SUPPLEMENTAL MATERIAL

Supplemental Methods

Cell culture and transfection

HEK293T cells (American Type Culture Collection, USA) were grown in Dulbecco's modified Eagle's medium supplemented with 2 mM-glutamine, 100 units/ml penicillin/streptomycin, and 10 % (v/v) heat-inactivated fetal bovine serum (FBS) at 37 °C and in an atmosphere of 5% CO_2. Cells stably expressing the HA tagged AT1R (2 ± 0.22 pmol/mg protein) were used as previously described[1] and YFP tagged AT1R (1 ± 0.31 pmol/mg protein) were a kind gift from Dr. Shenoy. In separate co-localization experiments, AT1R-YFP stable cells were transiently transfected with (2 µg DNA) of the plasmid containing Alix-mCherry (Addgene, plasmid 21504).

Exosomes isolation

Briefly, differential centrifugation at 4 °C was performed starting with a centrifugation at 1000 x g (15 min) followed by centrifugations at 12,000 x g (20 min) and 18,000–20,000 x g (20 min) to obtain a supernatant free of free floating cells and cellular debris. The supernatant was filtered by vacuum or gravity (Figure S1, Figure S2 A, B) through a 0.22 µm filter to remove cellular debris, apoptotic bodies and microvesicles. Purified exosomes were then collected by ultracentrifugation of the filtered solution at 100,000 x g (70 min) using a Ti70 rotor in a Beckman ultracentrifuge and resuspended in 100 µl of

1X PBS. No difference were observed for the size and quantity of exosomes isolated from serum compared to citrated plasma (Figure S2 C,D).

Nanoparticle Tracking Analysis (NTA)

NTA is based on the principle that the rate of Brownian movement of nanoparticles in solution is related to their size, and by tracking the movement of individual nanoparticles over time the particle diameter can be calculated.[2] Briefly, samples from the 100,000 x g centrifugation step were used for NTA, approximately 0.3 ml supernatant was loaded into the sample chamber of an LM10 unit (Nanosight, Ltd) and three videos of either 30 or 60 seconds were recorded of each sample.

AT1R radioligand binding assay

Briefly 50 µl of exosome suspension was incubated with a saturating concentration of radiolabeled AT1R antagonist [^{125}I]-SAR1-ILE8-ANGIOTENSIN II (2 nmol/L, Perkin Elmer NEX 248) in a buffer containing 50 mmol/L Tris-HCl pH 7.4, 150 mmol/L NaCl, 12.5 mmol/L Mg Cl^{2-}, 0.2% BSA. Specific binding was determined by subtracting non-specific binding in the presence of 10 µmol/L Telmisartan from total binding measured by the addition of assay buffer. After 90 minutes of incubation at RT, the binding assay was loaded onto chromatography columns (BioRad) containing 10 ml of Sephadex G50 medium resin (Sigma-Aldrich) and the exosomes were eluted by gravity with 5 ml of elution buffer (50 mmol/L Tris pH 7.4). Previous calibration experiments with Blue Dextran (2000 kDa) and Orange G (500 Da) showed that 5 ml was sufficient to completely elute the void volume containing the exosomes while retaining the free

radioligand (Figure 1B). Bound radioactivity was quantified using a Packard Cobra Gamma Counter. Specific radioactivity was converted to femtomole (fmol) of AT1R and normalized to 10^2 exosomes, then expressed as the number of receptors using the Avogadro constant: N° of AT1Rs in 1 fmol = $(6.23 \times 10^{23})/10^{-15}$.

Transmission Electron Microscopy and Immunogold labeling

The main marker of exosomes is their morphology, including oval-biconcave shape and size of 30 to 100 nm. To define their purity and quality, we examined exosome morphology using Transmission Electron Microscopy (T.E.M.). 5 µL of purified exosomes was loaded on a Formvar-coated copper grid (200 mesh) for 5 min and excess exosome suspension was blotted with filter paper. The grid was placed on a drop of 1% uranyl acetate solution in milli-Q H2O for 30 sec, and excess stain was removed. Exosomes were examined using a Philips/FEI T.E.M. CM12 transmission electron microscope at 80 kV.

For the immunogold-labeling the exosomes isolated by differential centrifugation were incubated overnight with a primary antibody 100 µl 1:5 dilution (Rabbit anti HA, Clontech and Mouse CD9, Millipore). Exosomes were then washed in PBS and centrifuged at 100 000 x g for 70 min in order to eliminate the excess of unbound antibody. The antibody labeled exosomes were loaded on Formvar-coated grid and fixed with 2% paraformaldehyde in PBS for 5 min. The exosome loaded grids were washed three times in PBS and incubated with Goat Blocking solution for 10 min and then with secondary antibody gold labeled Goat anti Rabbit (10 nm beads size) and

Goat anti Mouse (25 nm beads size) for 30 min. After 3 washes in PBS the grid was placed on a drop of 1% uranyl acetate solution in milli-Q H2O for 30 seconds, and excess stain was removed.

Exosomes transfer by confocal microscopy

Confocal microscopy was carried out as previously described [3] using a Zeiss 780 inverted laser scanning confocal microscope. HEK 293T cells stably transfected with the plasmids containing cDNAs encoding the AT1R-YFP and Alix-mCherry (ADDgene, Plasmid 21504) were used as positive control while not transfected HEK 293 served as the negative control. Cells were plated onto glass-bottom dishes, pre-coated with Collagen I (0.001 %), before observation on the confocal microscope. HEK 293 cells were treated overnight with exosomes derived from AT1R-YFP conditioned media of three different conditions: Osmotic Stretch (OSM), Angiotensin II (Ang II) 10µmol/L and No Stimulation (NS). In order to assess if AT1R was active once transferred to recipient cells, live cells were treated with Ang II (10 µmol/L) and images were collected 15 minutes after stimulation. Samples were visualized using single sequential line excitation filter at 514 and 568 nm and emission filter at 505–550 nm for YFP and mCherry detection respectively.

