ONLINE SUPPLEMENT

Adenosine Receptors Influence Hypertension in Dahl Salt-Sensitive Rats: Dependence on Receptor Subtype, Salt Diet, and Sex

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Running title: *Adenosine Receptors in Dahl SS Rats*

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Western Blotting for Adenosine Receptors. Kidneys were obtained from wildtype, A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats and were used to obtain tissue from the cortex, medulla, and junction between the two. Proteins were extracted using Tissue Protein Extraction Reagent (Pierce Biotechnology Inc; Rockford, IL). Total protein was measured (Pierce BCA Protein Assay Kit), and protein extracts were boiled for 5 minutes in Laemmli buffer. SDS-polyacrylamide-gel electrophoresis was performed on polyacrylamide gels (8-16%) with 30 µg of protein per lane. Proteins were transferred to PDVF membranes. Membranes were blocked in tris-buffered saline tween-20 containing 5% milk and probed with anti-A_1 receptor (rabbit polyclonal; 1:500; Sigma-Aldrich; St. Louis, MO; catalogue number A-268), anti-A_{2A} receptor (rabbit monoclonal; 1:1000; Abcam; Cambridge, MA; catalogue number ab169756), or anti-A_{2B} receptor (rabbit polyclonal; 1:1000; Origene; Rockville, MD; catalogue number TA349146) primary antibodies. Membranes were exposed to horseradish-peroxidase-conjugated-goat anti-rabbit antibody (1:2500; Pierce) and visualized with a Konica Minolta Medical Film Processor (Wayne, NJ) using luminal-based enhanced chemiluminescence substrate (Supersignal West Dura Extended Duration Substrate; Pierce).

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR) for Assessment of Adenosine Receptor RNA Expression. Total RNA was isolated from brains and kidneys of male and female wildtype, A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats (n=3 for each of the 8 sex/genotype combinations, total of 24 rats) using TRIZOL Reagent (Thermo Fisher Scientific; Waltham, MA) according to the manufacturer's instructions. The cDNA was synthesized using iScript™ cDNA synthesis kit (Bio-Rad). qPCR analysis was performed using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) in the Applied Biosystems QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). Primers were: for *Adora1* 5'-tgctgtggatcgatacctcc-3' (forward) and 5'-tcttgctctaccacactcagg-3' (reverse); for *Adora2a* 5'-tgtacatcacggtggagctg-3' (forward) and 5'-gatggtgatagcgaagggga-3' (reverse); for *Adora2b* 5'-tactttctggtgtccctggc-3' (forward) and 5'-cttgctcgtgttccagtgac-3' (reverse); for β-actin 5'-actcttccagccttccttc-3' (forward) and 5'-atctccttctgcatcctgtc-3' (revserse). Threshold cycle (Ct) for β-actin was subtracted from Ct for target to calculate $2^{\Delta Ct}$, and results were expressed as % of control with control being the $2^{\Delta Ct}$ for an arbitrarily chosen wildtype rat.

Acute Hemodynamic Effects of Intravenous Administration of Adenosine Receptor Agonists. Wildtype, A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats (approximately 12 weeks of age) were anesthetized with Inactin (90 mg/kg. i.p.), and body temperature was monitored with a rectal temperature probe and maintained at 37ºC with a temperature-controlled and heated surgical table and heat lamp. A polyethylene (PE)-240 cannula was placed in the trachea to facilitate respiration, and a PE-50 cannula was placed in the jugular vein for administration of adenosine receptor agonists and for an infusion of 0.9% saline (50 µl/min) to maintain hemodynamic stability. Next, a PE-50 cannula was inserted into the carotid artery for measurement of arterial blood pressure and heart rate using a pressure transducer (Micro-Med, Inc.; Louisville, KY). Also, a 1 mm or 1.5 mm transit-time flow probe was placed on the left renal artery or mesenteric artery, respectively, for measurement of renal (RBF) or mesenteric (MBF) blood flow

using a Transonic T402 transit-time flowmeter (Transonic Systems Inc; Ithaca, NY). Hemodynamic variables were recorded using the PowerLab data acquisition system and LabChart software (ADInstruments, Colorado Springs, CO).

