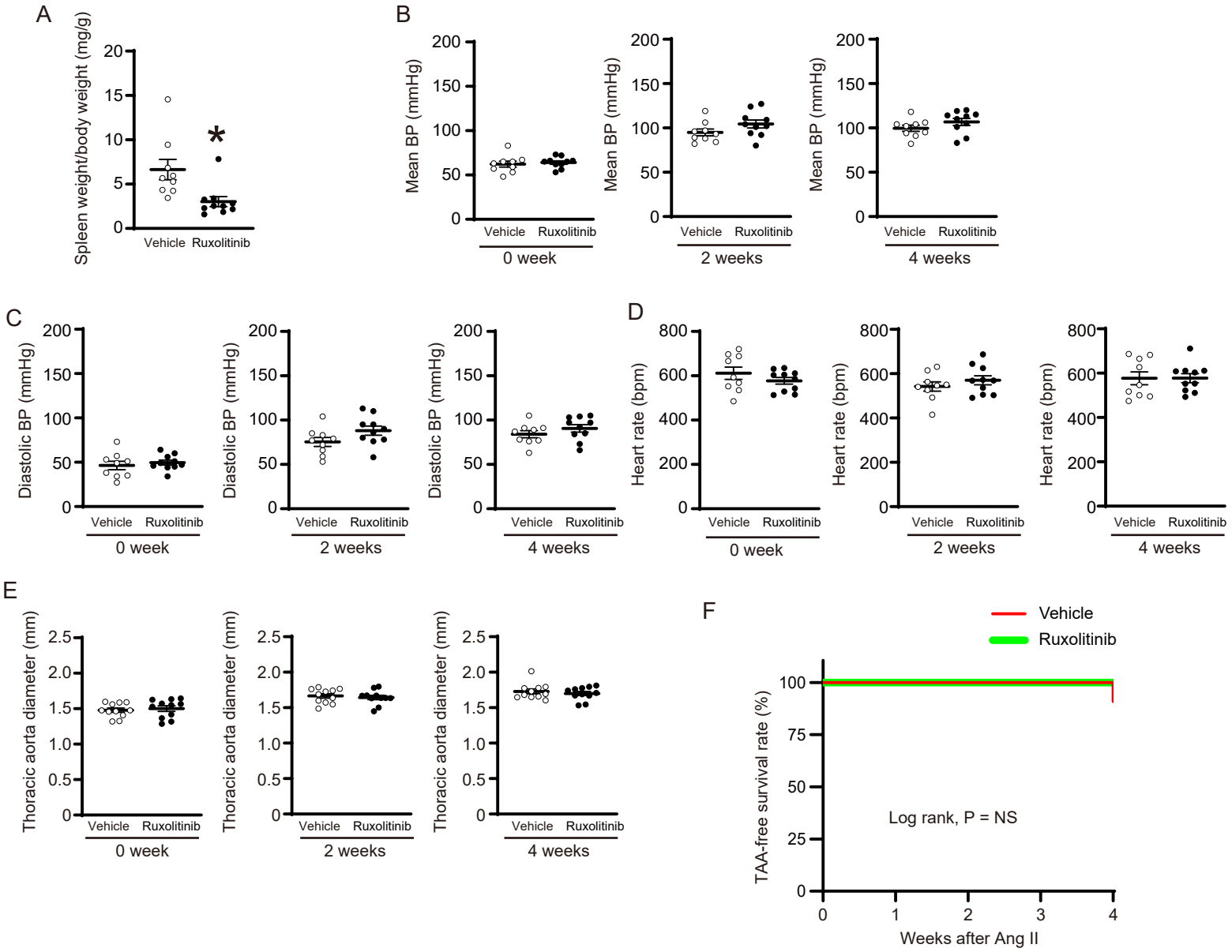

Figure S7. Effects of ruxolitinib on gravimetric and physiological parameters and incidence of thoracic aortic aneurysm (TAA) in angiotensin II (Ang II)-infused ApoE$^{-/-}$ recipients transplanted with JAK2^{V617F} bone marrow cells.

(A) Spleen weight/body weight 4 weeks after vehicle (0.5% methylcellulose) or ruxolitinib administrtion in Ang II-infused ApoE$^{-/-}$ recipients transplanted with JAK2^{V617F} bone marrow cells (n = 9–10). (B) Mean blood pressure (BP), (C) diastolic BP, and (D) heart rate at the baseline (n = 9–10), 2 weeks (n = 9–10), and 4 weeks (n = 9–10). (E) Thoracic aorta diameter at the baseline (n = 11 each), 2 weeks (n = 11 each), and 4 weeks (n = 11 each). (F) TAA-free survival rate (n = 11 each) with a log-rank test. All data are presented as mean ± SEM. *P < 0.05 versus the vehicle group by the unpaired Student’ s t-test.