Pressure overload

Animal studies were carried out according to approved protocols and animal welfare regulations of Duke University Medical Center's Institutional Review Boards. Mice were anesthetized with a mixture of ketamine (100 mg/kg) and xylazine (2.5 mg/kg). Chronic

pressure overload was induced by transverse aortic constriction (TAC) in WT mice for 1 week while in Sham operated mice the suture was passed around the aorta but not ligated as previously described.[4]

In vivo exosomes administration

In separate experiments, AT1R-KO mice were injected through tail vein with a range between 1.0-2.3 x 10^{11} exosomes isolated from AT1R stable expressing cells, and between 4.6-5.6 x 10^{13} exosomes isolated from the serum of wild type mice. Hemodynamic assessment was performed 24 hours after injection on mice at baseline and during AngII infusion (2 µg/kg/min).

An additional set of injections were performed on WT mice using ~5 x 10^{13} exosomes isolated from AT1R-YFP stable cells, then heart, skeletal muscle and mesenteric vessels were removed after 24 hours for histological analysis.

Hemodynamic study (blood pressure response)

After bilateral vagotomy, a polyethylene-50 (PE-50) catheter was inserted into the left axillary artery and connected to a Statham P23 Db pressure transducer (Gould Statham Instruments, Bayamon, Puerto Rico) for pressure monitoring. Blood pressure was recorded continuously with a pressure-recording system (MacLab, Millar Instruments, Houston, TX). Intravenous drug infusion was performed via the right jugular vein. Continuous infusion of AngII (2 µg/kg/min) was administered via the right jugular vein. Mean arterial pressure was recorded for 3 minutes before infusion (Basal)

and for 5 min after AngII infusion.

Histology

Wild type male mice (N=20) were injected through tail vein with a range between 4 to 5 x 10^{13} exosomes isolated from AT1R-YFP stable expressing cells. The day after the mice were sacrificed and tissues, after fixation in PFA (Paraformaldehyde) 4%, embedded in OCT compound and snap frozen prior to cryostat sectioning. For ex vivo confocal microscopy analysis, cryosections (7µm) of mice tissues, after ice cold fixation with PFA 4%, were immunostained for different markers using the following dilution of primary antibodies: Anti GFP (Invitrogen, A10262) 1:200, Anti Cardiac Troponin I 1:100 (AbCam ab19615), Anti PDGFRα (R&D systems AF1062) 20 µg/ml, Anti α-actin SMC (AbCam 5694) 1:50, Anti CD31 (BD Pharmigen 553370), 1:50.

Secondary antibodies dilution were used as follows : Anti chicken FITC (Invitrogen, A16131) 1:500, Anti mouse Alexa Fluor 568 (Invitrogen, A10037) 1:200, Anti goat Alexa Fluor 633 (Invitrogen, A21082) 1:200, Anti rat Alexa Fluor 633 (Invitrogen, A21094) 1:200.

Myocyte and Fibroblast Isolation

Adult myocytes were isolated as described previously.[5, 6] Following anesthesia, the heart was excised and the aorta was cannulated with a 20-gauge needle then mounted on the perfusion apparatus. The perfusion solution was composed of Joklik's minimum essential medium containing (in mmol/L) 113 NaCl, 4.7 KCL, 0.6 KH2PO2, 0.6 Na2PO4, 1.2 MgSO4, 0.5 MgCl2, 10 HEPES, 20 D-glucose, 30 taurine, 2.0 carnitine,

2.0 creatine, and 20 mM Ca21 at pH 7.4. The aorta was perfused for 2–3 min, then 150 units/ml of type-II collagenase (Worthington) was added and perfused for 15 min. The temperature of perfusate was maintained at 34 °C and all solutions were continuously bubbled with 95% 02, 5% CO2. LV tissue was separated from the great vessels, atria and right ventricle, minced, and allowed to digest in perfusate for 15 min. The digested heart was filtered through 200 mm nylon mesh, placed in a conical tube, and spun at 25 *x g* to allow viable myocytes to settle. The supernatant was centrifuged at 250 *x g* in order to pellet down the cardiac fibroblast. Cardiomyocytes and fibroblast were placed in confocal dishes for immunostaining experiments.

Western Blotting

Western blot analysis was performed as previously described [7]. Total cell lysate and exosome lysate were performed using NP-40 lysis buffer containing 20 mmol/L Tris (pH 7.4), 137 mmol/L NaCl, 1% NP-40, 20% glycerol, 10 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L Na3VO4, 10 mmol/L NaF, aprotinin (2.5 mg/ml), and leupeptin (2.5 mg/ml). Protein concentrations were assayed with Bio-Rad protein assay reagent, and 50 mg of protein was denatured by heating at 95°C for 5 min before resolving by SDS–polyacrylamide gel electrophoresis. Immunoblotting for ERK and phosphorylated ERK in HEK 293 cells after AngII (1μmol/L) stimulation was performed as previously described [8]. The following dilutions of primary antibody were used: total ERK (Millipore), 1:3000; ERK1/2 (Cell Signaling), 1:1200; CD9 (Millipore) 1:1000; Calnexin (AbCam) 1:1000; CD63 (Santa Cruz) 1:1000; GAPDH (Santa Cruz) 1:1000 ; β-arrestin 2 (Lefkowitz laboratory) 1:1000. Detection was carried out by ECL (Amersham

Biosciences). Densitometric analysis was performed with Bio-Rad Fluoro-S Multi-Image software.