Effects of Selective A_{2B} Receptor Agonist on Proliferation of Cardiac Fibroblasts from Wildtype, A_1KO, A_{2A}KO, and A_{2B}KO Rats. Cardiac fibroblasts (CFs) were isolated from hearts and cultured as previously described[1]. CFs from wildtype, A_1KO, A_{2A}KO, and A_{2B}KO rats were growth arrested for 48 hours in 0.25% fetal bovine serum, and then stimulated with 2.5% fetal bovine serum containing various concentrations of the selective A_{2B} receptor agonist Bay60-6583[2]. The medium was changed daily, and cells were counted after 4 days as previously described[3].

1. Dubey RK, Gillespie DG, Mi Z, Jackson EK. Exogenous and endogenous adenosine inhibits fetal calf serum–induced growth of rat cardiac fibroblasts: role of A_{2B} receptors. *Circulation*. 1997;96:2656-2666.

2. Schiedel AC, Lacher SK, Linnemann C, Knolle PA, Muller CE. Antiproliferative effects of selective adenosine receptor agonists and antagonists on human lymphocytes: evidence for receptor-independent mechanisms. *Purinergic Signal*. 2013;9:351-365.

3. Zhu X, Gillespie DG, Jackson EK. NPY_{1-36} and PYY_{1-36} activate cardiac fibroblasts: an effect enhanced by genetic hypertension and inhibition of dipeptidyl peptidase 4. *Am J Physiol Heart Circ Physiol*. 2015;309:H1528-1542.

A **Wildtype Genotyping Primers: Amplicon = 176 bp**

Forward Primer: ***TCGTGTCACTGGCGGTAGCT***
Reverse Primer: ***GTATCGATCCACAGCAATGG***
Wildtype sequence in Exon 1: 1406 **ccattgctgtgg**

1201 tgggctgtga aggtgaacca ggcacttcgc gatgccacct tctgcttca***T CGTGTCACTG***
1261 ***GCGGTAGCT***g atgtggccgt tggcgccctg gtcatcccac tggccatcct tatcaacatt
1321 gggccacaga cctacttcca cacctgcctc atggtggcct gccctgtcct catcctcacc
1381 cagagctcca ttctggctct gctcg***CCATT GCTGTGGATC GATAC***ctccg agtcaagatc
1441 cctctccggt gagtgcacag cagcccaagg tactctgtga aacccgatgt tgggtggctt
1501 gggggatgag ctagagaaga cagaagtgca taagccccca agtcagtcag gtattctctg
1561 gccatttgga tcccagccca catctccttc ctgggctctg tgtggggatg ctcagcagag
1621 ctgcggctgg agaagcagga gggggctggg tgtccagaga attaccatcc cgacagtgcc
1681 catgacctca gtgctgaagc ctctgcaacc agagggtctt ggggagcagg tgaagtaggg

B **A_1 Receptor Knockout Genotyping Primers: Amplicon = 286 bp**

Forward Primer: ***GCTCGATCCAATCGATACC***
Reverse Primer: ***CACTGAGGTCATGGGCACTG***
Deletion/Insertion in Exon 1: 1406 **atccaxxxxxxx**

1201 tgggctgtga aggtgaacca ggcacttcgc gatgccacct tctgcttcat cgtgtcactg
1261 gcggtagctg atgtggccgt tggcgccctg gtcatcccac tggccatcct tatcaacatt
1321 gggccacaga cctacttcca cacctgcctc atggtggcct gccctgtcct catcctcacc
1381 cagagctcca ttctggctct ***GCTCG****ATCCA* **xxxxxxx*****ATC GATACC***tccg agtcaagatc
1441 cctctccggt gagtgcacag cagcccaagg tactctgtga aacccgatgt tgggtggctt
1501 gggggatgag ctagagaaga cagaagtgca taagccccca agtcagtcag gtattctctg
1561 gccatttgga tcccagccca catctccttc ctgggctctg tgtggggatg ctcagcagag
1621 ctgcggctgg agaagcagga gggggctggg tgtccagaga attaccatcc cga***CAGTGCC***
1681 ***CATGACCTCA GTG***ctgaagc ctctgcaacc agagggtctt ggggagcagg tgaagtaggg

Figure S1. Denoted in blue letters are the wildtype sequence (A) and corresponding mutated sequence (B) in exon 1 in the A_1 receptor gene. CRIPSR targeting resulted in a 12 bp deletion and 5 bp insertion for a net 7-bp deletion in exon 1. Also shown are the forward and reverse PCR primers for genotyping, the sites in exon 1 targeted by the PCR primers (shown in bold upper case), and the amplicon size.

