

Supplementary Materials for

Endothelial extracellular vesicle miR-423-5p regulates microvascular homeostasis and renal function after ischemia-reperfusion injury

Francis Migneault^{1,2,3}, Hyunyun Kim^{1,2,4}, Alice Doreille¹, Shanshan Lan^{1,2,4}, Alexis Gendron^{1,4}, Marie-Hélène Normand^{1,2,3}, Annie Karakeussian Rimbaud¹, Martin Dupont¹, Isabelle Bourdeau¹, Éric Bonneil⁵, Julie Turgeon^{1,2}, Sylvie Dussault¹, Pierre Thibault^{2,5,6}, Mélanie Dieudé^{1,2,3,7}, Éric Boilard^{2,8}, Alain Rivard^{1,4}, Héloïse Cardinal^{1,2,4}, and Marie-Josée Hébert^{1,2,4}

¹Centre de Recherche, Centre Hospitalier de l'Université de Montréal (CRCHUM), Montréal, QC, Canada.

²Canadian Donation and Transplantation Research Program (CDTRP), AL, Canada.

³Département de Microbiologie, Infectiologie et Immunologie, Faculté de Médecine, Université de Montréal, Montréal, QC, Canada.

⁴Département de Médecine, Université de Montréal, Montréal, QC, Canada.

⁵Institute for Research in Immunology and Cancer, Montréal, QC, Canada.

⁶Department of Chemistry, Université de Montréal, Montréal, QC, Canada.

⁷Héma-Québec, Québec, QC, Canada.

⁸Centre de Recherche du, Centre Hospitalier Universitaire (CHU) de Québec-Université Laval, Département de Microbiologie et Immunologie Québec, QC, Canada.

Running title: miR-423 -5p and renal ischemia reperfusion

Corresponding author

Dr. Marie-Josée Hébert, marie-josée.hebert@umontreal.ca

CRCHUM, 900 St. Denis Street, Pavillon R, Room R12.412, Montreal, QC, Canada H2X 0A9. Telephone: (514) 890-8000 Extension 28479, Fax: (514) 412-7944.

DISCLOSURE

The authors of this manuscript have no conflicts of interest to disclose.

Supplemental Materials and Methods

Vesicle isolation. Briefly, conditioned serum-free media was centrifuged at 1200 x g for 15 min at 4 °C to remove dead cells and debris. The supernatant was exposed to centrifugation at 50,000 x g for 15 min at 4 °C to collect apoptotic bodies, followed by final centrifugation at 200,000 xg for 18 h at 4 °C to collect apoptotic exosome-like vesicles. Extracellular vesicles were resuspended in TRIzol Reagent (Life Technologies) to extract total RNA or D-PBS to assess proteasome activity.

Mimic miR-423 transfection. MATra-si Reagent was added to mimic miRNA to a concentration of 100 nM in Opti-MEM medium (Gibco) and incubated for 25 min at room temperature. The miRNA-bead mixture was added to the supernatant (Opti-MEM) of each well for a final concentration of 10 nM mimic miRNA. Then, the plate was placed on a magnet plate for 15 min and medium was changed after 30 min. After 48 h, the cells were processed according to the respective assays.

Wound healing assay. HUVECs were cultured on 12-well plates until confluence. The cells were mechanically injured using a P20/P200 pipette tip (three wounds per condition) as previously described (1). Wound closure was quantified with TScratch software (2) from the wound area measured after 6 h compared with the initial wound area for each wound.

Endothelial cell tube formation assay. Confluent HUVECs seeded onto 6-W plates were detached and reseeded at a cell density of 130 000 cells/well onto 24-W plates that had been precoated with growth factor-reduced Matrigel Matrix (Corning) and cultured at 37 °C for 6 h under their respective treatments. Each experiment was performed in duplicate for each condition. Capillary-like structures were captured (four fields per well) and quantified using the Angiogenesis analyzer for ImageJ (3).

Apoptosis level assessment. HUVECs were cultured in 6-well plates until they reached 90-95% confluence. Cells were rinsed twice with RPMI medium and then incubated for 4 h in either RPMI or complete M200 medium. After incubation, the cells were stained with Hoechst 33342 at a dilution of 1:10 000 (Molecular Probes) for 10 minutes. Just before analysis, propidium iodide was added to the culture medium to a final concentration of 5 µg/mL, with excitation set between $\lambda = 360\text{--}425$ nm. An investigator, who was blinded to the experimental conditions, assessed the proportions of normal, apoptotic, and necrotic adherent cells across eight random fields for each condition. Apoptotic cells were identified by their condensed nuclei (appearing bright blue) without any cell membrane permeability (indicated by red staining). The count of apoptotic cells was then divided by the total number of cells in each micrograph to calculate the percentage of apoptotic cells.

Proteasome activity assay. The proteasome activity assay was performed in white 96-well plates with clear bottoms using exosome-like vesicles purified from mouse serum (50 µg) and Proteasome-Glo Caspase-Like Cell-Based Assays (Promega) according to the manufacturer's instructions. The protein concentration was assessed using a BCA microdosage kit (Thermo Fisher). Briefly, exosome-like vesicles were isolated as described above and diluted in PBS to equal total protein concentration and volume and brought up to room temperature. Luminescent reagent was then added to the sample in equal volume, protected from light, and agitated at 450 rpm on an orbital shaker. Samples were incubated at room temperature for 10 min before reading on a Victor3 luminescence plate reader (PerkinElmer). Luminescence levels were normalized to protein levels and reported as activity per µL of serum.

Flow cytometric analyses of extracellular vesicles. Further details are provided in the Supplementary Methods section. Analyses were performed on a BD Canto II Special Order Research Product (BD Biosciences) equipped with a small particle option, as described previously (4, 5). The forward scatter (FSC) on this dedicated equipment is coupled to a

photomultiplier tube (PMT) with a 488 nm solid-state, 100 mW output blue laser and includes a 633 nm HeNe, 20 mW output red laser, a 405 nm solid-state diode, and a 50 mW output violet laser. The hs-FCM includes an FSC-PMT and a Fourier optical transformation unit, which reduces the background/noise and increases the angle of diffusion, thereby enhancing the detection of small-diameter particles. FCM performance tracking was performed daily before all analyses using the BD cytometer setup and tracking beads (BD Biosciences). Acquisition was performed at low speed (~10 $\mu\text{L}/\text{min}$), and to remain quantitative, a known quantity of (fluorescent) polystyrene microspheres (15 μm diameter: Polysciences) was added to each tube, and a constant number of beads detected on the basis of (auto)fluorescence was acquired for each sample throughout the study. Silica particles (Kisker Biotech GmbH & Co.) of known dimensions (100 nm, 500 nm and 1 μm in diameter) were used for instrument setup standardization. Samples were labeled in a total reaction volume of 100 μL at 37 $^{\circ}\text{C}$ for 60 min with the LWA 300 probe (125 nM) (Prof. Hermen Overkleeft, Department of Bioorganic Synthesis, Leiden University, Leiden, The Netherlands). Three microliters of Annexin V APC was added for 15 minutes at room temperature. Then, the sample was diluted by adding 100 μL of labeling buffer prior to analysis by hs-FCM. All samples were processed and analyzed by an investigator blinded to experimental conditions.

Cell lysis, protein isolation, and immunoblotting. To obtain the total extracted protein, cells were washed twice in ice-cold PBS before being lysed in lysis buffer at 4 $^{\circ}\text{C}$ for 15 minutes. The lysis buffer contained 1% Triton X-100, 150 mM NaCl, 5 mM EDTA, and 50 mM Tris (pH 7.5) and was supplemented with protease inhibitor (Calbiochem), phosphatase inhibitor cocktails and 1 mM phenylmethylsulfonyl fluoride (PMSF) (Sigma). The lysate was collected by scraping the cells with a rubber policeman and centrifuged at 12 000 $\times g$ for 10 min at 4 $^{\circ}\text{C}$. The protein concentration was evaluated with a BCA protein assay kit (Thermo Fisher) according to the manufacturer's instructions. The proteins were solubilized in sample buffer

(25 mM Tris·HCl, pH 6.8, 1% SDS, 0.1% bromophenol blue, 10% glycerol, and 2% β -mercaptoethanol) and incubated at 95 °C for 10 minutes before being subjected to SDS-polyacrylamide gel electrophoresis and transferred electrophoretically onto nitrocellulose membranes. The membranes were then blocked with 5% dried fat-free milk in Tris-buffered saline at pH 7.4 with 0.05% Tween 20 (TBST) for 1 h at room temperature and incubated overnight at 4 °C with specific primary antibodies in TBST plus 5% milk. The antibodies used for blotting were antibodies against PARP1 (9542; Cell Signaling Technology), TUBA1B (11224-1-AP; Proteintech), PLVAP (NB100-77668; Novus Biological), ACTB (a5441; Sigma–Aldrich), PECAM1 (AF3628-SP; R&D Systems), CD82 (ab66400; Abcam), CD81 (66866-1-IG; Thermo Fisher), SDCBP (SC-515538; Santa Cruz Biotechnology), PSMA3 (SC-67340, Santa Cruz Biotechnology and 11887-1-AP, Proteintech), HSPG2/LG3 (AF2364; R&D Systems or Polyclonal antibody against recombinant LG3; MediMabs), histone H3C1 (9715S; Cell Signaling). Following incubation, the membranes were washed with TBST and incubated with a goat anti-rabbit IgG linked to horseradish peroxidase (GE Healthcare) for 1 h, followed by Clarity Western ECL Substrate. The luminescence signal was recorded with a ChemiDoc XRS+ system (Bio-Rad Laboratories Inc.) and analyzed with Image Lab software (Bio-Rad Laboratories Inc.) to determine the intensity of each specific band.