β-arrestin 2 siRNA silencing in AT1R-YFP cells

β-arrestin-targeting siRNA 21-nucleotide sequence was customized and bought from Quiagen as follows: β-Arrestin1 siRNA: 5'AAAGCCTTCTGCGCGGAGAAT-3' ; β-Arrestin2 siRNA:5'AAGGACCGCAAAGTGTTTGTG-3', β-Arrestin1&2 siRNA:5'AAACCTGCGCCTTCCGCTATG-3' and Control siRNA: 5'-AATTCTCCGAACGTGTCACGT-3'. Cells were plated at 30 to 40% confluence in 10-cm dishes. siRNA (20 µg) was used with GeneSilencer Transfection reagent (Gene Therapy Systems). All experiments were performed 60 to 72 hours after siRNA transfection. Cells were serum-starved for 1 hour before stimulation.

Exosomes isolation from Citrate plasma or serum of wild type mice

Citrate plasma and serum were kept on ice upon blood collection to minimize platelets activation then stored at -80° C after centrifugation. Exosomes were purified, modifying previous methods [9, 10], by differential centrifugation at 4 °C, starting with a centrifugation at 1000 x g (15 min) and followed by centrifugations at 12,000 x g (20 min), 18,000–20,000 x g (20 min). The resulting supernatant was filtered through a 0.22 µm filter to obtain a solution free of cell debris, apoptotic bodies and microvesicles. Purified exosomes were then collected by ultracentrifugation at 100,000 x g (70 min) using a Ti70 rotor in a Beckman ultracentrifuge and resuspended in 100 µl of 1 X PBS.

Statistical analysis

Data are expressed as median with 1st and 3rd quartile. Statistical significance was determined by Kruskal-Wallis. Correction for multiple comparisons was made using a Dunn's correction. Analysis of blood pressure (Supplemental Material) comparing Basal vs Ang II was performed using Wilcoxon signed rank test for repeated measurements within each group, while comparison of % changes of hemodynamic parameters between 3 or more independent groups was assessed by Kruskal Wallis. QT-PCR data were analyzed by Mann-Whitney t test (Supplemental Material). A threshold value of $p<0.05$ was considered statistically significant. All analyses were performed with GraphPad Prism version 6.01.

Supplemental References

1. Wei H, Ahn S, Shenoy SK, Karnik SS, Hunyady L, Luttrell LM and Lefkowitz RJ. Independent beta-arrestin 2 and G protein-mediated pathways for angiotensin II activation of extracellular signal-regulated kinases 1 and 2. *Proc Natl Acad Sci U S A*. 2003;100:10782-7.
2. Dragovic RA, Gardiner C, Brooks AS, Tannetta DS, Ferguson DJ, Hole P, Carr B, Redman CW, Harris AL, Dobson PJ, Harrison P and Sargent IL. Sizing and phenotyping of cellular vesicles using Nanoparticle Tracking Analysis. *Nanomedicine*. 2011;7:780-8.
3. Naga Prasad SV, Barak LS, Rapacciuolo A, Caron MG and Rockman HA. Agonist-dependent recruitment of phosphoinositide 3-kinase to the membrane by beta-adrenergic receptor kinase 1. A role in receptor sequestration. *J Biol Chem*. 2001;276:18953-9.
4. Rockman HA, Ross RS, Harris AN, Knowlton KU, Steinhelper ME, Field LJ, Ross J, Jr. and Chien KR. Segregation of atrial-specific and inducible expression of an atrial natriuretic factor transgene in an in vivo murine model of cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 1991;88:8277-81.
5. Rockman HA, Choi DJ, Akhter SA, Jaber M, Giros B, Lefkowitz RJ, Caron MG and Koch WJ. Control of myocardial contractile function by the level of beta-adrenergic receptor kinase 1 in gene-targeted mice. *J Biol Chem*. 1998;273:18180-4.
6. Barki-Harrington L, Luttrell LM and Rockman HA. Dual inhibition of beta-adrenergic and angiotensin II receptors by a single antagonist: a functional role for receptor-receptor interaction in vivo. *Circulation*. 2003;108:1611-8.
7. Rakesh K, Yoo B, Kim IM, Salazar N, Kim KS and Rockman HA. beta-Arrestin-biased agonism of the angiotensin receptor induced by mechanical stress. *Sci Signal*. 2010;3:ra46.
8. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS, Smithies O and Rockman HA. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest*. 2006;116:1547-60.
9. Savina A, Furlan M, Vidal M and Colombo MI. Exosome release is regulated by a calcium-dependent mechanism in K562 cells. *J Biol Chem*. 2003;278:20083-90.
10. Gupta S and Knowlton AA. HSP60 trafficking in adult cardiac myocytes: role of the exosomal pathway. *Am J Physiol Heart Circ Physiol*. 2007;292:H3052-6.

Supplemental Figures and Tables:

Figure S1

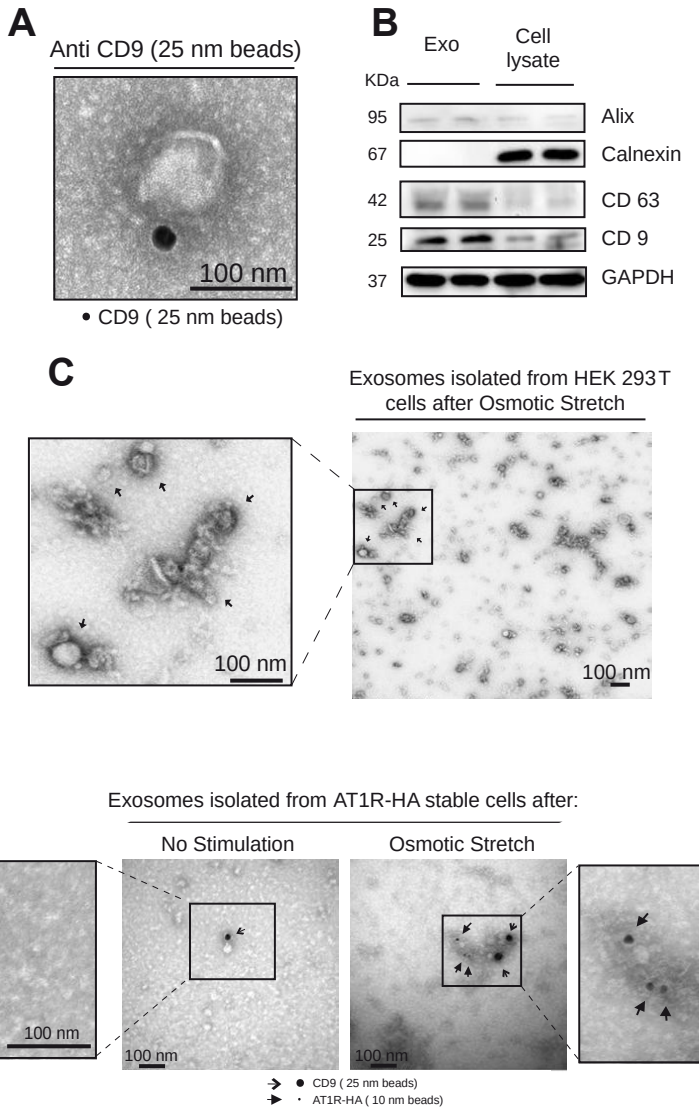

A
Anti CD9 (25 nm beads)
100 nm
• CD9 (25 nm beads)
B
Exo
Cell lysate
KDa
95
67
42
25
37
Alix
Calnexin
CD 63
CD 9
GAPDH
C
Exosomes isolated from HEK 293T cells after Osmotic Stretch
100 nm
100 nm
D
Exosomes isolated from AT1R-HA stable cells after:
No Stimulation
Osmotic Stretch
100 nm
100 nm
100 nm
100 nm
CD9 (25 nm beads)
AT1R-HA (10 nm beads)
E
Analysis of size distribution in exosomes with different conditions
Osmotic Stretch media; Mean size = 64.2 ± 0.7 (nm) (N=6)
Serum of mice after TAC 7 days; Mean size = 60.7 ± 0.7 (nm) (N=6)
Exosome Number (% of Total)
0
5
10
15
20
25
10
30
50
70
90
110
130
150
170
190
210
230
Diameter (nm)

