Supplemental Materials

Inhibition of Platelet-Derived Growth Factor Pathway Suppresses Tubulointerstitial Injury in Renal Congestion

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Short title: PDGFR pathway and renal congestion

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Supplementary Table S1: Primer information.

Symbol	primer set ID* or Forward primer / Reverse primer (5'-3')**
Rattus	
Abl1	GAACCGGGTGTCGGGTC / GGGCTATGAGGGACGATGGA
Abl2	CAGCACCAGCAGGGTTCAT / CTTTGCTTTCGAGGCAGAGC
Acta2 (αSMA)	RA060203
Actb	RA015375
Ccnd1	RA061296
Clk1	CCTGATCTGTCAGAGTGGAGAC / CGATTGAGCAGCTTCACAGTATC
Clk4	CCCATCCCAGCACACATGAT / CTTTAACGGCTTGCAGCGTC
Col1a1	RA065609
Col3a1	RA063380
Epha8	ACCCACGATGGTCAGTTCAC / GAATGTAGCCCAGGTCCGAG
Fn1	RA055827
Frk	GTGAGAGCCAGAAGGGTGAC / ACACACAGCCCATCACTTGT
Fyn	ATCTAGTCGTGGCAAAAGGTCA / GCACACAGCCCATTATCCAAG
Gak	CCGTGACCAGAGTGACTTCG / CAAATGCAAACCCTCCCTCG
Havcr1 (Kim1)	RA057664
Kit	AACAGCAAAGAGCAAATCCAG / CTCCTCGACAACCTTCCATTG
Lck	AGCTATGAGCCCTCCCATGA / TGTTTGCTTTCGCCACGAAG
Mapk8 (Jnk1)	CAGCCGTCTCCTTTAGGTGC / TCTGTATCCGAGGCCAGAGT
Mapk9 (Jnk2)	GGTGAAAGACCAGCCTTCAGA / TTGAGGCATCGAGACTGCTG
Mapk10 (Jnk3)	RA021650
Pdgfra	RA059727
Pdgfrb	RA048695
Plk4 (Stk18)	AACATGGCGGCGTGCAT / TGGACTCTCTGTACCATTCCAG
Rplp2	RA015377
Spp1 (Opn)	RA017345
Tagln (Sm22)	RA063024
Tnc	AACGAACTGCCCACATCTCG / TTCCGGTTCAGCTTCTGTGG
Vim	RA050877
Human	
ACTB	HA067803
COL1A1	HA181838
COL4A1	HA156982
FN1	HA219473
GAPDH	HA067812

*RA XXs and HA XXs were purchased from Takara Bio.

**Primers were designed by Primer3 (http://primer3.ut.ee) and synthesized by Integrated DNA Technologies.

Supplementary Table S2: Antibody information.

Antigen	Company	Catalog #	Clone	Host	WB	IHC	IF
ACTA2 (αSMA)	Cell Signaling	19245S	D4K9N	rabbit	1:1000		
ACTA2 (αSMA)	Dako	M0851	1A4	mouse		1:200*	
CD11B	Abcam	ab133357	EPR1344	rabbit			1:4000**
CD31 (PECAM1)	Santa Cruz	sc-1506		goat			1:100
CD68	BIO-RAD	MCA341R	ED1	mouse	1:1000		1:4000**
CD206	Cell Signaling	24595S	E6T5J	rabbit	1:1000		1:200**
CNN1 (Calponin)	Sigma-Aldrich	C2687	hCP	mouse		1:500*	
COL1A1	Novus Biologicals	NB600-408		rabbit		1:100*	
COL4	Abcam	ab6586		rabbit		1:100*	
FN1	Merck	F3648		rabbit	1:1000	1:400*	
GAPDH	Cell Sgnaling	2118S	14C10	rabbit	1:5000		
HAVCR1 (KIM1)	R&D system	AF3689		goat	1:1000	1:1250*	
NG2	Millipore	AB5320		rabbit			1:500**
PDGFRA	Cell Signaling	3174S	D1E1E	rabbit	1:1000	1:500*	
PDGFRB	Abcam	ab32570	Y92	rabbit	1:1000	1:100*	1:100**
PDGFRB	Cell Signaling	3175S	2B3	mouse			1:100**
p-PDGFRB (Thr751)	Cell Signaling	3161S		rabbit	1:1000		1:50*
SMAD2/3	Cell Signaling	8685S	D7G7	rabbit	1:1000		
p-SMAD2 (Ser465/467)/ SMAD3 (Ser423/425)	Cell Signaling	8828S	D27F4	rabbit	1:1000		
TAGLN (SM22)	Abcam	ab14106		rabbit		1:200*	
TGFB1	Abcam	ab215715	EPR21143	rabbit	1:1000		
TNC (Tenascin-C)	Abcam	ab108930	EPR4219	rabbit		1:500*	

*The antigens were retrieved by autoclave heating for 5 min in 10 mmol/L citrate buffer (pH6.0).

**The antigens were retrieved by autoclave heating for 5 min in 1.0 mmol/L ethylenediaminetetraacetic acid buffer (pH9.0).

Supplementary Table S3: Biological data of saline- and imatinib-treated rats.