Animal studies. Caspase-3-deficient mice (Casp-3 $-/-$) were bred from heterozygous Casp-3 $-/-$ (B6.129S1-C3tm1Flv/J) mice obtained from Jackson Laboratory (stock #006233; Bar Harbor, ME) and were 6-8 weeks old. These mice were viable and reached adulthood, but exhibited various abnormal cell arrangements and hyperplasias in the brain. The mice were housed in a 12-hour light/dark cycle and had access to food ad libitum. Ischemia-reperfusion injury (IRI) by unilateral renal artery clamping plus contralateral nephrectomy was performed as described.

Renal ischemia-reperfusion surgery procedures. Preparation before surgery: Sterile surgical instruments are prepared before the surgery. The body weight of each mouse is taken and

recorded on the surgical sheet. Then, the mouse is anesthetized with 2%-3% isoflurane and 1 L/min oxygen. Ophthalmic lubricant is administered to protect both eyes. Carprofen at a dosage of 10 mg/kg is administered subcutaneously to control pain. The fur on the abdomen region is removed using an electric shaver and cleaned with moisturized gauze. Depilatory cream is applied to the same region to clean the abdominal skin, and the cream is removed with cotton swabs. Surgery: Immediately after skin preparation, the mouse is placed on a homeothermic blanket. Surgery will not begin until the mouse is in deep anesthesia and does not respond to pain induced by a toe pinch. The surgical area is disinfected, and sterile surgical gloves are used to perform the surgery. A sterile surgical rodent drape is placed over the surgical area. Bupivacaine 0.25% (Marcaine, 2 mg/kg) is administered subcutaneously in the surgical wound area. A mid-abdominal incision is made with surgical scissors, and the abdominal cavity is exposed. The intestine is gently moved aside to expose the left kidney. The left renal pedicle is exposed using sterile cotton swabs. Dissection of the pedicle tissue is performed with ultra-fine-point tweezers to remove the tissue around the renal pedicle, exposing the blood vessels for renal pedicle clamping. A micro-aneurysm clamp is used to block blood flow to the kidney, inducing renal ischemia. Complete ischemia is indicated by a color change of the kidney from red to dark purple. After the pedicle clamping, the intestine is returned to its original position in the abdominal cavity. The surgical wound is covered with moist sterile gauze during the 30 or 60 minutes of ischemia. The mouse remains under anesthesia throughout renal artery clamping and the color of the left kidney is monitored during this period. After 30 or 60 minutes of ischemia, the micro-aneurysm clip is released from the left kidney to initiate reperfusion, indicated by the change in kidney color back to red. The nephrectomy of the right kidney is then performed. Immediately after wound closure, 0.5 mL of sterile saline and 0.3 mL of 2.5% dextrose are administered subcutaneously to each mouse. The body weight of the mouse is recorded on the surgical sheet. The animal is then kept in an incubator for recovery. The mouse

is returned to its housing cage with an isopad at the bottom after it regains full consciousness. Chocolate Ensure® mixed soft food is provided. Monitoring the success of renal ischemia-reperfusion: After clamping, the kidney color should change from red to dark purple, indicative of successful renal ischemia. The immediate color change at the very beginning requires careful observation. However, the kidney will transition to a deep dark purple color several minutes later. After removing the clips, the kidney color should change back to red to indicate reperfusion. Post-op care: Each mouse is examined twice daily for the first two days after surgery to assess its general condition, body weight, surgical wound, and food intake. Carprofen at a dosage of 10 mg/kg is administered subcutaneously once daily. Additionally, 0.3 mL of 2.5% dextrose and 0.5 mL of saline are given subcutaneously twice daily, depending on the hydration status of the mouse. Chocolate Ensure® mixed soft food is provided for the mouse each day. All data are recorded on the surgical sheet. The endpoint is a loss of 20% body weight.

Subcapsular injection procedures. After 30 minutes of ischemia, 50 µL of miRIDIAN microRNA mmu-miR-423-5p mimic or miRIDIAN microRNA Mimic negative control #1, mixed with *in vivo*-jetPEI reagent, was injected into the renal capsule using a 33-gauge, small hub RN 2-inch needle with a Hamilton syringe. The kidney was kept moist with warm sterile saline prior to the delivery of the miRNA. The needle of the syringe was inserted into the kidney capsule at the upper middle portion. The needle was then slowly moved from the upper end to the lower end along the inner surface of the renal capsule. The needle was pressed to slowly inject the miRNA, after which the syringe was carefully withdrawn, and the injection site was pressed with a cotton swab for 1 minute. Two days after injection, the kidney was harvested and cross-sectioned into four segments. The expression levels of miR-423-5p were measured by quantitative PCR in each quadrant to validate the diffusion of the miRNA throughout the kidney.

Cohort study of renal transplant patients.

Measurements. To identify other variables associated with PTC density and fibrosis, we collected data on recipient age, sex, height, weight, renal disease, number of previous transplantations, pretransplantation panel reactive antibodies, medication before and after transplantation, comorbidities, smoking habits, total ischemic time, induction and maintenance immunosuppressive protocols, allograft acute rejection occurring before or based on the post-transplantation biopsy defined per the Banff 2019 classification (6), and donor type, sex, age, height, weight, hypertension, diabetes, and cardiovascular disease. Donor comorbidities such as hypertension and diabetes were defined as the clinical diagnoses provided by the organ donation organization transplant coordinators to the transplant team. After cardiocirculatory arrest (DCD), all donors were maintained under controlled conditions (Maastricht category 3), awaiting death in the operating room. We had no access to information on the length of the agonal phase.

Renal tubular damage. Mice were euthanized at baseline, and at 2 or 21 days following renal ischemia-reperfusion injury (IRI) via cardiac puncture. The kidneys were collected, fixed in neutral buffered formalin (ChapTec), and embedded in paraffin. Samples were sectioned into 4 μm slices and automatically stained with hematoxylin-eosin (H&E). Renal tubular damage was assessed on the H&E stained sections using the following criteria: tubular dilatation, loss of brush border, formation of luminal casts, tubular necrosis, and infiltration by polynuclear neutrophils. The extent of damaged tubules was quantified as a tubular injury score (TIS), with the following scale: 0 (no damaged tubules; normal kidney), 1 (< 10% damaged tubules; mild injury), 2 (11-25% damaged tubules; moderate injury), 3 (26-49% damaged tubules; severe injury), 4 (50-75% damaged tubules; high severe injury), and 5 (> 75% damaged tubules; extensive injury).

Renal tubular epithelial cell culture. PT-2 tubular epithelial cells (TECs) (kind gift of A. Jevnikar, Western University, London, Ontario, Canada) is a human renal tubular cell line isolated and cloned from centrifuged urine, which was obtained from a transplant recipient undergoing acute rejection (7). PT-2 cells were cultured in K1 medium constituted with following products: Dulbecco's modified Eagle's medium (DMEM): Hams F12 (50:50) (Invitrogen-Gibco), 5% fetal bovine serum (Invitrogen), hormone mix (5 µg/mL insulin, 1.25 ng/mL prostaglandin E1, 34 pg/mL triiodothyronine, 5 µg/mL transferrin, 1.73 ng/mL sodium selenite and 18 ng/mL of hydrocortisone) and 25 ng/mL epidermal growth factor. PT-2 cells were grown in normal medium until confluence, then exposed to serum-free medium for 24 hours to generate apoptotic exosome-like vesicles.

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Supplementary Table 1. List of microRNAs expressed in ApoExo, ApoBodies and endothelial cells in normal (HUVEC_N) or serum-starved (HUVEC_SS) conditions.