A **Wildtype Genotyping Primers: Amplicon = 394 bp**

Forward Primer: ***ACGTCTTCGGGGAGCTCTC***
Reverse Primer: ***AACAGGCGAAGAAGAGGCA***
Wildtype sequence in Exon 2: 8715 **ctgctcccaccatgggctcctcggtgtacatcacgg tggagctggccatcgctgtgctggccatcctgggcaacgtgctcgtgtgctgggccgtgtgg**

8461 cactgagact ctctctttcc aggtatctca gaaccctgaa gccgagagga gccaggccag
8521 atcccctgga gaagtcatga ttttgggaga tgcagagtcg tctgtgaga***A CGTCTTCGGG***
8581 ***GAGCTCTC***tc tgggaacgct cgtggctctt gtgaggaaag ggctgcgggg cttgggtggg
8641 tggacggctg ggctgcagct atggaccgag agctggccca ggcctgcatc cctgctgagc
8701 ctgcccaagt gtgg**ctgctc ccaccatggg ctcctcggtg tacatcacgg tggagctggc**
8761 **catcgctgtg ctggccatcc tgggcaacgt gctcgtgtgc tgggccgtgt gg**atcaacag
8821 taacctgcag aacgtcacca acttctttgt ggtatcgctg gcggcggctg acattgcagt
8881 gggtgtgctc gccatcccct tcgctatcac catcagcacc ggcttctgcg ccgcctgcca
8941 cggc***TGCCTC TTCTTCGCCT GTT***ttgtcct ggtcctcacg cagagttcca tctttagcct
9001 cttggctatc gccatcgacc gctacatcgc catccgaatt ccactccggt gagccagggg

B **A_{2A} Knockout Genotyping Primers: Amplicon = 296 bp**

Forward Primer: ***ACGTCTTCGGGGAGCTCTC***
Reverse Primer: ***AACAGGCGAAGAAGAGGCA***
Deletion in Exon 2: 8715 **xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx**

8461 cactgagact ctctctttcc aggtatctca gaaccctgaa gccgagagga gccaggccag
8521 atcccctgga gaagtcatga ttttgggaga tgcagagtcg tctgtgaga***A CGTCTTCGGG***
8581 ***GAGCTCTC***tc tgggaacgct cgtggctctt gtgaggaaag ggctgcgggg cttgggtggg
8641 tggacggctg ggctgcagct atggaccgag agctggccca ggcctgcatc cctgctgagc
8701 ctgcccaagt gtgg**xxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx**
8761 **xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xx**atcaacag
8821 taacctgcag aacgtcacca acttctttgt ggtatcgctg gcggcggctg acattgcagt
8881 gggtgtgctc gccatcccct tcgctatcac catcagcacc ggcttctgcg ccgcctgcca
8941 cggc***TGCCTC TTCTTCGCCT GTT***ttgtcct ggtcctcacg cagagttcca tctttagcct
9001 cttggctatc gccatcgacc gctacatcgc catccgaatt ccactccggt gagccagggg

Figure S2. Denoted in blue letters are the wildtype sequence (A) and corresponding mutated sequence (B) in exon 2 in the A_{2A} receptor gene. CRIPSR targeting resulted in a 98-bp deletion in exon 2. Also shown are the forward and reverse PCR primers for genotyping, the sites in exon 2 targeted by the PCR primers (shown in bold upper case), and the amplicon size. Note: Exon 1 and part of exon 2 are in the UTR region of the A_{2A} gene and the 98-bp deletion spans the junction between the UTR and the initial part of the protein coding region.

A **Wildtype Genotyping Primers: Amplicon = 323 bp**

Forward Primer: ***ACACAACCCCGGTAGAGGA***
Reverse Primer: ***GATGGAGCTCTGTGTGAGCA***
Wildtype sequence in Exon 1: 96 **gctggcccggccatgcagctagagacgcaggacgcgctgta cgtggcgctggagctggttatcgccgcgctggcagtggcgggcaacgtgctggtgtgcgctgcggtgggagcct cgagtgctttacagacccccaccaactactttctggtgtccctggcg**