Figure S2

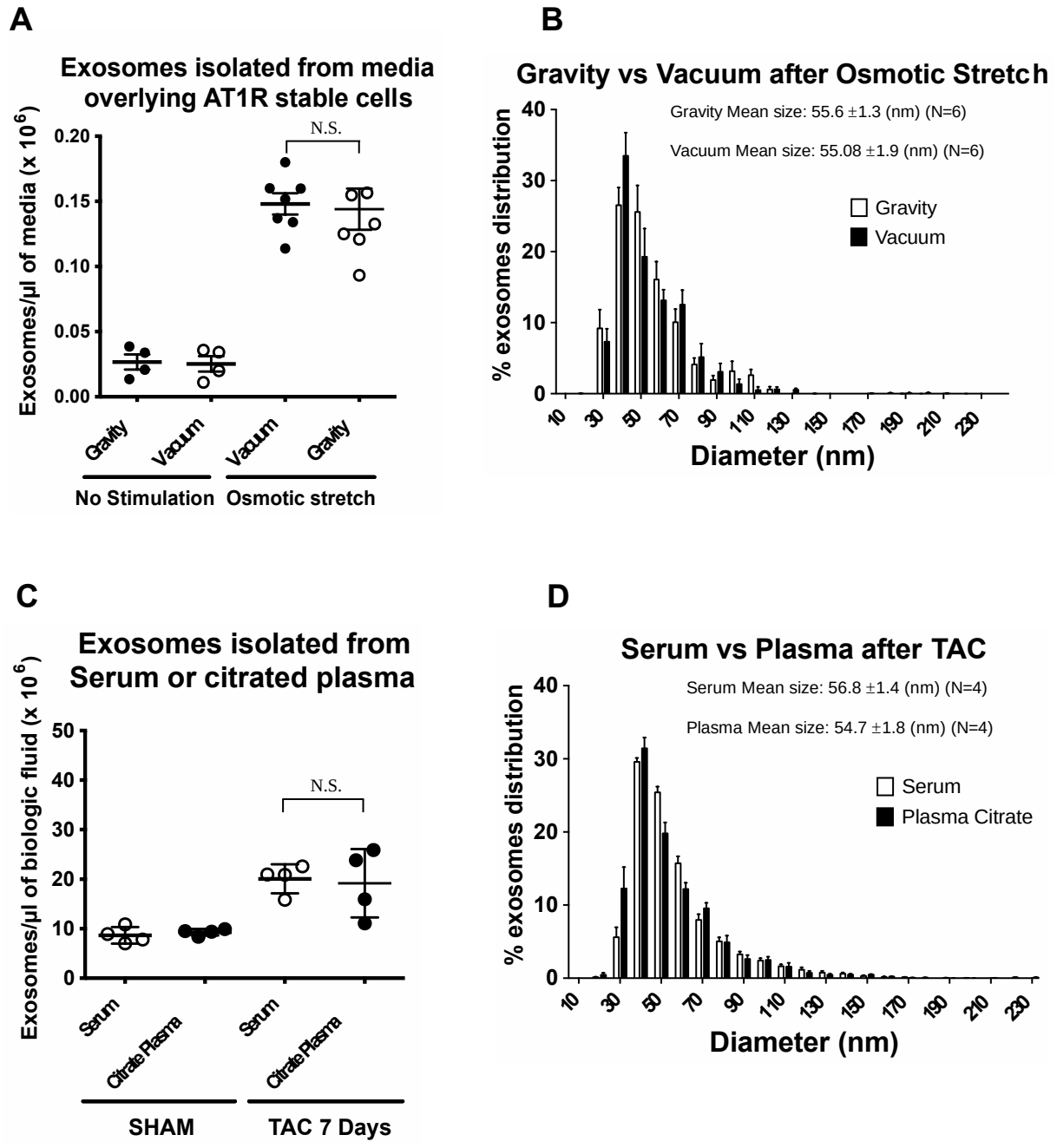

A
Exosomes isolated from media overlying AT1R stable cells
Exosomes/µl of media (x 10^6)
0.20
0.15
0.10
0.05
0.00
N.S.
Gravity
Vacuum
Vacuum
Gravity
No Stimulation
Osmotic stretch
B
Gravity vs Vacuum after Osmotic Stretch
Gravity Mean size: 55.6 ±1.3 (nm) (N=6)
Vacuum Mean size: 55.08 ±1.9 (nm) (N=6)
% exosomes distribution
40
30
20
10
0
Gravity
Vacuum
10
30
50
70
90
110
130
150
170
190
210
230
Diameter (nm)
C
Exosomes isolated from Serum or citrated plasma
Exosomes/µl of biologic fluid (x 10^6)
50
40
30
20
10
0
N.S.
Serum
Citrate Plasma
Serum
Citrate Plasma
SHAM
TAC 7 Days
D
Serum vs Plasma after TAC
Serum Mean size: 56.8 ±1.4 (nm) (N=4)
Plasma Mean size: 54.7 ±1.8 (nm) (N=4)
% exosomes distribution
40
30
20
10
0
Serum
Plasma Citrate
10
30
50
70
90
110
130
150
170
190
210
230
Diameter (nm)

Figure S3

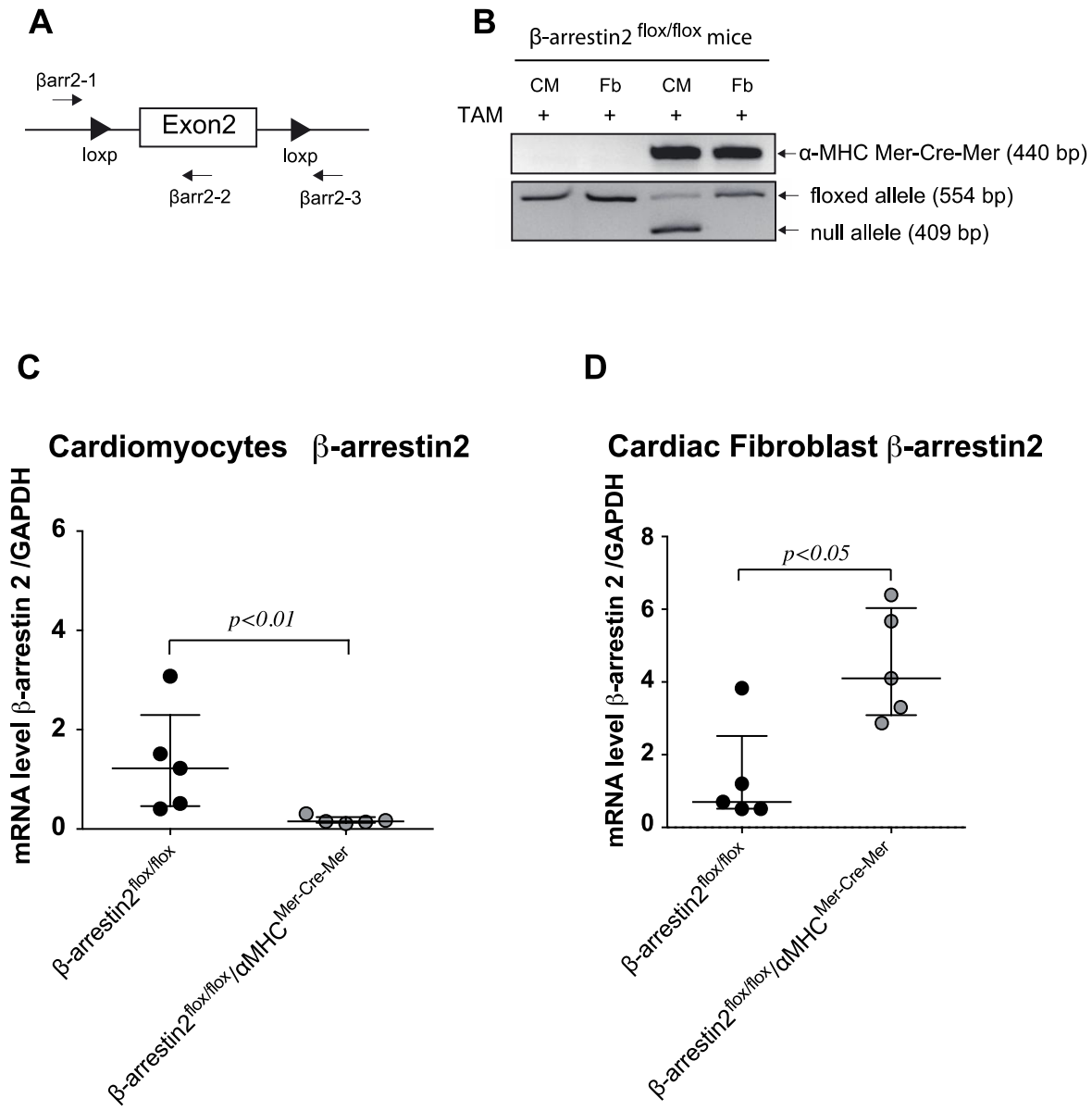

A
βarr2-1
Exon2
loxp
loxp
βarr2-2
βarr2-3
B
β-arrestin2 flox/flox mice
CM
Fb
CM
Fb
TAM
+
+
+
+
α-MHC Mer-Cre-Mer (440 bp)
floxed allele (554 bp)
null allele (409 bp)
C
Cardiomyocytes β-arrestin2
mRNA level β-arrestin 2 /GAPDH
p<0.01
β-arrestin2flox/flox
β-arrestin2flox/flox/αMHCMer-Cre-Mer
D
Cardiac Fibroblast β-arrestin2
mRNA level β-arrestin 2 /GAPDH
p<0.05
β-arrestin2flox/flox
β-arrestin2flox/flox/αMHCMer-Cre-Mer