Rats	Saline	Imatinib
serum	n=7	n=6
TP (g/dL)	5.30 ± 0.17	5.57 ± 0.11
Alb (g/dL)	3.41 ± 0.12	3.43 ± 0.10
BUN (mg/dL)	24.94 ± 1.80	19.48 ± 1.19*
Cre (mg/dL)	0.33 ± 0.01	0.33± 0.01
AST (IU/L)	129.00 ± 28.02	123.83 ± 25.66
ALT (IU/L)	46.71 ± 7.53	43.67 ± 6.63
γ-GTP (IU/L)	< 3	< 3
ChE (IU/L)	< 5	< 5
T-Cho (mg/dL)	71.57 ± 4.34	71.33 ± 6.04
urine	n=8	n=8
Cre (mg/dL)	8.10 ± 2.17	8.01 ± 1.11
Alb (μg/dL)	165.06 ± 43.20	188.54 ± 93.93
NAG (IU/L)	7.76 ± 1.55	9.11 ± 1.67

TP, total protein; Alb, albumin; BUN, blood urea nitrogen; Cre, creatinine; AST, aspartate transaminase; ALT, alanine transaminase; γ-GTP, gamma-glutamyltransferase; ChE, cholinesterase; T-Cho, total cholesterol; NAG, n-acetylglucosaminidase. Values are mean ± SEM. *P<0.05 vs Saline-treated rats by Mann Whitney U-test.

Supplementary Table S4: List of imatinib-binding protein kinases [37].

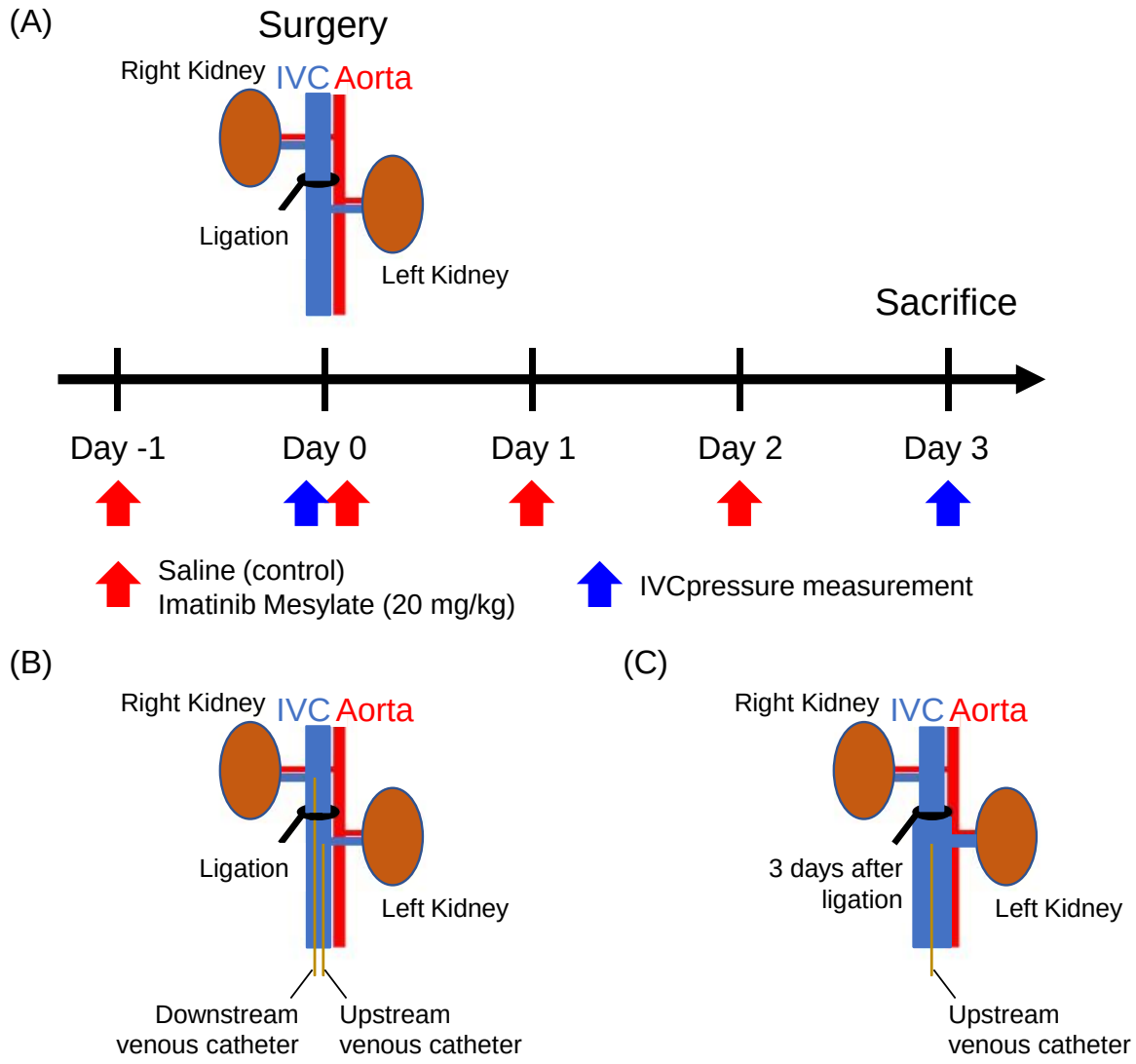
		Cx**		OM**	
Gene symbol	Kd (μM)*	Fold Change	p-value	Fold Change	p-value
ABL1	0.0022	1.12	0.13	1.11	0.24
ABL2	0.013	0.96	0.65	0.95	0.27
CLK1	4.5	1.11	0.05	1.15	0.06
CLK4	4.2	1.01	0.65	0.97	0.65
EPHA8	2.1	0.93	0.23	0.97	0.72
FRK	3.5	1.21	0.04	1.60	0.02
FYN	5.5	1.24	0.12	1.34	0.04
GAK	3.6	0.97	0.64	0.95	0.28
MAPK8 (JNK1)	3.2	1.09	0.12	1.18	0.02
MAPK9 (JNK2)	5.2	0.96	0.47	0.97	0.48
MAPK10 (JNK3)	3.3	0.85	0.26	1.49	0.003
KIT	0.83	0.98	0.86	1.27	0.04
LCK	0.062	1.13	0.31	1.11	0.21
PDGFRB	0.028	2.23	0.0004	3.15	0.002
STK17A***	2.8	---	---	---	---
PLK4 (STK18)	9	2.34	0.02	1.68	0.06

* Binding constant for the interaction between imatinib and human protein kinases; Kd < 1 μM indicates high affinity [37].