hsa-miR-654-5p	22	61	73	66	88	109	55	77	0.3	0.9	0.3	0.6	0.4	0.8	FAUX	FAUX	0.5
hsa-miR-425-5p	20	63	133	73	119	79	83	134	0.1	0.9	0.1	0.5	0.2	0.5	FAUX	FAUX	0.4
hsa-miR-548a-5p	27	52	41	19	47	48	34	63	0.7	2.8	0.6	1.1	0.8	0.8	FAUX	FAUX	1.1
hsa-miR-410-3p	16	62	170	99	726	264	99	265	0.0	0.6	0.0	0.2	0.2	0.2	FAUX	FAUX	0.2
hsa-miR-299-3p	22	42	56	42	115	115	21	68	1.2	0.5	0.1	1.0	0.8	0.8	FAUX	FAUX	0.7
hsa-miR-195-5p	32	43	39	26	42	18	11	6	0.8	1.7	0.8	2.4	2.9	6.8	FAUX	FAUX	2.6
hsa-miR-1380-3p	21	51	39	26	27	46	40	38	0.5	1.8	0.8	1.1	0.9	1.3	FAUX	FAUX	1.0
hsa-miR-4846-5p	32	162	165	26	28	20	22	24	1.2	2.4	1.2	1.6	1.8	1.8	FAUX	FAUX	1.8
hsa-miR-484	16	54	80	82	119	115	94	110	0.2	0.7	0.1	0.5	0.2	0.5	FAUX	FAUX	0.4
hsa-miR-152-3p	34	37	24	16	54	54	37	41	0.4	1.4	2.2	0.6	0.7	0.9	FAUX	FAUX	1.0
hsa-miR-193b-3p	32	39	67	113	103	46	20	19	0.5	0.7	0.5	0.5	0.6	2.0	FAUX	FAUX	1.0
hsa-miR-135-5p	55	13	6	0	17	0	14	14	8.5	1335.0	3.2	1335.0	3.9	1.0	FAUX	FAUX	447.8
hsa-miR-32-3p	12	13	9	9	15	29	19	24	1.9	0.9	2.4	1.1	1.6	1.6	FAUX	FAUX	2.1
hsa-miR-548c-5p	25	43	34	14	39	41	26	53	0.7	3.1	0.6	1.0	0.9	0.8	FAUX	FAUX	1.2
hsa-miR-548b-5p	25	43	34	14	39	41	26	53	0.7	3.1	0.6	1.0	0.9	0.8	FAUX	FAUX	1.2
hsa-miR-548a-5p	25	43	34	14	39	41	26	53	0.7	3.1	0.6	1.0	0.9	0.8	FAUX	FAUX	1.2
hsa-miR-29b-3p	19	49	19	19	52	26	19	8	2.0	1.0	0.9	2.5	2.4	2.4	FAUX	FAUX	1.7
hsa-miR-454-3p	36	21	265	102	204	211	211	214	0.1	0.3	0.2	0.2	0.1	0.1	FAUX	FAUX	0.2
hsa-miR-29b-1-5p	25	41	21	10	19	19	48	36	2.5	2.2	1.2	2.1	0.5	1.2	FAUX	FAUX	1.6
hsa-miR-309a-5p	26	38	16	33	29	40	17	38	1.6	1.2	0.9	0.9	1.5	1.0	FAUX	FAUX	1.2
hsa-miR-342-5p	41	28	17	35	17	19	36	0.6	0.8	1.2	2.5	1.2	1.2	1.2	FAUX	FAUX	1.2
hsa-miR-455-5p	21	42	149	185	230	188	116	109	0.1	0.2	0.1	0.2	0.2	0.4	FAUX	FAUX	0.2
hsa-miR-105	18	46	26	25	17	15	15	15	0.7	1.8	0.7	2.6	1.3	5.1	FAUX	FAUX	2.0
hsa-miR-339-3p	15	47	16	12	22	8	10	10	0.9	4.0	0.7	5.6	4.5	2.9	FAUX	FAUX	2.0
hsa-miR-154-5p	7	54	39	66	72	65	9	25	0.2	0.8	0.1	0.8	0.7	2.1	FAUX	FAUX	0.8
hsa-miR-146b-5p	29	37	83	56	157	53	0.4	0.8	0.5	0.6	0.2	0.6	0.6	0.8	FAUX	FAUX	0.5
hsa-miR-89a-3p	18	42	97	66	76	63	28	63	0.2	0.6	0.2	0.9	0.6	0.7	FAUX	FAUX	0.5
hsa-miR-431-5p	15	44	71	63	47	272	203	61	0.2	0.7	0.1	0.2	0.2	0.2	FAUX	FAUX	0.3
hsa-miR-548a-3p	44	11	7	9	16	46	6	10	6.8	1.2	2.8	0.2	7.0	1.1	FAUX	FAUX	3.2
hsa-miR-485-5p	15	40	117	18	18	18	20	18	1.3	0.8	2.2	2.1	0.8	2.1	FAUX	FAUX	1.4
hsa-miR-1304-3p	25	30	52	12	52	194	68	103	0.8	2.6	0.5	0.2	0.4	0.3	FAUX	FAUX	0.8
hsa-miR-425-5p	18	37	36	33	29	21	33	33	0.5	1.1	0.6	1.8	0.5	1.1	FAUX	FAUX	0.9
hsa-miR-213b-3p	9	28	44	19	139	245	19	153	0.3	0.4	0.2	0.4	0.2	0.4	FAUX	FAUX	0.6
hsa-miR-576-3p	22	21	21	20	14	23	31	16	1.1	3.3	1.1	2.3	1.0	1.9	FAUX	FAUX	1.8
hsa-miR-100b-5p	15	37	45	154	26	14	33	35	0.2	0.9	0.1	1.4	0.3	0.4	FAUX	FAUX	0.5
hsa-miR-214-5p	16	34	97	28	28	24	27	0.4	0.7	1.2	0.8	1.2	1.3	1.0	FAUX	FAUX	1.0
hsa-miR-452-5p	15	36	83	38	99	40	50	67	0.2	0.9	0.2	0.9	0.3	0.5	FAUX	FAUX	0.5
hsa-miR-4792	23	177	342	1	242	1	61	1	0.1	0.1	0.1	0.1	0.1	29.3	FAUX	FAUX	1.9
hsa-miR-505-3p	19	30	31	42	33	42	24	24	0.6	0.7	0.6	0.7	0.8	1.2	FAUX	FAUX	0.8
hsa-miR-450a-2-3p	22	23	11	2	12	10	8	19	2.0	9.9	1.9	2.4	2.8	1.2	FAUX	FAUX	3.4
hsa-miR-365a-5p	16	29	6	14	16	16	17	16	1.0	3.6	1.0	1.0	0.5	1.7	FAUX	FAUX	1.6
hsa-miR-127-5p	11	33	31	21	136	75	14	44	0.3	1.6	0.1	0.4	0.2	0.5	FAUX	FAUX	0.7
hsa-miR-12a-1-5p	16	26	2	14	48	17	18	36	10.5	1.8	0.6	1.2	0.5	1.8	FAUX	FAUX	2.6
hsa-miR-548l	14	6	27	6	14	25	10	13	2.2	1.3	2.2	1.1	0.9	1.9	FAUX	FAUX	1.8
hsa-miR-2082-5p	19	28	19	28	10	13	0.5	1.0	0.5	1.6	0.5	1.6	0.5	2.3	FAUX	FAUX	1.1
hsa-miR-542-5p	20	28	30	17	6	19	0.6	0.7	1.0	2.1	1.1	1.1	1.1	1.4	FAUX	FAUX	1.4
hsa-miR-21c	11	26	16	14	39	19	10	5	0.6	1.8	0.3	1.3	1.0	5.0	FAUX	FAUX	1.7
hsa-miR-100b-3p	11	26	13	9	10	10	14	20	0.8	2.7	1.0	2.6	0.7	1.3	FAUX	FAUX	1.5
hsa-miR-301b-3p	9	26	27	26	12	34	8	24	0.4	1.3	0.3	3.2	1.1	0.6	FAUX	FAUX	1.3
hsa-miR-464a-5p	8	28	8	5	4	0	2	0	1.0	3.0	2.1	2799.0	5.2	2799.0	FAUX	FAUX	928.2
hsa-miR-673a-5p	16	18	3	14	12	14	13	14	3.0	6.7	3.4	3.8	1.2	3.8	FAUX	FAUX	1.8
hsa-miR-628-5p	16	17	13	5	5	1	6	1.3	1.5	1.1	3.4	20.5	1.6	2.6	FAUX	FAUX	5.7
hsa-miR-769-3p	15	18	29	52	21	43	37	41	0.5	0.3	0.7	0.4	0.4	0.4	FAUX	FAUX	0.5
hsa-miR-139-3p	14	24	10	16	15	15	12	0.6	0.6	0.9	1.2	1.2	1.2	1.2	FAUX	FAUX	1.0
hsa-miR-215-5p	12	21	2	12	2	15	2	15	7.2	1133.0	1.0	2.8	0.9	1.4	FAUX	FAUX	355.0
hsa-miR-210-3p	13	19	67	45	72	109	39	49	0.2	0.4	0.2	0.3	0.4	0.4	FAUX	FAUX	0.3
hsa-miR-652-5p	11	13	20	13	14	14	9	10	1.4	0.8	0.4	0.4	1.1	2.0	FAUX	FAUX	1.2
hsa-miR-7705	11	20	8	12	8	9	17	27	1.3	1.7	1.3	2.2	0.6	0.8	FAUX	FAUX	1.3
hsa-miR-92-3p	7	5	23	5	12	4	6	4	1.0	0.6	4.2	2.8	1.8	2.3	FAUX	FAUX	3.4
hsa-miR-30c-1-3p	16	13	3	9	4	9	13	6	5.0	1.4	4.2	1.5	1.2	2.1	FAUX	FAUX	2.6
hsa-miR-30c-1p	13	17	13	12	21	12	6	9	1.0	1.4	0.6	1.4	2.0	1.9	FAUX	FAUX	1.4
hsa-miR-1200-3p	7	28	12	38	16	56	13	14	0.3	0.4	0.7	0.4	0.5	1.6	FAUX	FAUX	0.6
hsa-miR-548a-5p	20	9	3	16	9	17	12	10	6.1	0.5	2.2	0.5	0.7	0.9	FAUX	FAUX	2.0
hsa-miR-548k	20	9	3	16	9	17	12	10	6.1	0.5	2.2	0.5	0.7	0.9	FAUX	FAUX	2.0
hsa-miR-22-5p	11	18	23	12	37	12	7	11	0.5	1.5	1.3	1.5	1.5	1.5	FAUX	FAUX	1.5
hsa-miR-651b-5p	21	7	29	28	8	4	9	61	0.7	0.2	2.7	1.6	2.2	0.1	FAUX	FAUX	1.3
hsa-miR-673-3p	13	14	11	9	23	66	57	11	1.5	0.6	0.2	0.3	0.3	0.3	FAUX	FAUX	0.7
hsa-miR-2110	13	12	5	14	5	9	10	11	2.6	0.9	2.5	1.4	1.1	1.1	FAUX	FAUX	1.6
hsa-miR-1268a	12	11	7	0	8	7	18	1.8	1.1	1133.0	1.5	2.0	1.6	0.6	FAUX	FAUX	186.8
hsa-miR-505-5p	9	13	8	7	3	1	5	1.2	3.6	6.4	2.6	11.7	0.7	0.7	FAUX	FAUX	4.6
hsa-miR-7641	9	13	65	61	170	36	129	119	0.1	0.2	0.1	0.4	0.1	0.1	FAUX	FAUX	0.2
hsa-miR-671-5p	9	14	50	58	74	54	64	91	0.1	0.2	0.1	0.2	0.1	0.2	FAUX	FAUX	0.2
hsa-miR-758-3p	8	14	18	19	54	37	17	20	0.5	0.8	0.2	0.4	0.5	0.7	FAUX	FAUX	0.5
hsa-miR-3679-5p	7	16	11	9	3	3	8	10	0.6	1.7	2.7	4.5	0.9	1.5	FAUX	FAUX	2.0
hsa-miR-424-5p	9	23	9	23	43	4	8	20	0.6	0.4	0.3	2.1	1.6	0.4	FAUX	FAUX	0.9
hsa-miR-374a-3p	11	11	42	14	177	31	35	91	0.2	0.8	0.1	0.4	0.3	0.1	FAUX	FAUX	0.3
hsa-miR-548p-5p	8	13	11	12	12	14	10	5	1.4	0.7	0.9	0.8	0.8	2.6	FAUX	FAUX	1.2
hsa-miR-548q-5p	8	11	13	12	10	13	9	5	1.1	0.8	1.0	0.9	0.9	2.6	FAUX	FAUX	1.2
hsa-miR-4645-3p	9	11	13	2	10	11	9	6	0.7	4.7	0.9	1.0	1.1	1.8	FAUX	FAUX	1.7
hsa-miR-1813-3-3p	8	12	2	2	20	2	6	20	5.0	1.2	0.4	5.9	1.3	1.2	FAUX	FAUX	3.2
hsa-miR-178f	7	13	7	0	34	24	34	36	1.1	1335.0	0.2	0.6	0.3	0.4	FAUX	FAUX	222.9
hsa-miR-481-5p	12	8	0	1	1	0	3	1	1771.0	0.8	4.5	11.1	1171.0	3.1	FAUX	FAUX	393.6
hsa-miR-1197	9	10	9	5	3	6	15	6	0.6	1.1	1.8	2.9	1.7	0.7	FAUX	FAUX	1.5
hsa-miR-1260c	11	8	294	66	375	192	280	351</									