1 gggacgcgcg gtctcggcgc tgtggccatg cctggcggca ccttagcggc tgtcctgagc
61 ccg***ACACAAC CCCGGTAGAG GA***ctccccgg gcccg**gctgg cccggccatg cagctagaga**
121 cgcaggacgc gctgtacgtg gcgctggagc tggttatcgc cgcgctggca gtggcgggca
181 acgtgctggt gtgcgctgcg gtgggagcct cgagtgcttt acagaccccc accaactact
241 ttctggtgtc cctggcgacg gcggacgtgg ctgtgggact cttcgccatc ccctttgcca
301 tcaccatcag cctgggcttc tgcacggact ttcacagctg cctcttcctc gcctgcttcg
361 tgctgg***TGCT CACACAGAGC TCCATC***ttta gcctcttggc ggtggctgtc gaccggtatc
421 tggccattcg cgtcccgctc aggtgagact aatctttctt gccttggcca ggttaaagtt

B **A_{2B} Knockout Genotyping Primers: Amplicon = 161 bp**

Forward Primer: ***ACACAACCCCGGTAGAGGA***
Reverse Primer: ***GATGGAGCTCTGTGTGAGCA***
Deletion in Exon 1: 96 **xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx**

1 gggacgcgcg gtctcggcgc tgtggccatg cctggcggca ccttagcggc tgtcctgagc
61 ccg***ACACAAC CCCGGTAGAG GA***ctccccgg gcccg**xxxxx xxxxxxxxxx xxxxxxxxxx**
121 **xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx**
181 **xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx xxxxxxxxxx**
241 **xxxxxxxxxx xxxxxxx**acg gcggacgtgg ctgtgggact cttcgccatc ccctttgcca
301 tcaccatcag cctgggcttc tgcacggact ttcacagctg cctcttcctc gcctgcttcg
361 tgctgg***TGCT CACACAGAGC TCCATC***ttta gcctcttggc ggtggctgtc gaccggtatc
421 tggccattcg cgtcccgctc aggtgagact aatctttctt gccttggcca ggttaaagtt

Figure S3. Denoted in blue letters are the wildtype sequence (A) and corresponding mutated sequence (B) in exon 1 in the A_{2B} receptor gene. ZFNs targeting resulted in a 162-bp deletion in exon 1. Also shown are the forward and reverse PCR primers for genotyping, the sites in exon 1 targeted by the PCR primers (shown in bold upper case), and the amplicon size.