** Changes in gene expression in the congested kidney compared to the control kidney in the cortex (Cx) and outer medulla (OM) according to our previous microarray data set (GEO accession number: GSE114031) [14].

*** Not encode in rat.

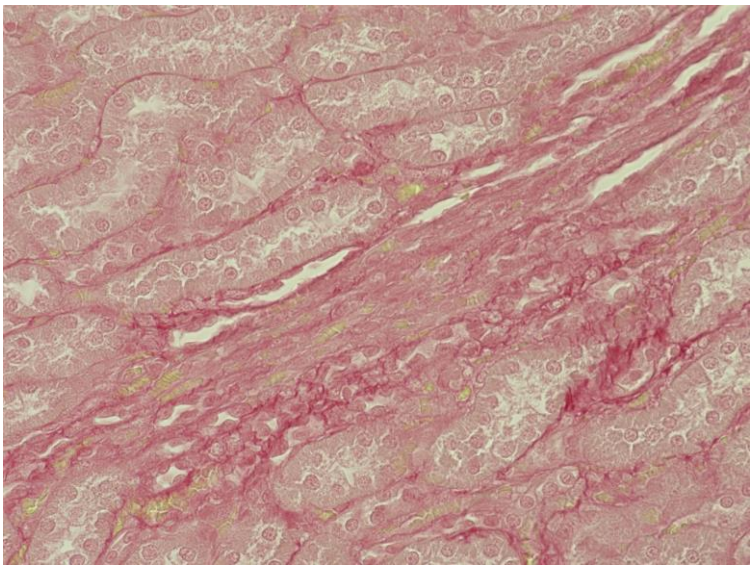
Supplementary Figure S1.



Supplementary Figure S1: Experimental design overview. (A) Male Sprague-Dawley rats were divided into two groups: imatinib-treated group (n = 12) and saline-treated group (n = 12). The inferior vena cava (IVC) between the renal veins was ligated on a temperature-controlled (38°C) surgical table (Day 0). Intraperitoneal administration of imatinib mesylate (20 mg/kg) or saline was performed daily from Day -1 to Day 2 (red arrow). IVC pressure was measured during IVC ligation and on day 3 (blue arrow). Tissues were collected after blood and urine sampling and the rats were euthanized under deep anesthesia (Day 3). No anatomical vein abnormalities were observed, and no rats died during the experimental period. (B) Schematic diagram of IVC pressure measurement during the IVC ligation. The catheters were detained for upstream (junction of the left renal vein and the vena cava) and downstream (junction of the right renal vein and the vena cava) IVC pressure measurements. (C) Schematic diagram of IVC pressure measurement at the end of the experimental period. Because of complete ligation of the IVC between renal veins, the catheter was left in place only for upstream IVC measurement.

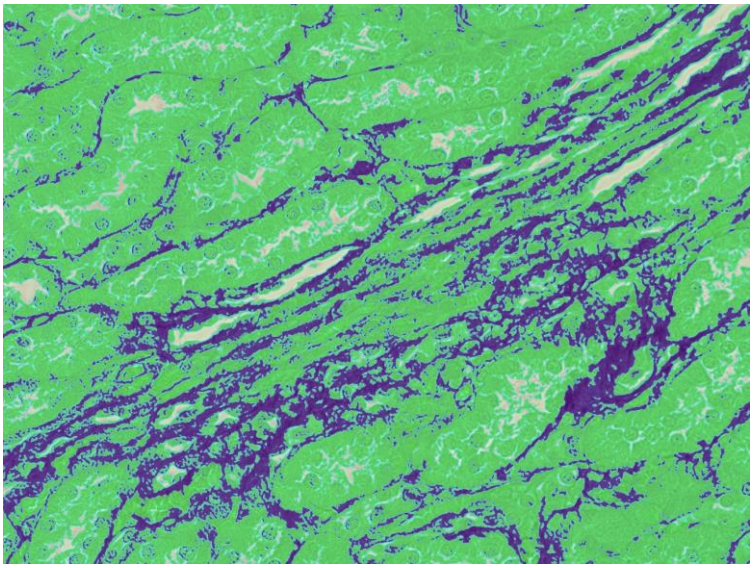
Supplementary Figure S2.

(A)



(40 x)