Supplementary Table 2a. Associations between recipient, donor and procedure characteristics on peritubular capillary density on the post-transplant biopsy in univariable analyses.

Recipient/ Donor/ Procedure characteristics	Difference (95 % CI) in %	p-value
miR-423-5p levels (per 1 natural log higher)	0.8 (0.2, 1.4)	0.01
Recipient age	-0.2 (-0.6, 0.3)	0.53
Recipient female sex	0.5 (-0.7, 1.7)	0.41
Recipient African American race	-1.3 (-3.0, 0.4)	0.13
Cause of chronic kidney disease Other (reference)		
Diabetes	0.4 (-1.3, 2.1)	0.60
Autoimmune disease	0.2 (-2.3, 2.7)	0.88
Hypertension/vascular	0.2 (-1.8, 2.2)	0.85
Glomerular disease	0.4 (-0.9, 1.6)	0.58
Time on dialysis pre-transplant (per 1-month higher)	0.0 (0.0, 0.0)	0.85
Pre-emptive transplantation	1.3 (-0.7, 3.4)	0.20
Previous transplantations	-0.5 (-2.0, 1.0)	0.49
Recipient diabetes	0.5 (-0.8, 1.7)	0.48
Recipient coronary artery disease	-0.2 (-2.0, 1.5)	0.78
Recipient body mass index (per 1 unit higher)	0.0 (-0.1, 0.1)	0.47
Recipient active smoking at transplantation	0.5 (-1.0, 2.1)	0.48
Recipient statin use at transplantation	1.0 (-0.1, 2.2)	0.06
Recipient statin use 6 months post transplantation	0.3 (-0.8, 1.4)	0.58
Recipient RAS blockers use at transplantation	-0.6 (-1.8, 0.7)	0.35
Recipient RAS blockers use 6 months post transplantation	0.1 (-1.3, 1.6)	0.85
Pre-transplant PRA > 0% (versus 0%)	-0.1 (-1.6, 1.5)	0.92
Peak historical PRA > 0% (versus 0%)	-0.5 (-1.6, 0.7)	0.42
HLA mismatches (reference 0-2)		
3-4	0.6 (-3.6, 4.8)	0.77
5-6	0.9 (-3.4, 5.2)	0.67
Thymoglobulin induction	-0.1 (-1.3, 1.0)	0.81
Total ischemic time (per 1-hour higher)	0.0 (-0.1, 0.1)	0.86
Donor type (reference neurologically deceased)		
Living donor	-0.6 (-3.0, 1.8)	0.61
Donor after cardiac arrest	0.8 (-0.6, 2.2)	0.25
Use of hypothermic machine perfusion	1.1 (0.0, 2.2)	0.06
Donor age (per 10-year higher)	-0.1 (-0.5, 0.3)	0.53
Donor creatinine (per 10 µmol/L higher)	0.1 (-0.1, 0.3)	0.33
Female donor sex (versus male)	-0.5 (-1.6, 0.6)	0.39

Donor positive smoking history (versus negative or unknown)	0.3 (-0.9, 1.4)	0.65
Donor peripheral vascular disease	1.0 (-1.4, 3.4)	0.41
Donor hypertension	-0.7 (-2.1, 0.8)	0.37
Donor diabetes	-1.1 (-3.1, 1.0)	0.30
Time to biopsy in days	0.0 (0.0, 0.0)	0.17
Rejection before the post-transplant biopsy	0.2 (-1.0, 1.4)	0.69
Peritubular capillary density on the pre-transplant biopsy (per 1% higher)	0.1 (0.0, 0.3)	0.12

Supplementary Table 2b. Associations between recipient, donor and procedure characteristics on peritubular capillary density on the post-transplant biopsy in the initial multivariable model

Clinical characteristics	Adjusted difference in %, 95% CI	p-value
miR-423-5p levels 1 month post-transplant (per 1 natural log higher)	1.0 (0.4, 1.5)	0.001
Female recipient sex (reference male)	1.2 (-0.1, 2.4)	0.06
Peritubular capillary density on the pre-implantation biopsy (per 1% higher)	0.1 (-0.0, 0.3)	0.11
Use of hypothermic pump during transportation	1.0 (-0.0, 2.0)	0.06
Use of thymoglobulin as induction	0.5 (-0.7, 1.7)	0.42
African American ethnicity	-0.1 (-1.8, 1.6)	0.89
Use of statins at transplant	1.0 (0.0, 2.0)	0.04

Supplementary Table 3a. Associations between recipient, donor and procedure characteristics on interstitial fibrosis on the post-transplant biopsy in univariable analyses.

Recipient/ Donor/ Procedure characteristics	Difference (95 % CI) in %	p-value
miR-423-5p levels 1 month post transplant (per 1 natural log higher)	-4.8 (-8.8, -0.9)	0.02
Recipient age	-0.1 (-0.3, 0.1)	0.40
Recipient female sex	1.2 (-3.9, 6.3)	0.64
Recipient African American race	6.6 (-0.3, 13.5)	0.06
Cause of chronic kidney disease Other (reference)		
Diabetes	-5.0 (-11.6, 1.7)	0.14
Autoimmune disease	-5.4 (-15.5, 4.8)	0.29
Hypertension/vascular	-0.5 (-8.5, 7.5)	0.90
Glomerular disease	0.7 (-4.7, 6.1)	0.79
Time on dialysis pre-transplant (per 1-month higher)	0.1 (0.0, 0.1)	0.04
Pre-emptive transplantation	-2.6 (-11.2, 6.0)	0.55
Previous transplantations	2.6 (-4.1, 9.4)	0.43
Recipient diabetes	-3.8 (-9.2, 1.6)	0.16
Recipient coronary artery disease	0.9 (-6.3, 8.1)	0.80
Recipient body mass index (per 1 unit higher)	-0.15 (-0.6, 0.3)	0.51
Recipient active smoking at transplantation	1.8 (-4.6, 8.2)	0.57
Recipient statin use at transplantation	-1.9 (-6.8, 2.9)	0.42
Recipient statin use 6 months post transplantation	-3.2 (-7.8, 1.5)	0.17
Recipient RAS blockers use at transplantation	1.5 (-3.6, 6.6)	0.56
Recipient RAS blockers use 6 months post transplantation	-0.5 (-6.6, 5.6)	0.88
Pre-transplant PRA > 0% (versus 0%)	-3.7 (-10.0, 2.6)	0.25
Peak historical PRA > 0% (versus 0%)	1.5 (-3.2, 6.3)	0.52
HLA mismatches (reference 0-2)		
3-4	1.6 (-14.6, 17.7)	0.85
5-6	7.1 (-9.5, 23.7)	0.40
Thymoglobulin induction	-0.8 (-5.6, 4.0)	0.74
Total ischemic time (per 1-hour higher)	0.07 (-0.53, 0.40)	0.77
Donor type (reference neurologically deceased)		
Living donor	0.6 (-9.3, 10.5)	0.90
Donor after cardiac arrest	-3.4 (-9.3, 2.4)	0.25
Use of hypothermic machine perfusion	4.4 (-0.3, 9.1)	0.07
Donor age (per 10-year higher)	0.2 (0.1, 0.3)	0.01
Donor creatinine (per 10 µmol/L higher)	0.0 (-0.07, 0.1)	0.93
Female donor sex (versus male)	-1.8 (-6.5, 2.9)	0.45