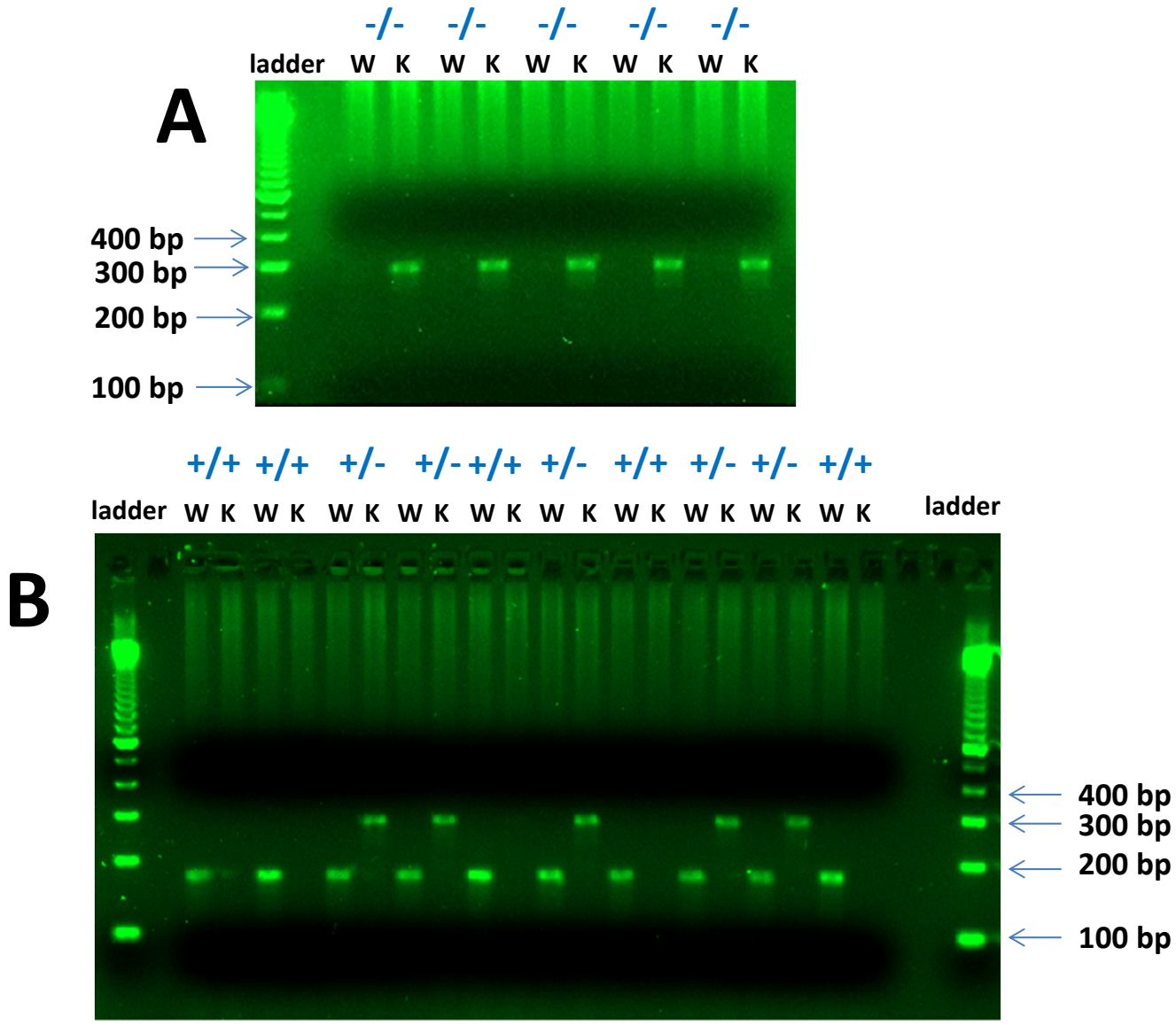
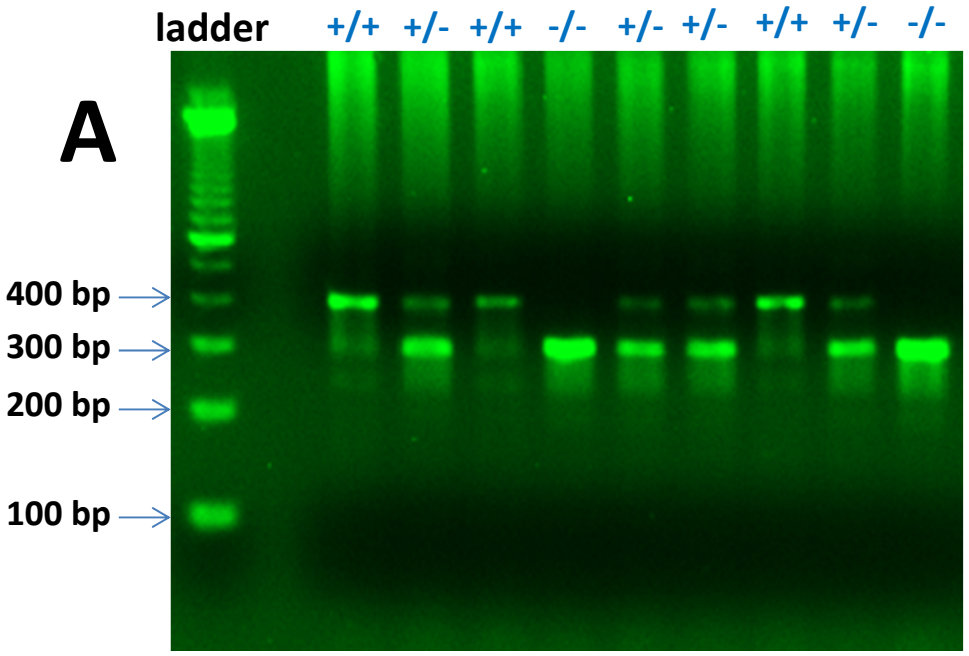

A
-/- -/- -/- -/- -/-
ladder W K W K W K W K W K
400 bp
300 bp
200 bp
100 bp
B
+/+ +/+ +/- +/- +/+ +/- +/+ +/- +/- +/+
ladder W K W K W K W K W K W K W K W K W K W K ladder
400 bp
300 bp
200 bp
100 bp

Figure S4. Figure displays typical genotyping results for wildtype and A_1KO Dahl SS rats using agarose gel electrophoresis of PCR amplicons stained with ethidium bromide and imaged using a Bio-Rad Gel Doc XR+ System. (A) Knockout rats (-/-) yielded a 286-bp band only. (B) Wildtype rats (+/+) yielded a 176-bp band only, and heterozygous rats (+/-) produced both the 176 and 286 bands.