Figure S4

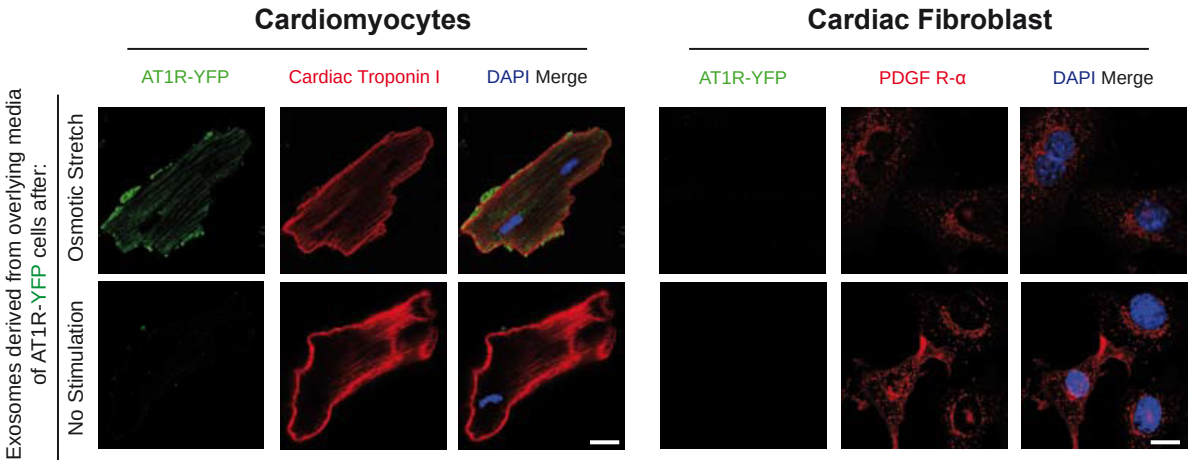
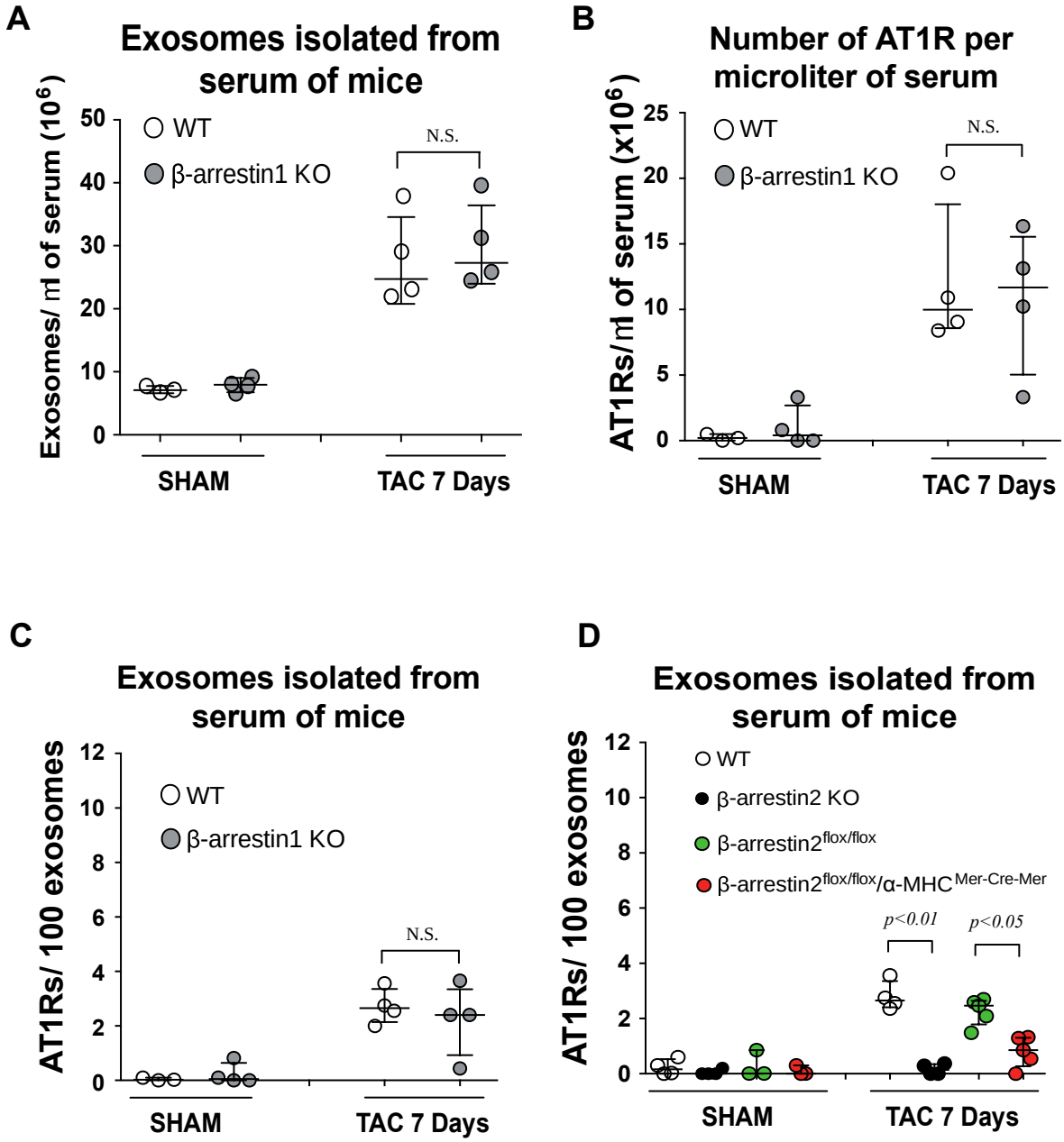