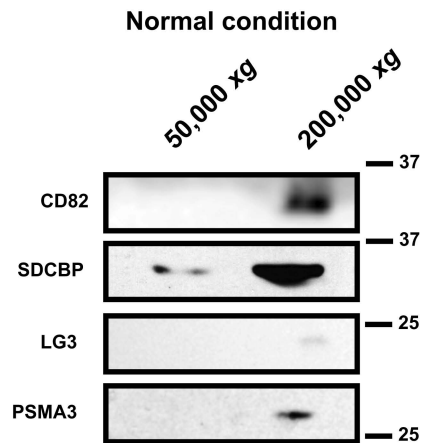
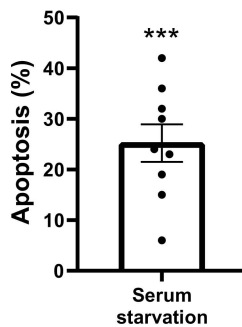
Donor positive smoking history (versus negative or unknown)	2.5 (-2.2, 7.3)	0.28
Donor peripheral vascular disease	-2.7 (-12.5, 7.1)	0.58
Donor hypertension	4.0 (-2.0, 10.0)	0.18
Donor diabetes	1.2 (-7.4, 9.8)	0.78
Time to biopsy in days	-0.1 (-0.2, 0.1)	0.31
Rejection before the post-transplant biopsy	6.8 (-1.1, 14.7)	0.08
Peritubular capillary density on the pre-transplant biopsy (per 1% higher)	0.2 (-0.4, 0.8)	0.44
Fibrosis on the pre-implantation biopsy (per 1% higher)	0.6 (0.1, 1.1)	0.03

RAS: renin-angiotensin system; PRA: panel reactive antibodies

Supplementary Table 3b. Associations between recipient, donor and procedure characteristics on interstitial fibrosis on the post-transplant biopsy in the initial multivariable model

Clinical characteristics	Adjusted difference in %, 95% CI	p-value
miR-423-5p levels 1 month post-transplant (per 1 natural log higher)	-4.6 (-8.6, -0.9)	0.02
Female recipient sex (reference male)	4.8 (-3.1, 12.7)	0.23
Time on dialysis before transplantation (per 1 month higher)	0.1 (-0.0, 0.2)	0.09
Donor age (per 10 year higher)	3.3 (0.8, 5.8)	0.01
Rejection before/on the post-transplant biopsy	7.0 (-0.2, 14.3)	0.056
African American ethnicity	-4.6 (-16.7, 7.5)	0.45
Thymoglobulin as induction	0.6 (-7.9, 9.1)	0.89
Use of hypothermic pump during organ transportation	1.1 (-6.3, 8.5)	0.76
Fibrosis on the pre-implantation biopsy (per 1% higher)	-0.0 (-0.1, 0.1)	0.98

Figure S1. Apoptotic exosome-like vesicles (ApoExos) from endothelial cells, but not from renal tubular epithelial cells, are enriched in miR-423-5p. (A) Representative immunoblots of CD82, SDCBP, LG3 and PSMA3 in large (centrifugation at 50,000 xg) and small (centrifugation at 200,000 xg) EV fractions purified from endothelial cells under normal condition for 4 h. (B) Evaluation by Hoescht 33342 and Propidium iodide (HO/PI) staining of apoptosis in renal tubular epithelial cells serum-starved (SS) for 48 h. P value was obtained by one sample t test (*** P < 0.001), n = 9. Representative immunoblots of SDCBP, PSMA3, LG3, and histone H3C1 in large (centrifugation at 50,000 xg) and small (centrifugation at 200,000 xg) EV fractions purified from apoptotic renal tubular epithelial cells serum starved for 48 h. (C) MiR-423-5p, let-7b-5p and let-7c-5p expression in large and small EV fractions derived from apoptotic endothelial cells or apoptotic renal tubular epithelial cells. Expression of miRNAs was measured by quantitative RT-PCR and the result is presented as relative copies expression of miRNA per ng of RNA \pm SEM after normalization with cel-miR-39; n = 3. P values were obtained by one-way ANOVA and Bonferroni post hoc test (* P < 0.05, ** P < 0.01).

A**B**

Serum starvation
Tubular Epithelial cells

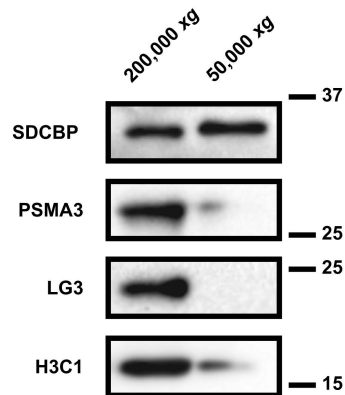
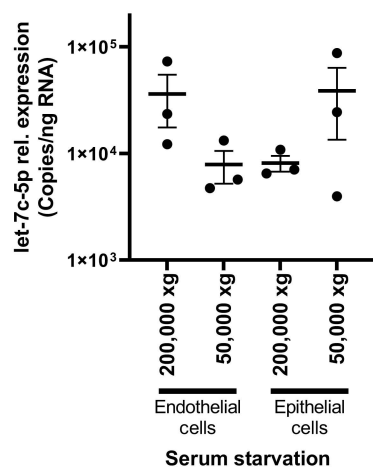
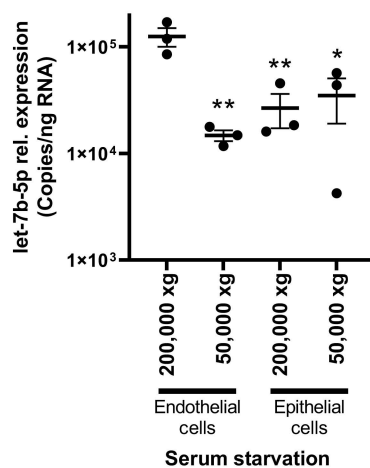
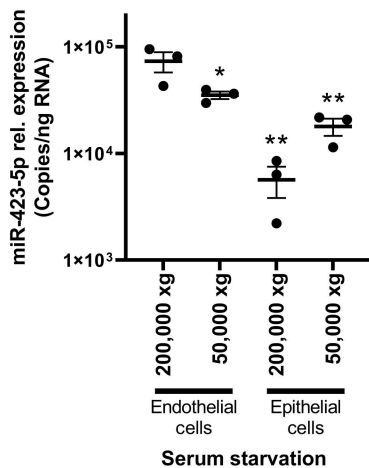
**C**

Figure S2. Circulating miR-423-5p, let-7b-5p and let-7c-5p in mice after renal IRI.

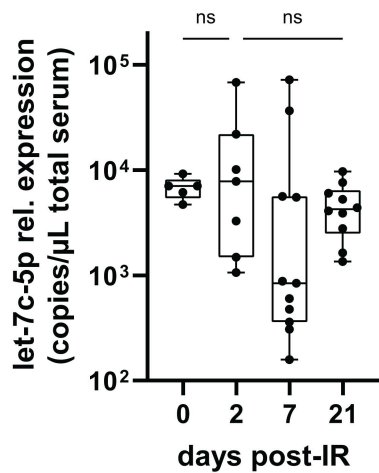
(A) Quantification of let-7c-5p serum levels presurgery or 2, 7 and 21 days post IRI. Let-7c-5p was measured by quantitative RT-PCR, and the result is presented as relative copy expression of miRNA per μL of total serum \pm SEM after normalization with cel-miR-39; $n \geq 5$. Values are the mean \pm SEM. P values were obtained by one-way ANOVA and Bonferroni post hoc test.

(B) Correlation matrix of miRNAs mouse serum levels. P values were obtained by Spearman correlation and r is represented by heatmap.

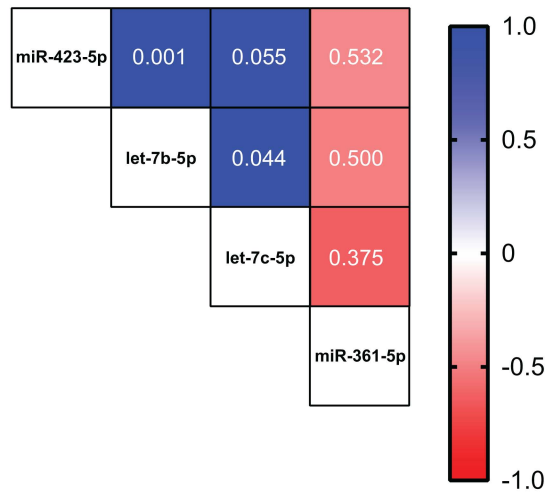
(C) Quantification of miR-361-5p serum levels presurgery or 2, 7 and 21 days post IRI. MiR-361-5p was measured by quantitative RT-PCR, and the results are presented as relative copy expression of miRNA per μL of total serum \pm SEM after normalization with cel-miR-39; $n \geq 4$. Values are the mean \pm SEM.

(D) Quantification of let-7c-5p serum levels in wild-type mice at baseline. Serum was treated or not with RNase A (0.025 mg/mL) with or without Triton X-100 (0.1 %) for 20 min at 37 °C. Expression of let-7c-5p was measured by quantitative RT-PCR and the result is presented as relative copies of miRNA per μL of total serum \pm SEM. $n = 3$ for each condition. P values were obtained by one-way ANOVA and Tukey post hoc test (****, $P < 0.0001$).