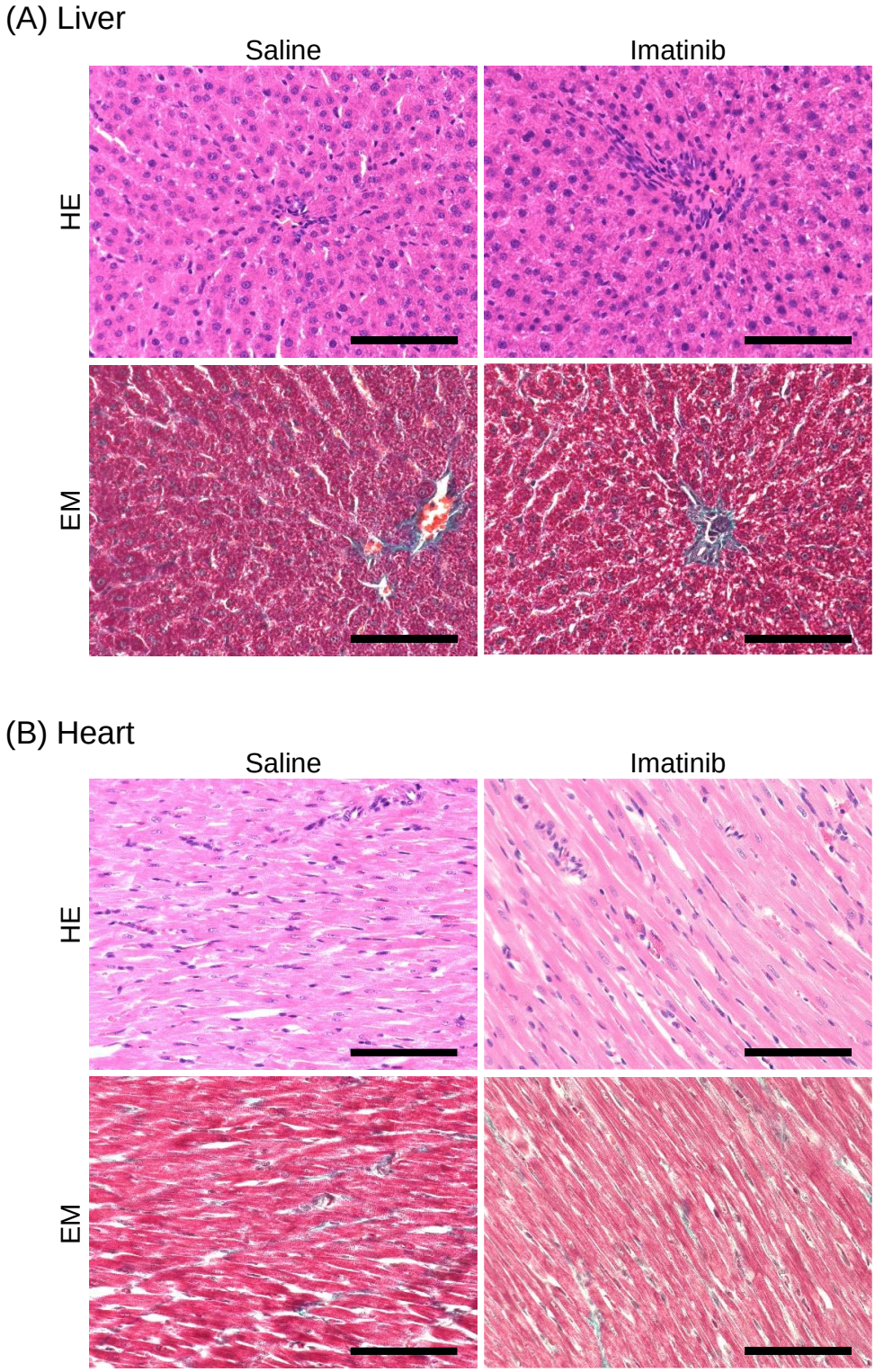
(B)



(40 x)

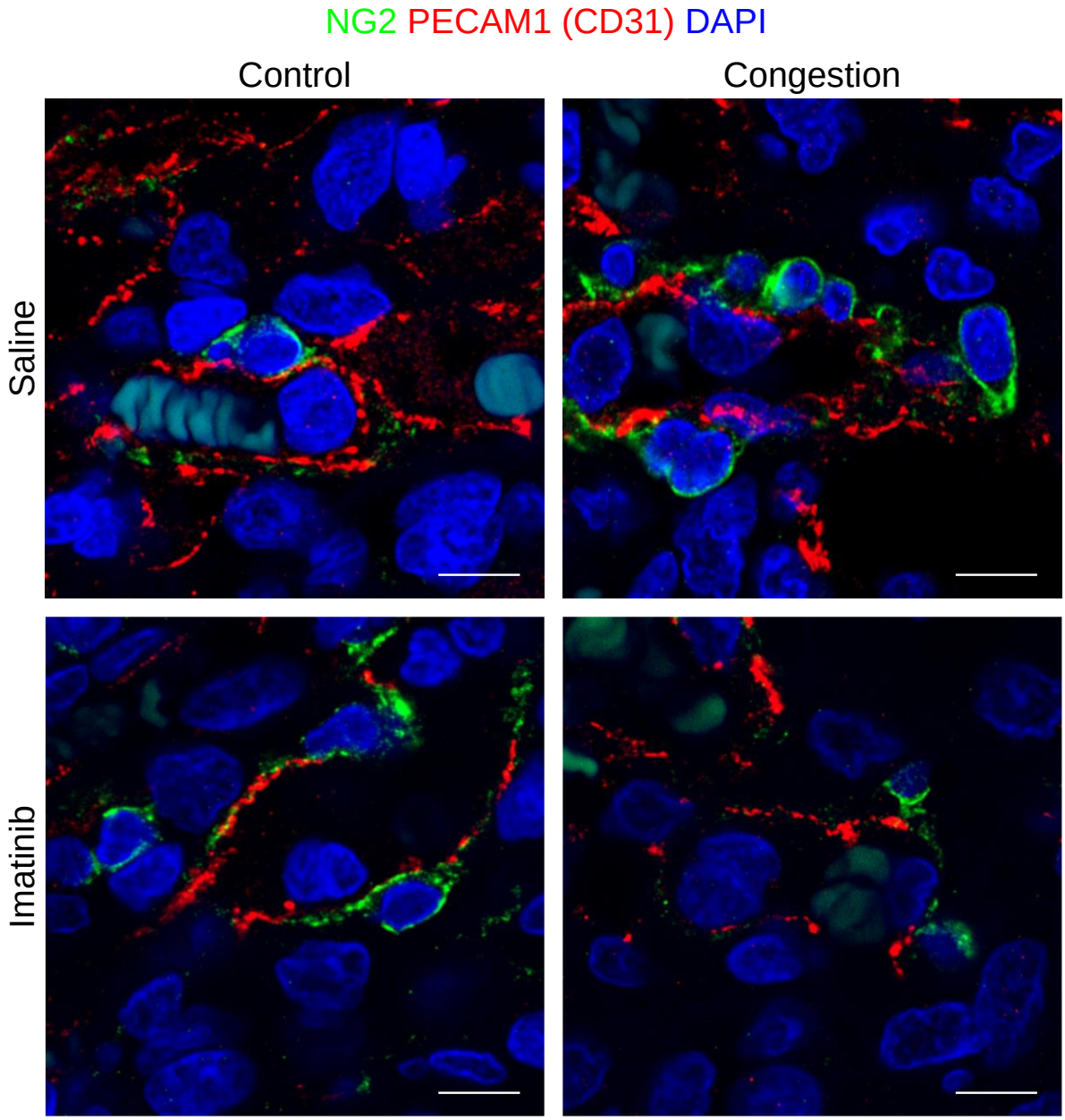
Supplementary Figure S2: Picrosirius Red Staining and evaluation of the collagen positive area. (A) At least two fields around the vasa recta in the outer stripe of the outer medulla from 1 section were randomly selected from each slide and captured at 40x magnification. (B) The threshold was set in an arbitrarily selected region in the right kidney of the saline-treated group. The ratio of the collagen positive area per all fields [(blue)/(blue + green)x100 (%)] was automatically calculated by Hybrid Cell Count BZ-H3C application.