Cardiomyocytes
Cardiac Fibroblast
AT1R-YFP
Cardiac Troponin I
DAPI Merge
AT1R-YFP
PDGF R-α
DAPI Merge
Exosomes derived from overlying media of AT1R-YFP cells after:
Osmotic Stretch
No Stimulation

Figure S5


A
Exosomes isolated from serum of mice
Exosomes/ ml of serum (10^6)
WT
β-arrestin1 KO
N.S.
SHAM
TAC 7 Days
B
Number of AT1R per microliter of serum
AT1Rs/ml of serum (x10^6)
WT
β-arrestin1 KO
N.S.
SHAM
TAC 7 Days
C
Exosomes isolated from serum of mice
AT1Rs/ 100 exosomes
WT
β-arrestin1 KO
N.S.
SHAM
TAC 7 Days
D
Exosomes isolated from serum of mice
AT1Rs/ 100 exosomes
WT
β-arrestin2 KO
β-arrestin2flox/flox
β-arrestin2flox/flox/α-MHCMer-Cre-Mer
p<0.01
p<0.05
SHAM
TAC 7 Days

Supplemental Figure Legends

Figure S1: Characterization of exosomes isolated from cell media and mice serum.

A) Isolated exosomes show CD9 on the vesicle surface following immune-gold labeling and visualization with T.E.M. (scale bar is 100 nm). **B)** Biochemical characterization of exosome and cell lysate blotted for the typical exosome markers Alix, CD63, CD9 and the cellular marker as Calnexin. **C)** Exosomes derived from conditioned HEK 293T cells showed the typical cup shape and ranged in size between 30 and 100 nm (scale bare = 100 nm). **D)** T.E.M. was used to identify CD9 and AT1Rs on the surface exosomes derived from conditioned media overlying HEK 293T AT1R-HA stable transfected cells before and after osmotic stretch. Exosomes derived from HEK 293T AT1R-HA stable transfected cells after osmotic stretch exhibited both CD9 (25 nm) and AT1R-HA (10 nm) on their surface. **E)** Size distribution analysis of exosomes isolated from media of cells after Osmotic stretch and from serum of mice after TAC 7 days. Exosomes isolated from different conditions showed a similar size distribution with a peak at around 60 nm. Data expressed as mean ± SEM.

Figure S2: Different methods of isolation do not affect exosomes concentration and size distribution.

A) Exosomes isolated from AT1R-HA stable expressing cells using Gravity or Vacuum filtration step showed similar concentration in no stimulation (~30.000 exosomes/µl) and after osmotic stretch condition (~150.000 exosomes/µl). **B)** Exosomes size distribution analysis showed a similar range size distribution of exosomes isolated with gravity

filtration step and exosomes isolated with vacuum filtration step. **C)** Exosomes isolate from citrate plasma showed a concentration similar to exosomes isolated from serum without citrate in SHAM and TAC condition. **D)** Circulating exosomes isolated from citrate plasma or serum showed a similar size distribution.

Figure S3: β-arrestin2$^{flox/flox}$ mice characterization:

A) PCR strategy to detect allele recombination after Tamoxifen treatment of β-arrestin 2$^{flox/flox}$ /αMHC $^{Mer\text{-}Cre\text{-}Mer}$. The recombination primers were used as follows: βarr2-1: 5'-GCTCCCTAACCAGGCAAGAG-3', βarr2-2: 5'-TGGGAAAGGACCCCAGGTAA-3', βarr2-3: 5'-ACGGAGGGTCGGTACACATA-3'. Floxed gene band is 554 bp and null gene band is 409 bp. **C)** Representative recombination PCR of cardiomyocytes (CM) and Fibroblast (Fb) showing the presence of null allele only in CM of β-arrestin 2$^{flox/flox}$ /αMHC $^{Mer\text{-}Cre\text{-}Mer}$ but not β-arrestin 2$^{flox/flox}$ after Tamoxifen treatment. **C)** Cardiomyocytes specific knock out of β-arrestin 2$^{flox/flox}$ /αMHC $^{Mer\text{-}Cre\text{-}Mer}$ mice was confirmed by Real time PCR using Taqman probes showing ~80% reduction of β-arrestin2. **D)** Real Time PCR shows upregulation of β-arrestin2 in cardiac fibroblasts of β-arrestin 2$^{flox/flox}$ /αMHC $^{Mer\text{-}Cre\text{-}Mer}$. Data expressed as median with 1st and 3rd quartile. Statistical significance was determined by Mann-Whitney t-Test.

Figure S4: Circulating AT1Rs deposit in cardiomyocytes rather than fibroblast

Cardiomyocytes and cardiac fibroblasts were isolated from wild type hearts 24 hours after the intravenous injection of exosomes and immunostained for different cellular markers as indicated. AT1R green fluorescence was detected using GFP antibody

recognizing YFP. Cardiomyocytes were detected using cardiac troponin I (left), while fibroblast were positively stained for PDGFR-α (right). Consistent with the histology data, only cardiomyocytes were positively stained for YFP-AT1R. Cardiomyocytes were fixed and processed for the staining the day after of cell isolation. Cardiac fibroblasts were fixed and stained after 4 days in culture in order to obtain a sufficient cell confluence to perform confocal microscopy. Scale bar = 15 µm; N= 4.