Genotyping of A_{2A} Colony



Genotyping of A_{2B} Colony

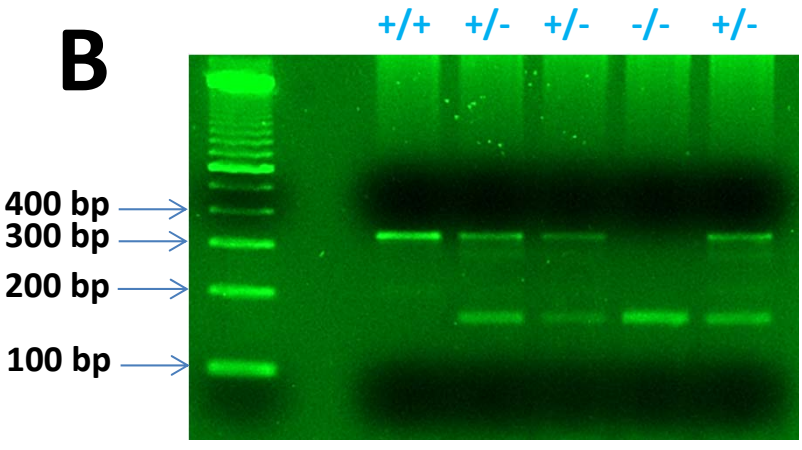


Figure S5. Panel A displays typical genotyping results for wildtype and A_{2A}KO Dahl SS rats using agarose gel electrophoresis of PCR amplicons stained with ethidium bromide and imaged using a Bio-Rad Gel Doc XR+ System. Knockout rats (-/-) yielded a 296-bp band only, wildtype rats (+/+) yielded a 394-bp band only, and heterozygous rats (+/-) produced both the 176-bp and 286-bp bands. Panel B displays typical genotyping results for wildtype and A_{2B}KO Dahl SS rats using the same method. Knockout rats (-/-) yielded a 161-bp band only, wildtype rats (+/+) yielded a 323-bp band only, and heterozygous rats (+/-) produced both the 161-bp and 323-bp bands.