Supplementary Figure S3.



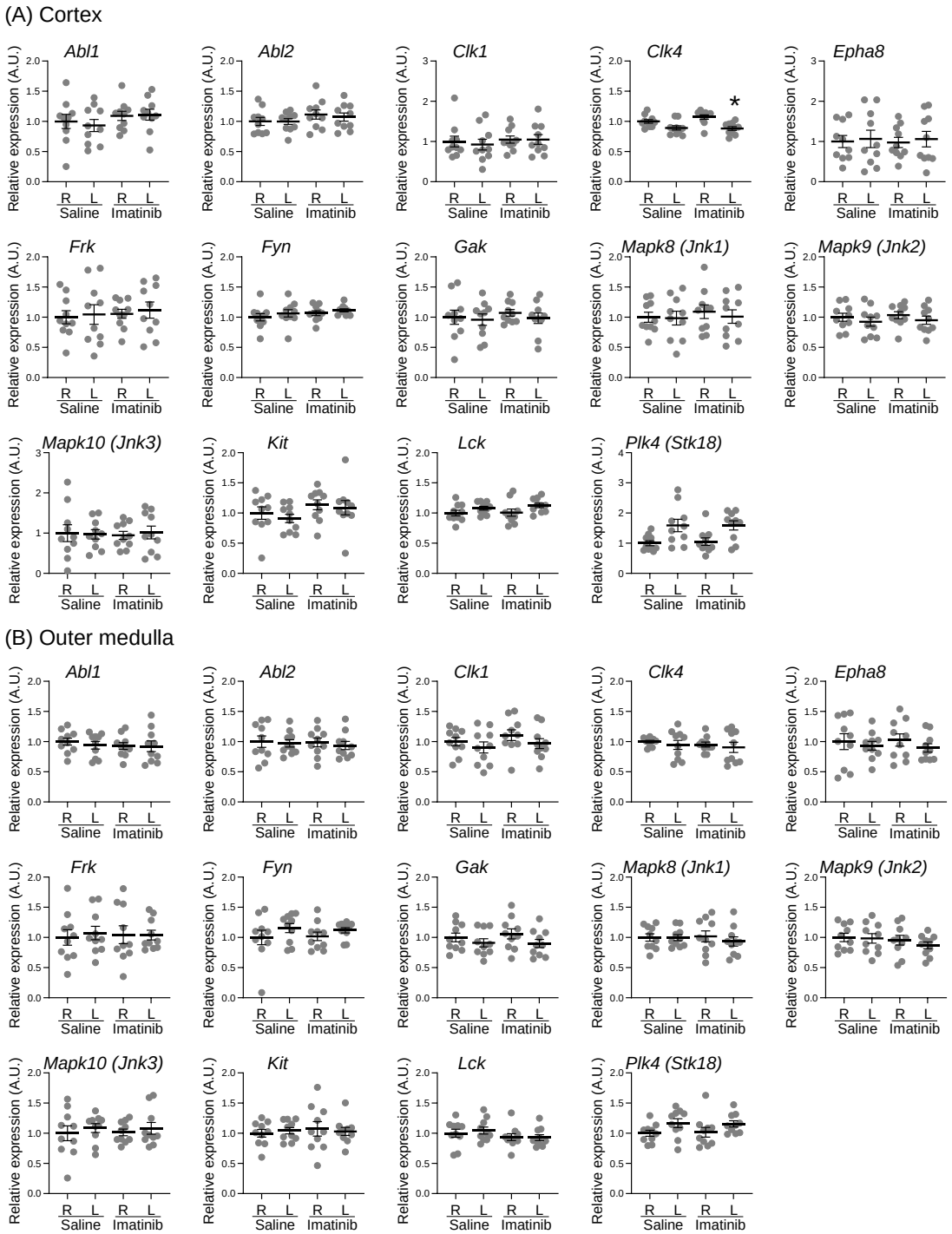
Supplementary Figure S3: Toxicity of imatinib in rat liver and heart. Representative images of rat liver (A) and heart (B) stained with hematoxylin-eosin (HE) and Elastica-Masson (EM). These organs were not affected by treatment with 20 mg/kg imatinib. Scale bar = 100 μm.

Supplementary Figure S4.