Figure S5: β-arrestin1 deletion has no effect on circulating exosomes containing AT1Rs secretion after TAC

A) Exosomes concentration of circulating exosomes in serum of β-arrestin1 KO was similar to exosomes isolated from serum of WT mice. **B)** The amount of circulating AT1Rs within exosome secreted after TAC in serum was similar between β-arrestin1 KO and WT mice suggesting that the isoform 1 is not important for extracellular trafficking of AT1R. **C)** AT1R density in exosomes of β-arrestin 1 KO after TAC was similar to AT1R density in WT exosomes after TAC. **D)** Exosomes isolated from β-arrestin2 KO, global and cardiac specific, showed a blunted AT1R density after 1 week TAC suggesting that the absence of β-arrestin 2 impaired the AT1R packaging in exosomes during mechanical stress and circulating AT1Rs are secreted mostly by cardiomyocytes. Data expressed as median with 1^{st} and 3^{rd} quartile. Statistical significance was determined by Kruskal-Wallis with Dunn's post hoc test. p = NS β-arrestin$2^{flox/flox}$ /αMHC $^{Mer\text{-}Cre\text{-}Mer}$ vs. β-arrestin2 KO

Table S1. Hemodynamic parameters

Genotype		WT	AT1R-KO				
				Exosomes isolated from AT1R stable cells		Exosomes isolated from WT mice serum	
		No Exosomes	No Exosomes	NS	Osmotic Stretch	Sham	TAC
		N=4	N=5	N=4	N=8	N=4	N=7
Systolic BP (mm Hg)	Basal	130.1 (111.3-134.6)	135.6 (126.9-145.8)	128.4 (116.6-146.5)	133.1 (118.9-134.7)	138.2 (126.2-145.4)	134.3 (128.9-139.1)
	Ang II	179.5 (179.5-183.2)	146.4 (135.8-159.8)	140.4 (128.9-157.5)	168.7 (146.7-179.0)*	147.6 (138.1-154.7)	172.5 (165.1-174.0)*
% increase of SBP		41.7 (41.7-53.6)	7.6 (5.0-11.3)	7.6 (7.0-8.7)	28.9 (22.3-36.4)**†	7.3 (4.3-12.9)	28.6 (22.3-28.9)**††
Diastolic BP (mm Hg)	Basal	109.7 (93.2-118.2)	107.6 (95.4-117.9)	114.7 (111.1-117.3)	102.8 (100.5-107.1)	121.1 (110.0-124.0)	101.9 (98.6-103.6)
	Ang II	159.2 (148.1-163.1)	116.7 (103.3-130.3)	124.8 (121.4-125.5)	118.5 (108.5-121.5)*	130.0 (121.1-130.9)	109.4 (108.1-113.2)*
% increase of DBP		47.2 (47.2-54.8)	10.7 (8.3-13.4)	6.4 (6.0-7.9)	14.3 (8.4-23.2)	7.6 (5.7-10.3)	8.2± (6.4-10.0)
Mean BP (mm Hg)	Basal	117.1 (99.3-124)	119.0 (114.7-122.6)	124.1 (119.0-128.2)	116.7 (107.1-122.8)	126.8 (116.4-131.1)	113.2 (110.0-115.4)
	Ang II	164.7 (156.8-169.2)	126.3 (119.9-135.8)	130.5 (124.6-135.8)	137.2 (126.6-134.9)	135.8 (125.9-138.0)	129.8 (126.8-131.6)

	% increase of MBP	29.4 (29.4-33.5)	10.9 (8.0-12.2)	5.6 (-3.4-1.5)	13.5 (10.5-21.0)**†	5.0 (4.7-6.3)	13.1 (12.1-14.4)**
Heart Rate (BPM)	**Basal**	341.6 (300.1-374.0)	403.3 (345.8-459.8)	376.8 (368.8-397.8)	392.0 (342.9-412.1)	403.5 (368.5-426.3)	381.5 (347.5-382.2)
	Ang II	445.4 (422.6-467.8)	400.0 (335.0-465.7)	377.9 (362.5-400.2)	404.6 (346.7-412.5)	407.4 (372.2-427.7)	389.7 (351.5-396.5)
	% increase of HR	35.1 (35.1-39.6)	-0.6 (-1.2-0.6)	-0.5 (-3.4-1.5)	1.4 (-1.7-2.6)	0.4 (-0.2-0.8)	2.4 (1.3-2.8)

Table S1: Hemodynamic parameter changes before and after Ang II infusion.

Hemodynamic parameters of WT mice and AT1R-KO mice used in the in vivo exosome injection experiments. Peripheral blood pressure was recorded for 3 minutes before AngII infusion (Basal) and for 5 minutes after AngII infusion. Data expressed as median with 1st and 3rd quartile. * $p<0.05$ Basal vs. AngII determined by Wilcoxon signed rank test for repeated measurements in each independent group. ** $p<0.05$ vs control group AT1R-KO (No Exo), † $p<0.05$ AT1R-KO (NS) vs. AT1R-KO (Osmotic Stretch), †† $p<0.05$ AT1R-KO (SHAM) vs. AT1R-KO (TAC), statistical significance determined by Kruskal-Wallis with Dunn's test for multi comparison.

Table S2. AT1R density in tissues after exosome injection

Genotype	AT1R-KO					WT
		Exosomes Donors				
Recipient Tissue	No Exosomes	Exosomes isolated from AT1R stable cells after:		Exosomes isolated from WT mice serum after:		No Exosomes
		NS	Osmotic Stretch	Sham	TAC	
	N=5	N=4	N=8	N=4	N=7	N=4
Heart (fmol/mg)	N.D.	N.D.	8.3±2.0	N.D.	8.9±1.9	15.0±4.0
Skeletal Muscle (fmol/mg)	N.D.	N.D.	10.8±2.4	N.D.	8.8±2.4	19.0±2.5
Kidney (fmol/mg)	N.D.	N.D.	N.D.	N.D.	N.D.	12.5±3.3
Lung (fmol/mg)	N.D.	N.D.	N.D.	N.D.	N.D.	7.7±1.1

N.D.= Not Detectable

Table S2: Distribution of AT1R in membrane fractions of AT1R-KO mice derived from tissue biopsy: Heart, kidney, lung and skeletal muscle were removed following injection of exosomes and AT1R density was determined by radioligand binding. AT1Rs were detected in the heart and skeletal muscle, but not the lung or kidney of AT1R KO mice. Mean ± SEM.