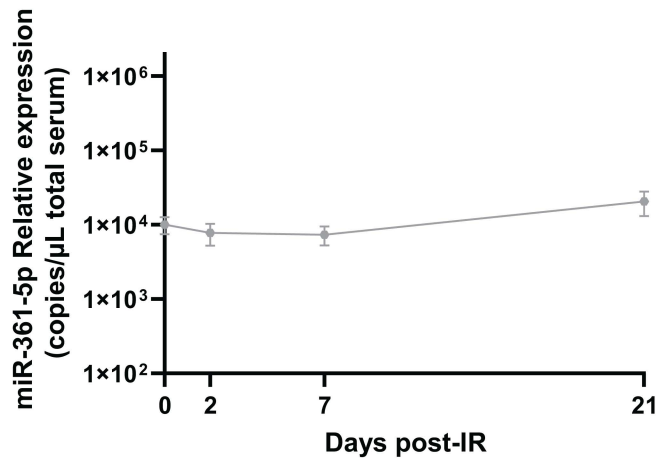
A



B



C



D

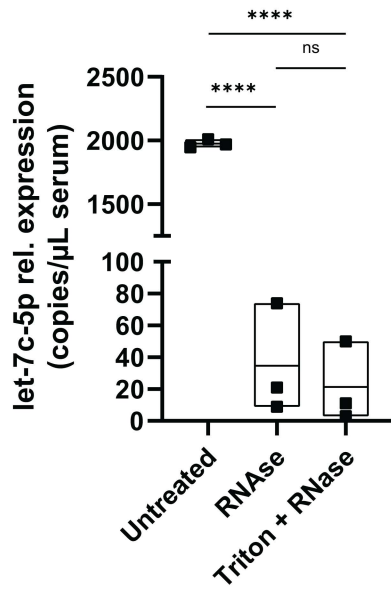


Figure S3. At a distance from IRI, lower miR-423-5p serum levels correlate with more severe microvascular rarefaction and renal fibrosis. (A) Blood urea nitrogen (BUN) concentration in mice at baseline or 1, 2, 7, or 21 days after 30-min (IR30) and 60-min (IR60) renal ischemia–reperfusion injury (IRI); n = 5 for each condition. (B) Quantification of miR-423-5p serum levels presurgery or 1, 2, 7 and 21 days post IRI. MiR-423-5p was measured by quantitative RT–PCR, and the result is presented as relative copy expression of miRNA per μL of total serum \pm SEM after normalization with cel-miR-39; n \geq 6. Values are the mean \pm SEM. (C) Quantification of cleaved caspase-3-positive peritubular capillaries (PTCs) in renal sections at baseline, 1 and 2 days post-surgery in mice that underwent 30 min or 60 min or renal artery clamping; n \geq 3. Representative images of cleaved caspase-3 immunohistochemistry in renal sections (corticomedullary junction) presurgery and at 1 day post-IRI. Scale bar: 50 μm (D) Quantification of phosphorylated Receptor-interacting serine/threonine-protein kinase 3 (RIPK3)-positive peritubular capillaries (PTCs) in renal sections at baseline, 1 and 2 days post-surgery in mice that underwent 30 min or 60 min of renal artery clamping; n \geq 3. Representative images of pRIPK3 immunohistochemistry in renal sections (corticomedullary junction) at baseline and 1 day post-IRI. P values compared to baseline (time 0) [*] or between IR30 and IR60 [#]. Scale bar: 50 μm (E) Mean tubular injury scores of ten randomly chosen high-power fields in renal cortical sections at baseline and 2 days post-IRI in mice that underwent 30 min and 60 min IRI or 2 days post-sham operation with contralateral nephrectomy. Representative haematoxylin and eosin (H&E) stained renal sections showing tubular injury. n = 3-6 for each condition. Scale bar: 50 μm (F) Quantification of miR-423-5p serum levels at baseline, 2 days after the sham operation with contralateral nephrectomy, and at 2 and 21 days following the sham operation without contralateral nephrectomy. The expression of miR-423-5p was measured by quantitative RT–PCR, and the result is presented as relative copies of miR-423-5p per μL of total serum \pm SEM after normalization with cel-miR-39; n \geq 3. (G) Representative

immunoblots of different protein markers in large (centrifugation at 50,000 xg) and small (centrifugation at 200,000 xg) extracellular vesicles purified from mouse serum at baseline and 2 days post-sham operation with contralateral nephrectomy. PLVAP is a marker of endothelial-derived extracellular vesicles, PSMA3 and LG3 are markers of apoptotic exosome-like vesicles, ACTB is a general marker of extracellular vesicles and CD82 is an exosome marker, n = 3. All P values were obtained by one-way ANOVA and the Bonferroni post hoc test (* P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001).

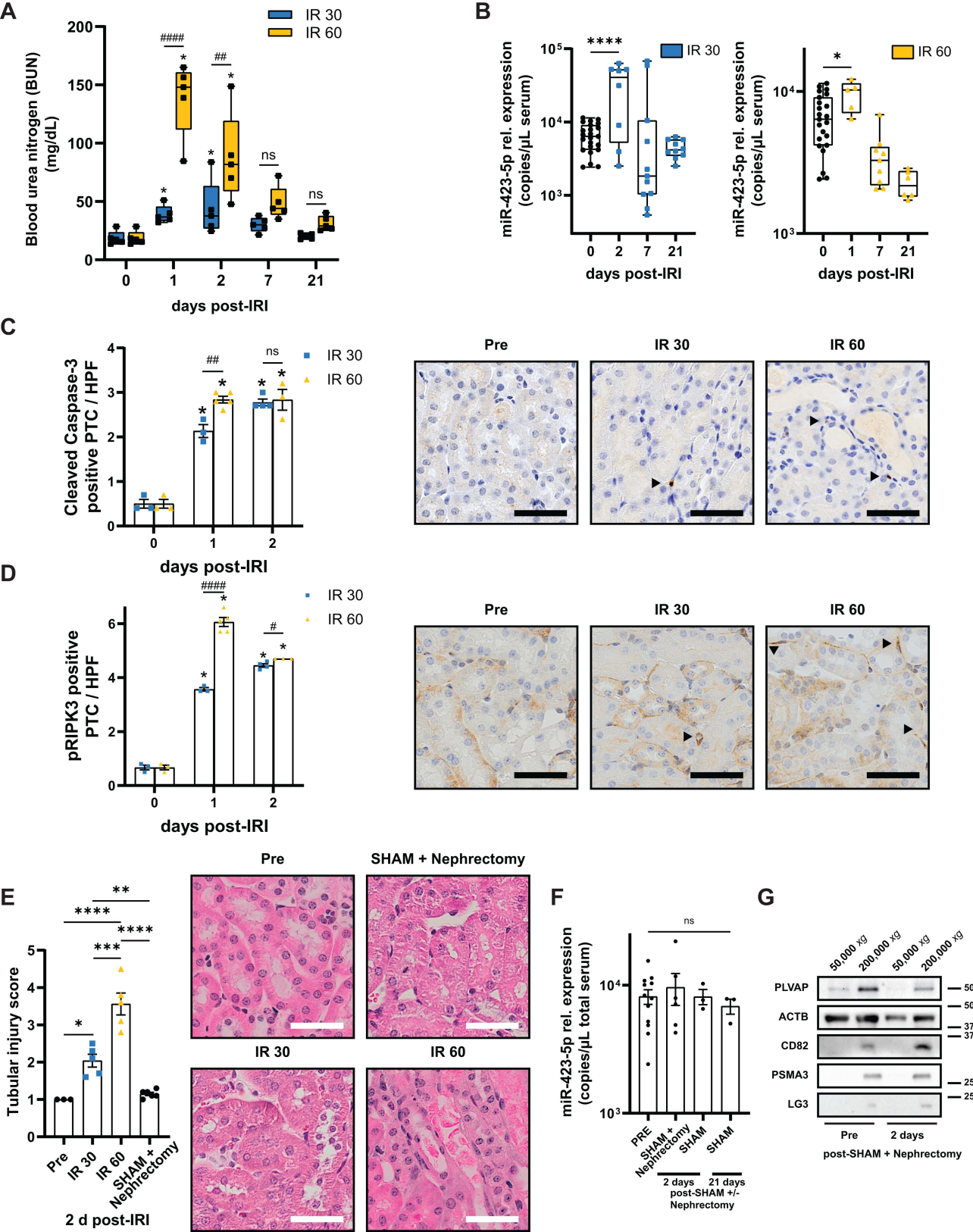


Figure S4. Serum levels of miR-423-5p 21 days after renal ischemia-reperfusion injury (IRI) are independent of caspase-3 activation. (A) Kaplan-Meier survival curves of wild-type (WT) or caspase-3 knockout (Casp-3 $-/-$) mice that underwent 30 min or 60 min of renal artery clamping; $n \geq 23$. P values were obtained by log-rank test (** $P < 0.01$). (B) Quantification of miR-423-5p serum levels 21 days after 60-min renal artery clamping in WT or Casp-3 $-/-$ mice. Expression of miR-423-5p was measured by quantitative RT-PCR and the result is presented as relative copies of miR-423-5p per μL of total serum \pm SEM after normalization with cel-miR-39. (C) Quantification of PLVAP-positive peritubular capillaries (PTC) per tubule with representative images of PLVAP immunohistochemistry in renal sections (corticomedullary junction) 21 days after 60 of renal artery clamping in WT or Casp-3 $-/-$ mice. Scale bar: 50 μm (D) Quantification of Sirius Red within PTC areas in the corticomedullary junction of renal sections in WT or Casp-3 $-/-$ mice 21 days after renal artery clamping for 60 min IRI with representative images of Sirius Red staining; $n = 5$. P values were obtained by unpaired t test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$). Scale bar: 50 μm (E) *Left:* Evaluation by Hoescht 33342 and Propidium iodide (HO/PI) staining of apoptosis in HUVECs in normal or serum-starved condition and treated with the pan-caspase inhibitor zVAD-Fmk (ZVAD) or vehicle (Ctrl) for 4 h. P values were obtained by one-way ANOVA and the Bonferroni post hoc test (** $P < 0.01$, **** $P < 0.0001$), $n = 3$. *Right:* MiR-423-5p expression in small extracellular vesicle fractions derived from apoptotic endothelial cells (ApoExo) or in large extracellular vesicle fractions derived from healthy endothelial cells (MVs) treated with ZVAD or its vehicle (Ctrl) for 4 h. The expression of miRNAs was measured by quantitative RT-PCR, and the results are presented as the relative copy expression of miRNA per mL of supernatant \pm SEM after normalization to cel-miR-39; $n = 3$. P values were obtained by unpaired t test (* $P < 0.05$).