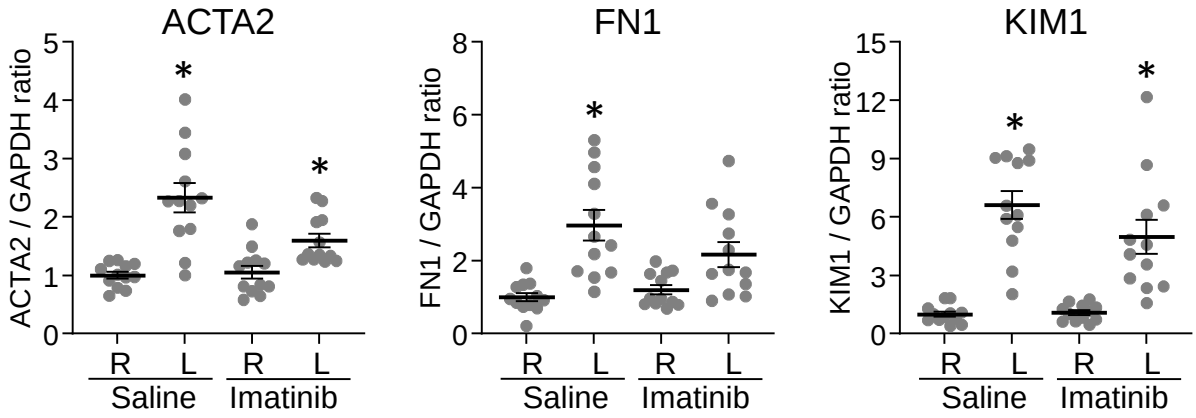
Supplementary Figure S4: Immunofluorescence analysis of pericyte detachment in the descending vasa recta. Immunofluorescence of NG2 (pericyte, green) and PECAM1 (CD31) (vascular endothelium, red). Nuclei were stained with Hoechst 33342 (blue). Scale bar = 10 μm.

Supplementary Figure S5.



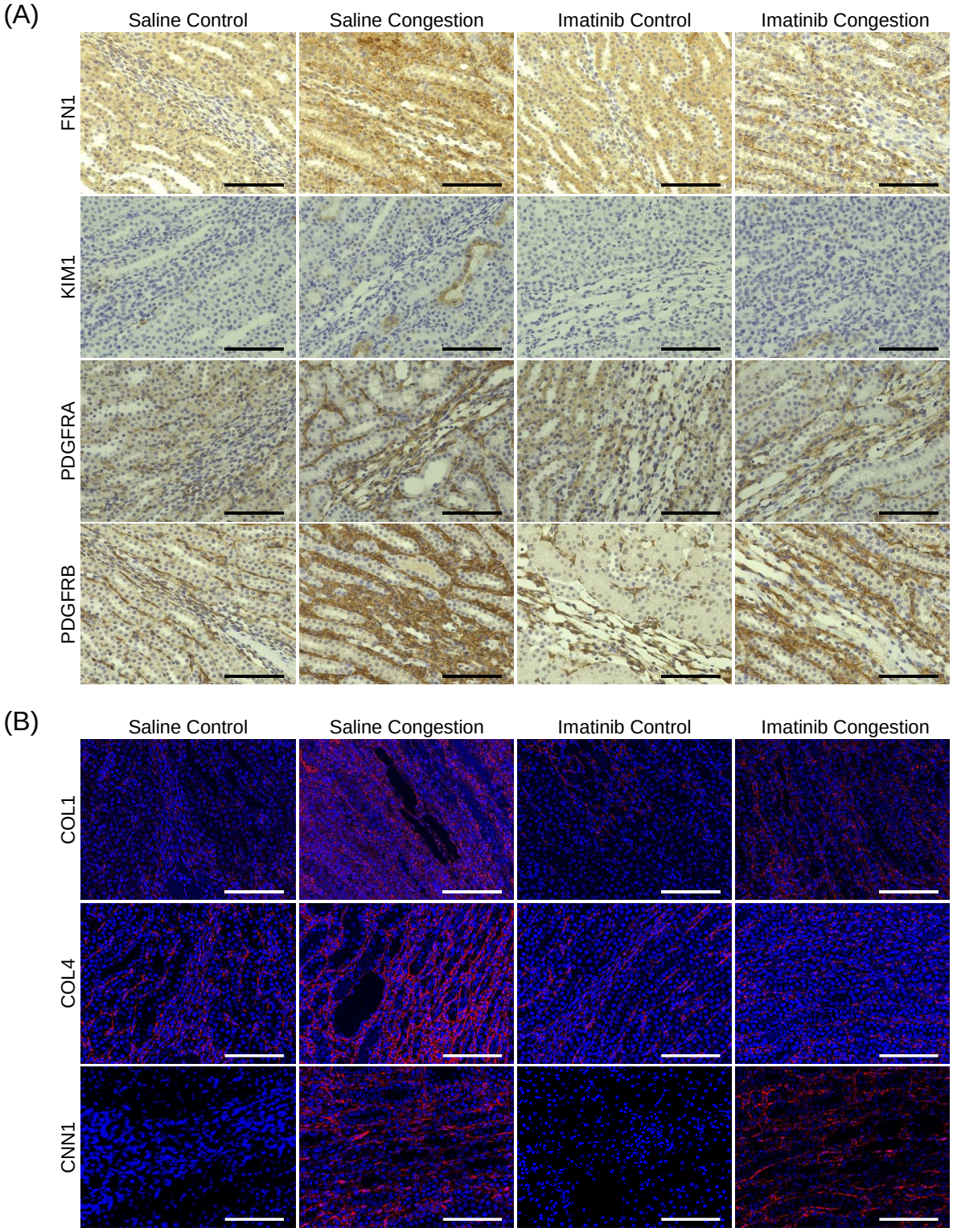
Supplementary Figure S5: mRNA expression in the cortex and outer medulla. The mRNA expression level of imatinib-binding protein kinases in the cortex (a) and outer medulla (b) was assessed by qPCR. The relative mRNA expression levels were normalized to *Rplp2 (ribosomal protein lateral stalk subunit P2)*. The data show individual values and mean±SEM; n = 10 per group. *P < 0.05 versus the right kidney in each group by Steel-Dwass test. R: right kidney; L: left kidney.

Supplementary Figure S6.