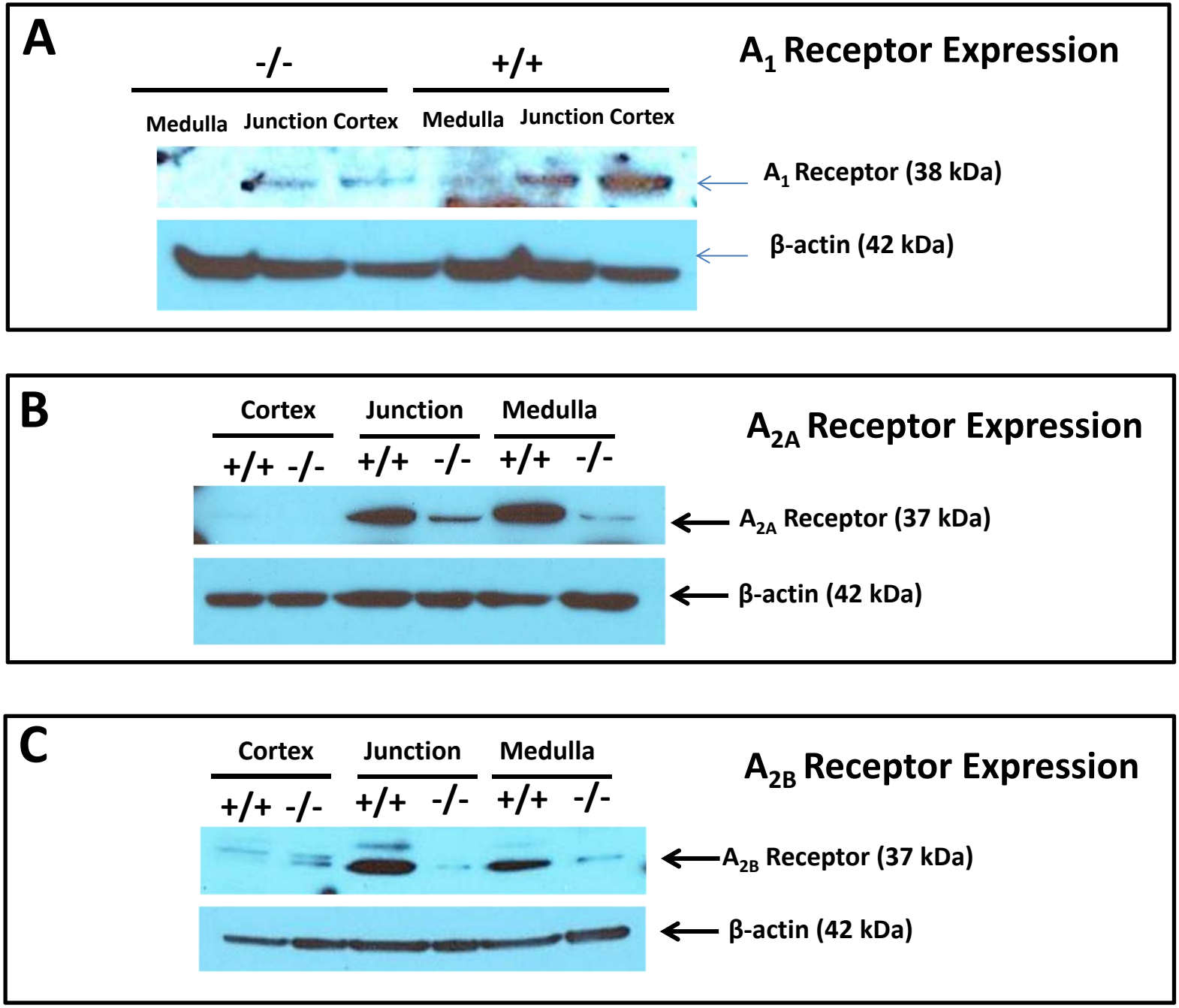

A
-/-
+/+
Medulla
Junction Cortex
Medulla
Junction Cortex
A1 Receptor Expression
A1 Receptor (38 kDa)
β-actin (42 kDa)
B
Cortex
Junction
Medulla
+/+ -/-
+/+ -/-
+/+ -/-
A2A Receptor Expression
A2A Receptor (37 kDa)
β-actin (42 kDa)
C
Cortex
Junction
Medulla
+/+ -/-
+/+ -/-
+/+ -/-
A2B Receptor Expression
A2B Receptor (37 kDa)
β-actin (42 kDa)

Figure S6. Figure demonstrates reduced expression (compared to wildtype (+/+) Dahl SS rats) of A_1, A_{2A}, and A_{2B} receptors in A_1, A_{2A}, and A_{2B} knockout (-/-) Dahl SS rats (panels A, B, and C, respectively). Western blots were performed on kidney tissue obtained from the cortex, juxtamedullary region, and medulla.