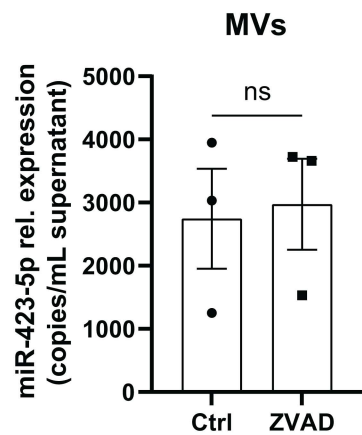
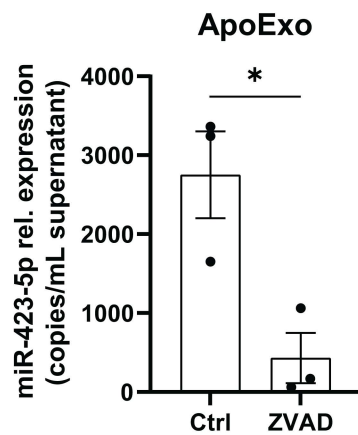
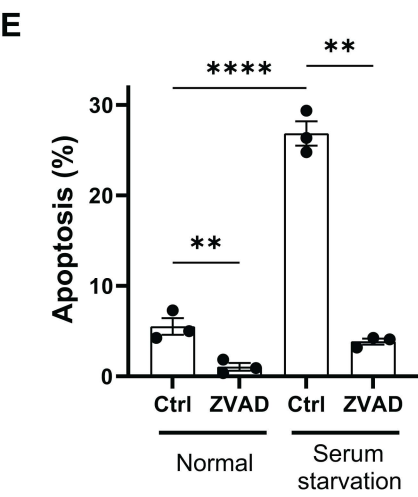
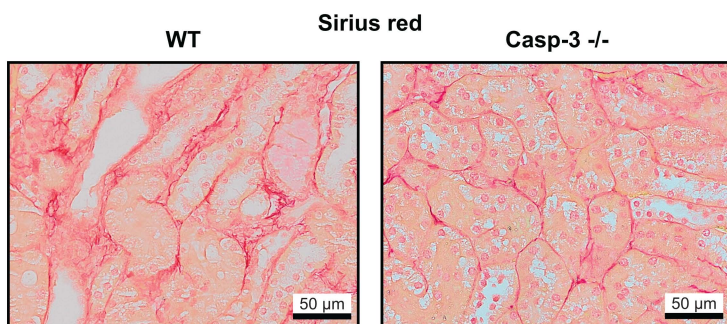
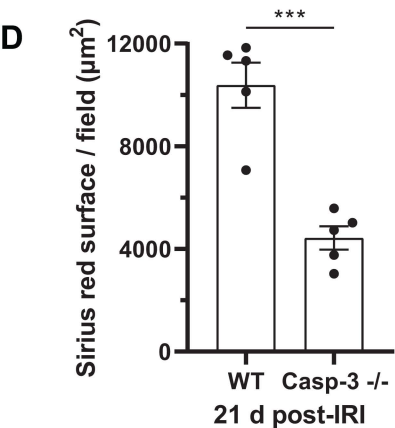
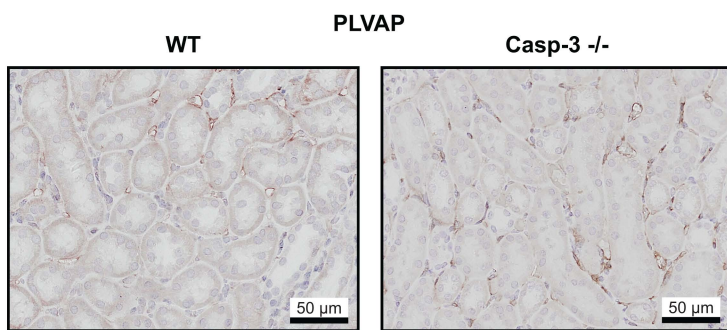
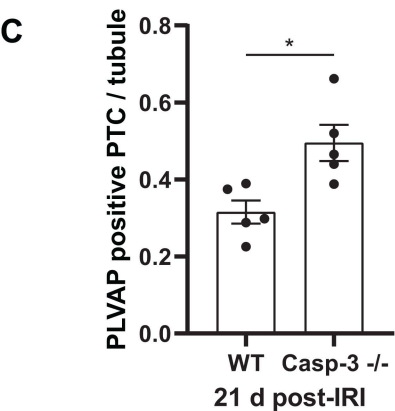
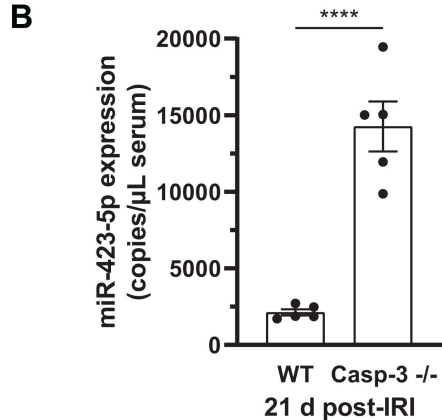
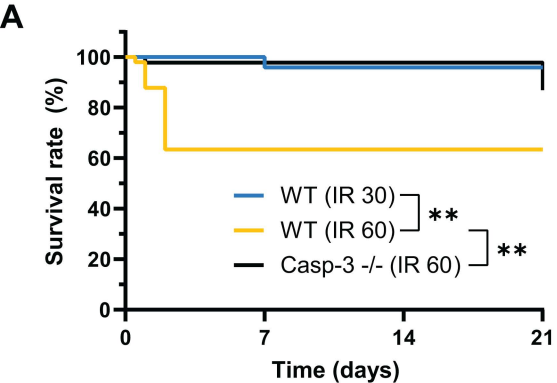


Figure S5. MiR-423-5p serum levels in human renal transplant patients. (A) The correlation matrix of miRNAs serum levels, measured in total serum from transplanted patients, was analyzed. P values were obtained by Spearman correlation, and the correlation coefficient (r) is represented by a heatmap. (B) Expression levels of miR-423-5p, let-7b-5p and let-7c-5p in total serum and extracellular vesicles purified from the same amount of patients' serum; n = 5-6. (C) Distribution of miR-423-5p in natural logarithm among the delayed graft function (DGF) patients; n = 51. (D) Relative distribution of miR-423-5p expression in large EV fraction (centrifugation at 50,000 xg) and small EV fraction (centrifugation at 200,000 xg) from the serum of six randomly chosen DGF patients at 8-10 days and one-month post-transplantation; n = 6. MiR-423-5p serum level was measured by quantitative RT-PCR and normalized with cel-miR-39. P values were obtained by paired t test (* P < 0.05).