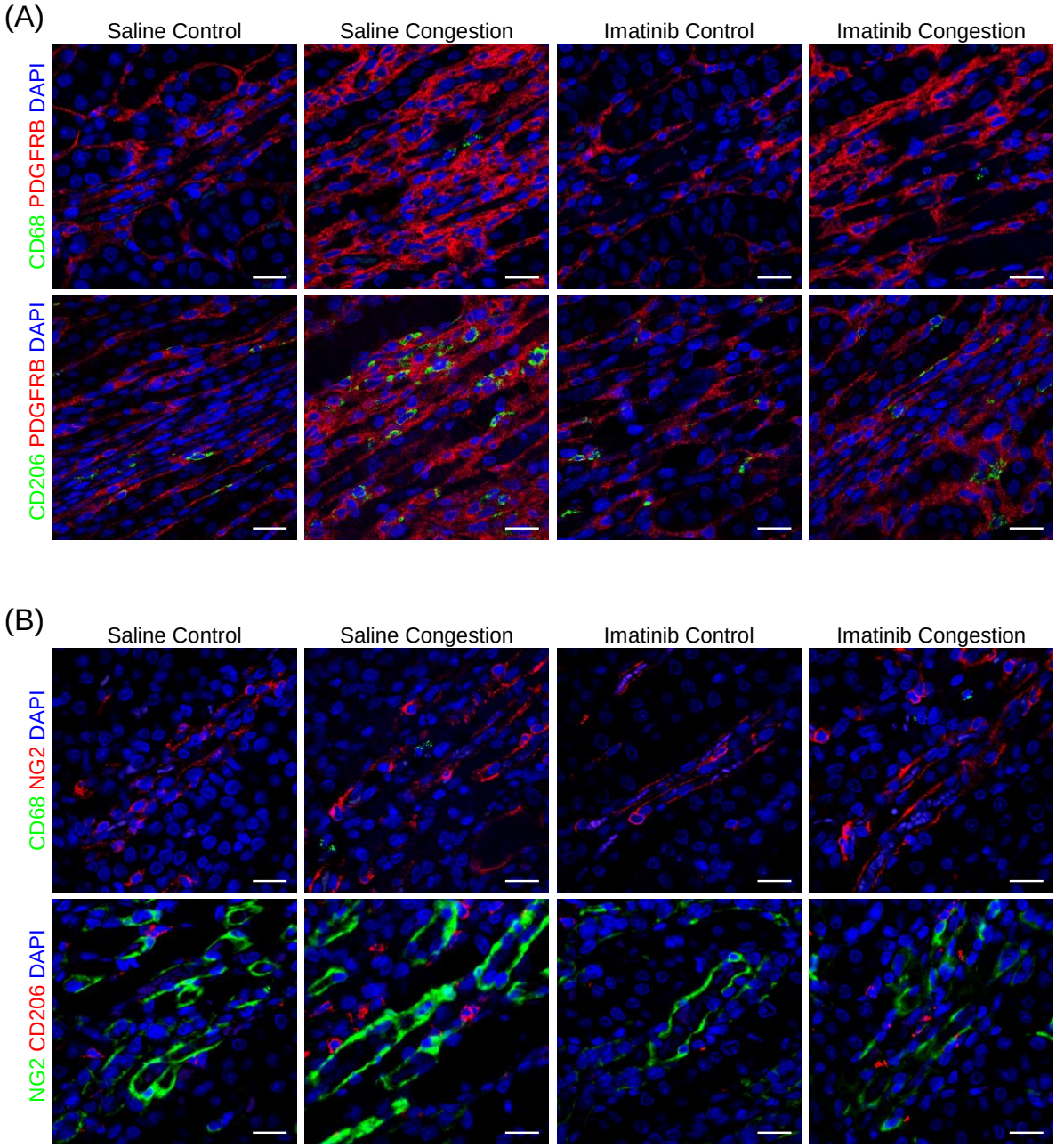
Supplementary Figure S6: Effect of imatinib treatment on protein expression in the cortex. Western blotting analysis of ACTA2, FN1, and KIM1 in the cortex. The data show individual values and mean±SEM; n = 12 per group. *P < 0.05 vs the right kidney in each group. R: the right kidney; L: the left kidney.

Supplementary Figure S7.



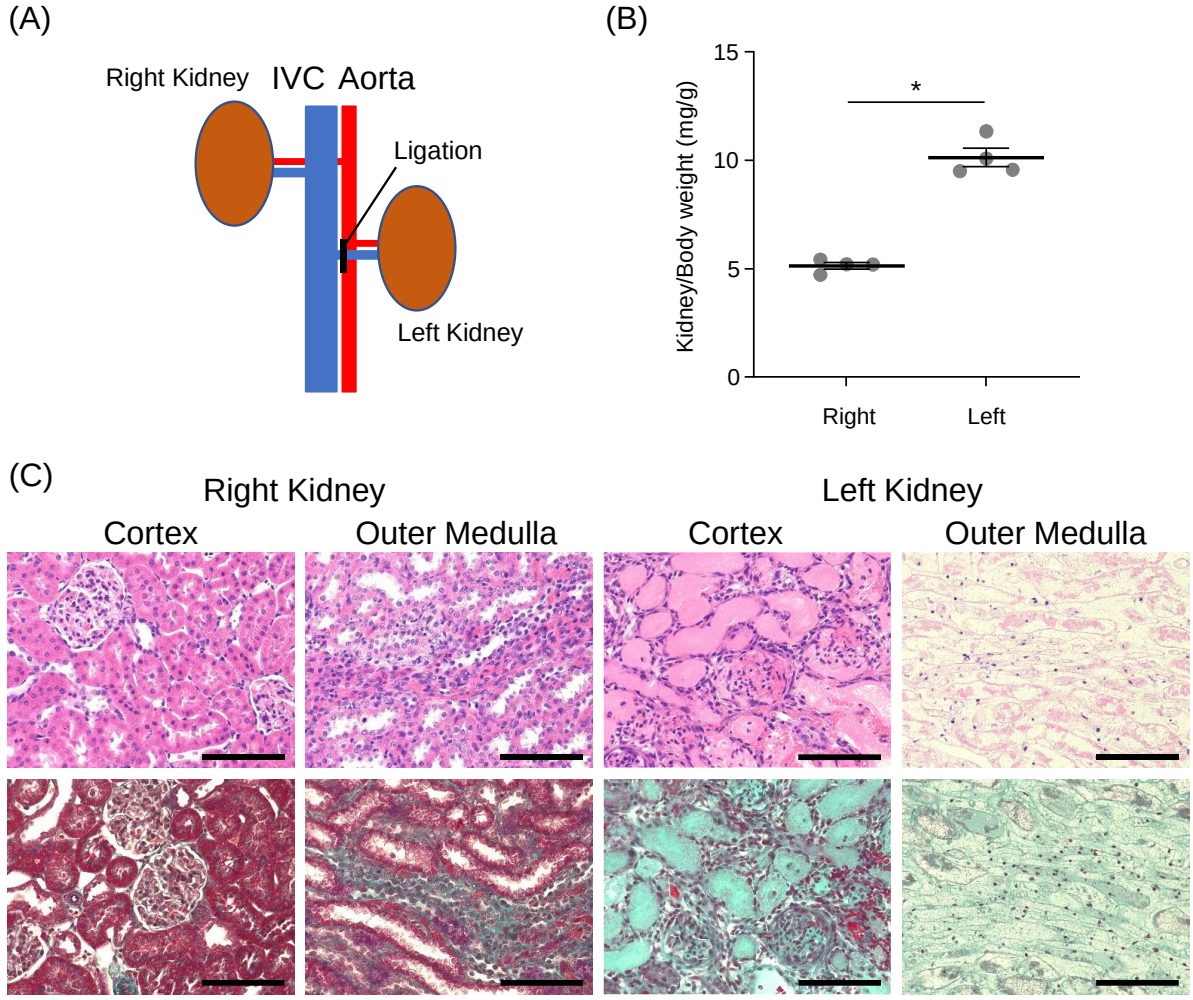
Supplementary Figure S7: Histological analysis of area surrounding the vasa recta in the outer medulla. (A) Immunostaining with FN1, KIM1, PDGFRA, and PDGFRB around the vasa recta. (B) Immunofluorescence of COL1A1, COL4, and CNN (red) around the vasa recta. Nuclei were stained with Hoechst 33342 (blue). Scale bar = 100 μm.

Supplementary Figure S8.



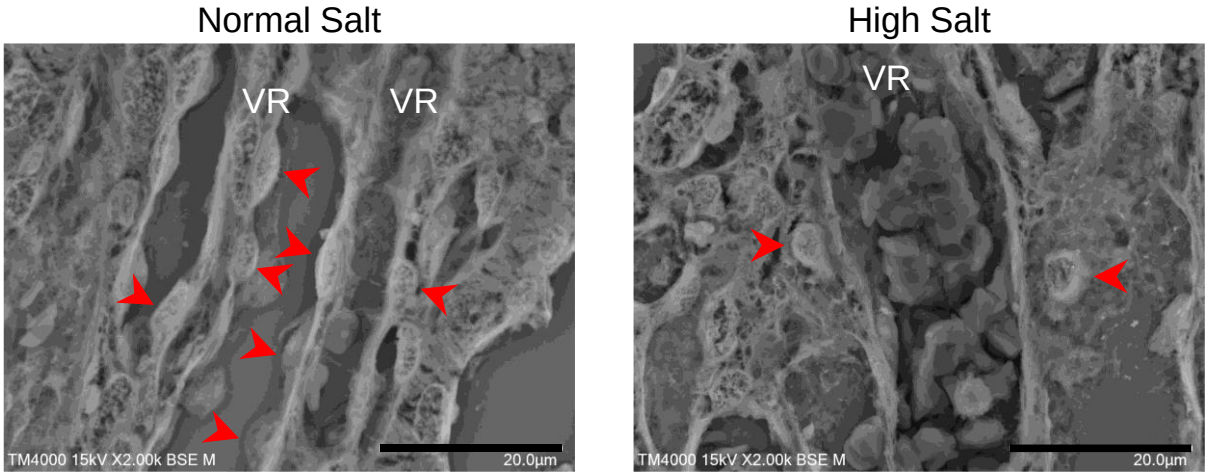
Supplementary Figure S8: Double-labeling immunofluorescence stainings of macrophage markers (CD68 and CD206) with PDGFRB (A) or NG2 (B) around the vasa recta of imatinib- and saline-treated rats. Nuclei were stained with Hoechst 33342 (blue). Scale bar = 100 µm.

Supplementary Figure S9.



Supplementary Figure S9: Left renal vein ligation model in male Sprague-Dawley ras (6 weeks old). (A) Schema of total occlusion of the left renal vein. (B) Weight of the renal vein-occluded kidneys increased almost two-fold compared to that of the contralateral control kidneys (n = 4). The weight of the kidney was normalized to body weight. The data shows individual values and mean±SEM. *P < 0.05 by Mann-Whitney U test. (C) Representative images of hematoxylin-eosin (HE) and elastica-masson (EM) staining in the kidneys. The left kidney had severe necrotic damage. Scale bar = 100 μm.

Supplementary Figure S10.



Supplementary Figure S10: Representative images of pericytes (red arrowhead) in the descending vasa recta (VR) of Dahl Salt-Sensitive rats (Dahl SS/mcwi) by low-vacuum scanning electron microscopy. Dahl SS/mcwi rats were fed normal salt (0.4% NaCl) or high salt (4% NaCl) diet for 4 weeks. Scale bar = 20 µm.