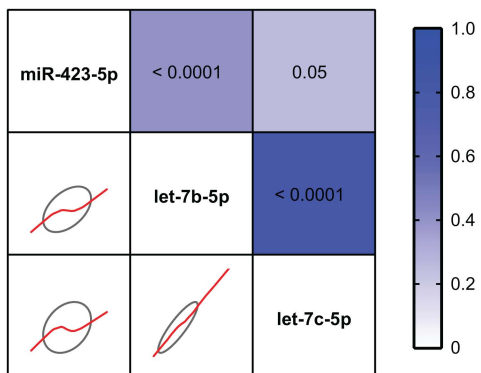
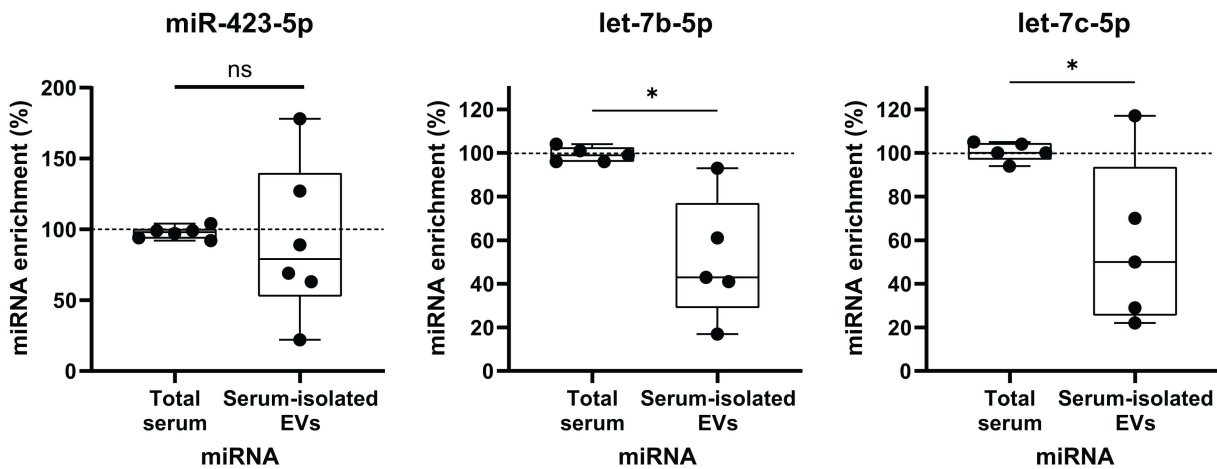
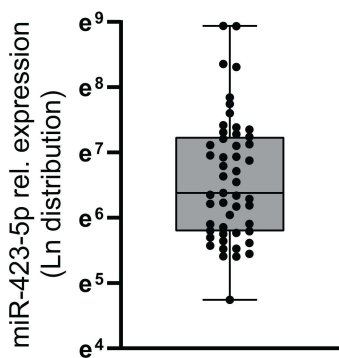
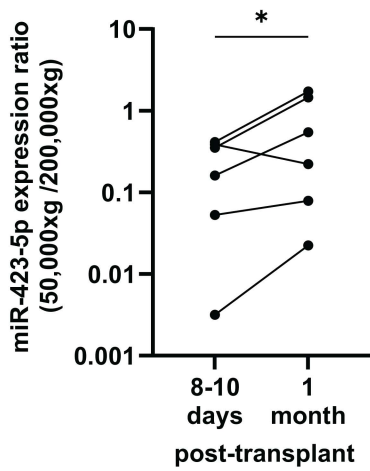
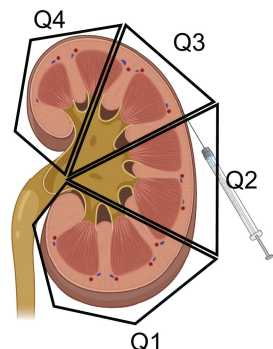
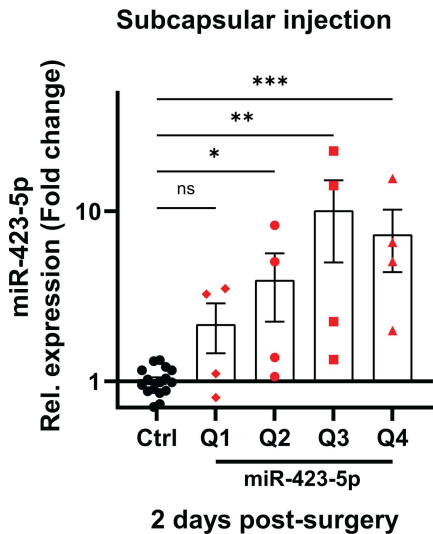
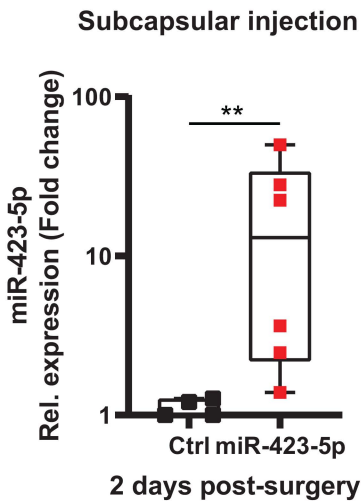
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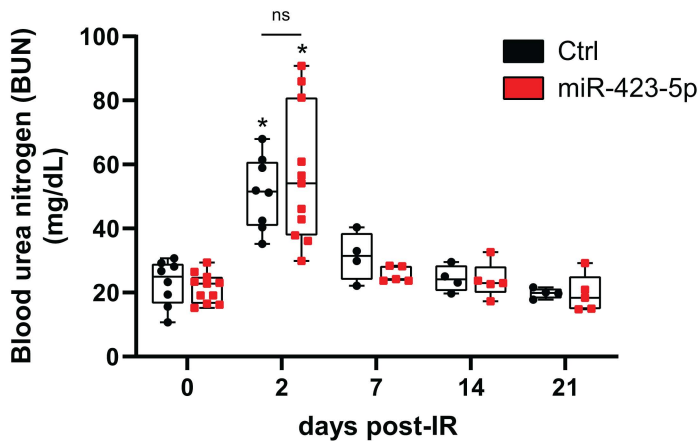
Figure S6. Subcapsular injection of miR-423-5p increases renal expression levels.

(A) Total (*Left*) or sectioned (*Right*) renal expression of miR-423-5p following subcapsular injection of Ctrl or miR-423-5p mimics 2 days post-surgery. Expression of miR-423-5p in the whole kidney (*Left*) or in the four kidney sections (Q1-4) (*Right*), was measured by quantitative RT-PCR and the result is presented as expression fold change \pm SEM. Each quadrant corresponds to a portion of the kidney obtained through cross-sectional cuts. P value was obtained by unpaired t test (*Left*) and by one-way ANOVA and the Bonferroni post hoc test (* P < 0.05, ** P < 0.01, *** P < 0.001), n \geq 4. (B) Blood urea nitrogen (BUN) concentration at baseline or 2, 7, 14 and 21 days after 30-min renal ischemia–reperfusion injury (IRI) in mice with subcapsular injection of Ctrl or miR-423-5p mimics. P value was obtained by one-way ANOVA and the Bonferroni post hoc test (* P < 0.05); n \geq 4 for each condition. (C) Mean tubular injury scores of ten randomly chosen high-power fields in mice renal cortical sections at 2 days post-IRI from mice that underwent 30 min IRI with subcapsular injection of Ctrl or miR-423-5p mimics. P value was obtained by unpaired t test; n = 4-6 for each condition.

A



B



C

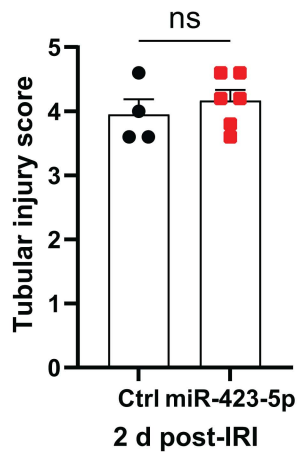


Figure S7. MiR-423-5p promotes endothelial migration and angiogenesis. (A) Endothelial cells were transfected with Ctrl or miR-423-5p mimics (10 nM). Forty-eight hours post-transfection, miR-423-5p expression was measured by quantitative RT-PCR and presented as relative copies expression of miR-423-5p \pm SEM after normalization with cel-miR-39; n = 3. (B) Endothelial cells were transfected with Ctrl or miR-423-5p mimics (10 nM). The expression of *HIF1A* and *VEGFA* mRNAs was measured by quantitative RT-PCR 48 h post-transfection. The results are presented as the relative expression of mRNA compared to cells transfected with mimic Ctrl (Ctrl) \pm SEM after normalization with *HPRT1*; n = 6 for each condition. P values were obtained by unpaired t test (* P < 0.05, ** P < 0.01). (C) Endothelial cells transfected with Ctrl or miR-423-5p mimics and: (top) mechanically injured and percentage of open wound areas at 0 h was measured with TScratch software. Data are shown as % of the open wound area. (bottom) Capillary-like structures were quantified after 6 h on Matrigel. Angiogenic activity is assessed by quantifying the number of nodes, junctions and segment length per field \pm SEM. n = 4 for each condition. P values obtained by unpaired t-test. Scale bar: 200 μ m

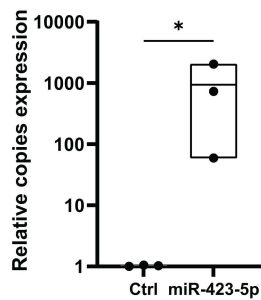
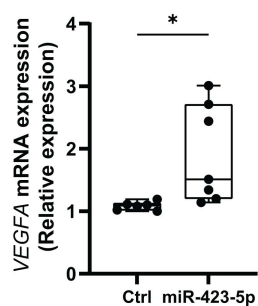
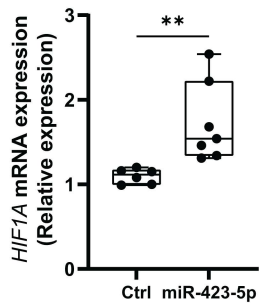
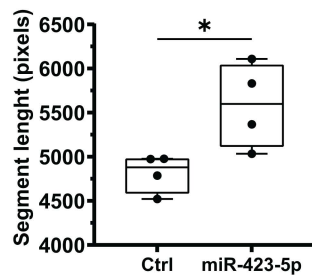
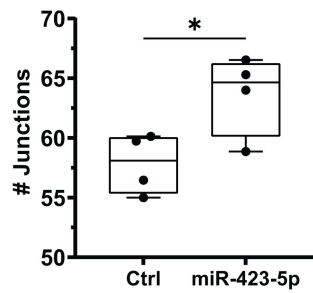
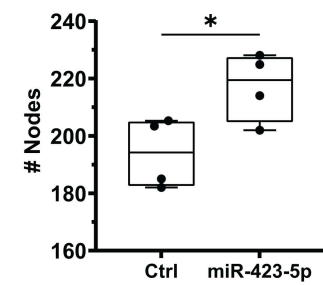
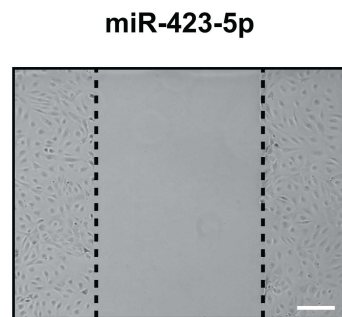
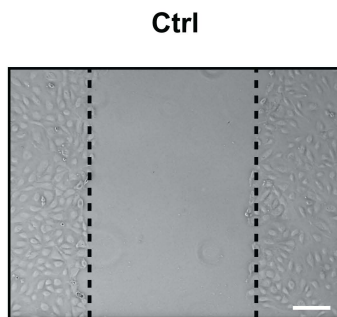
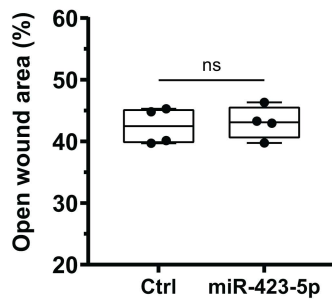
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Figure S8. MiR-423-5p expression levels are increased following intramuscular injection.

Expression of miR-423-5p in ischemic muscle following intramuscular injection of Ctrl or miR-423-5p mimics at 3-days post-surgery. Expression of miR-423-5p was measured by quantitative RT-PCR and the result is presented as expression fold change \pm SEM. P value was obtained by unpaired t test (** P < 0.01); n = 3.

Intra-muscular injection