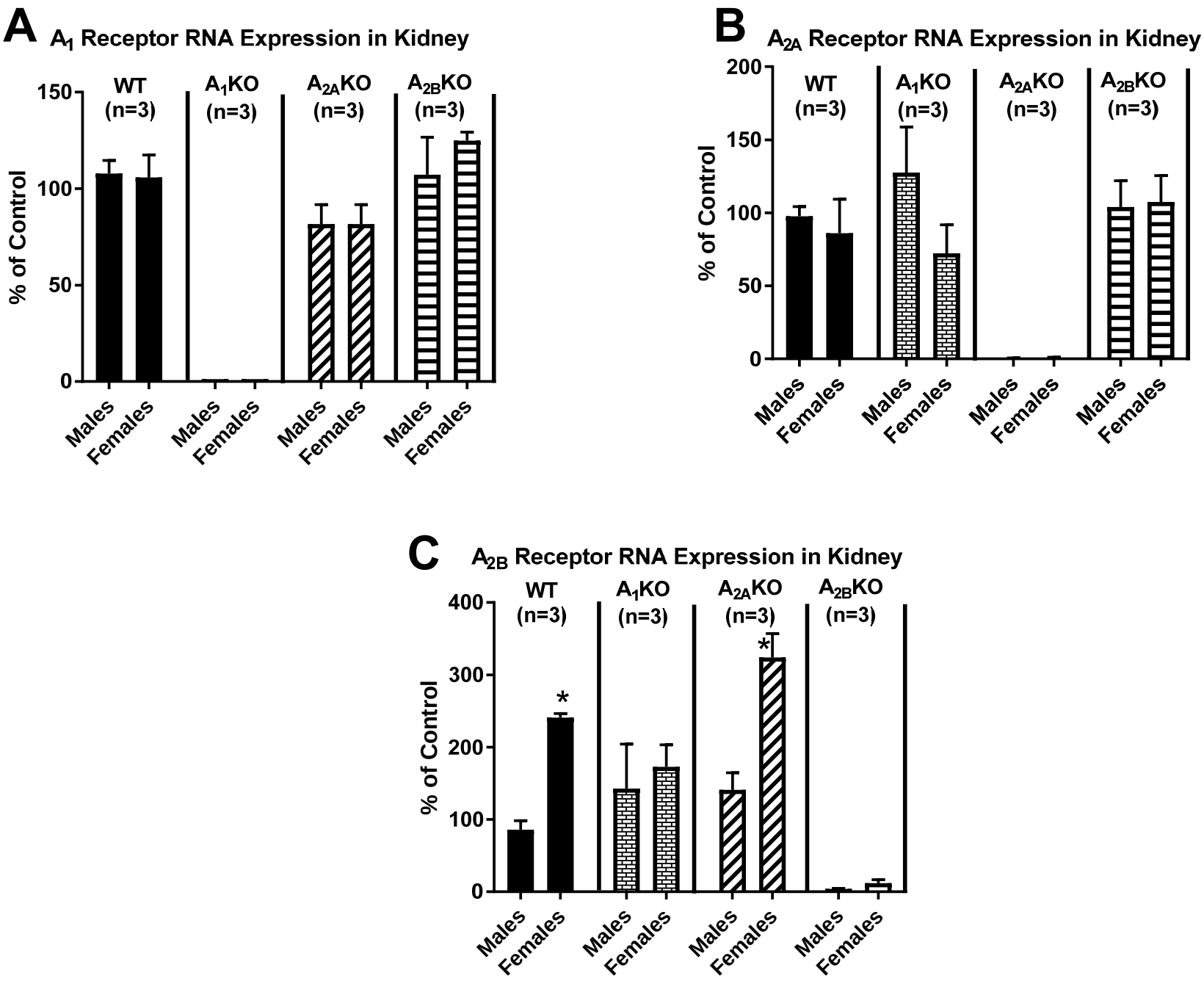

A
A1 Receptor RNA Expression in Kidney
% of Control
150
100
50
0
WT (n=3)
A1KO (n=3)
A2AKO (n=3)
A2BKO (n=3)
Males
Females
B
A2A Receptor RNA Expression in Kidney
% of Control
200
150
100
50
0
WT (n=3)
A1KO (n=3)
A2AKO (n=3)
A2BKO (n=3)
Males
Females
C
A2B Receptor RNA Expression in Kidney
% of Control
400
300
200
100
0
WT (n=3)
A1KO (n=3)
A2AKO (n=3)
A2BKO (n=3)
*
*
Males
Females

Figure S7. Figure summaries A_1 (A), A_{2A} (B), and A_{2B} (C) receptor mRNA expression in kidney tissue from male and female wildtype (WT), A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats. Total RNA was isolated from kidneys of wildtype, A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats (n=3 for each of the 8 sex/genotype combinations, total of 24 rats) using TRIZOL Reagent (Thermo Fisher Scientific; Waltham, MA) according to the manufacturer's instructions. The cDNA was synthesized using iScript^{TM} cDNA synthesis kit (Bio-Rad). qPCR analysis was performed using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) in the Applied Biosystems QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). Primers were: for *Adora1* 5'-tgctgtggatcgatacctcc-3' (forward) and 5'-tcttgctctaccacactcagg-3' (reverse); for *Adora2a* 5'-tgtacatcacggtggagctg-3' (forward) and 5'-gatggtgatagcgaagggga-3' (reverse); for *Adora2b* 5'-tactttctggtgtccctggc-3' (forward) and 5'-cttgctcgtgttccagtgac-3' (reverse); for β-actin 5'-actcttccagccttccttc-3' (forward) and 5'-atctccttctgcatcctgtc-3' (revserse). Threshold cycle (Ct) for β-actin was subtracted from Ct for target to calculate $2^{\Delta Ct}$, and results were expressed as % of control with control being the $2^{\Delta Ct}$ for an arbitrarily chosen wildtype rat. Values are means ± SEM. *Indicates $P<0.05$ compared with corresponding males of same genotype.

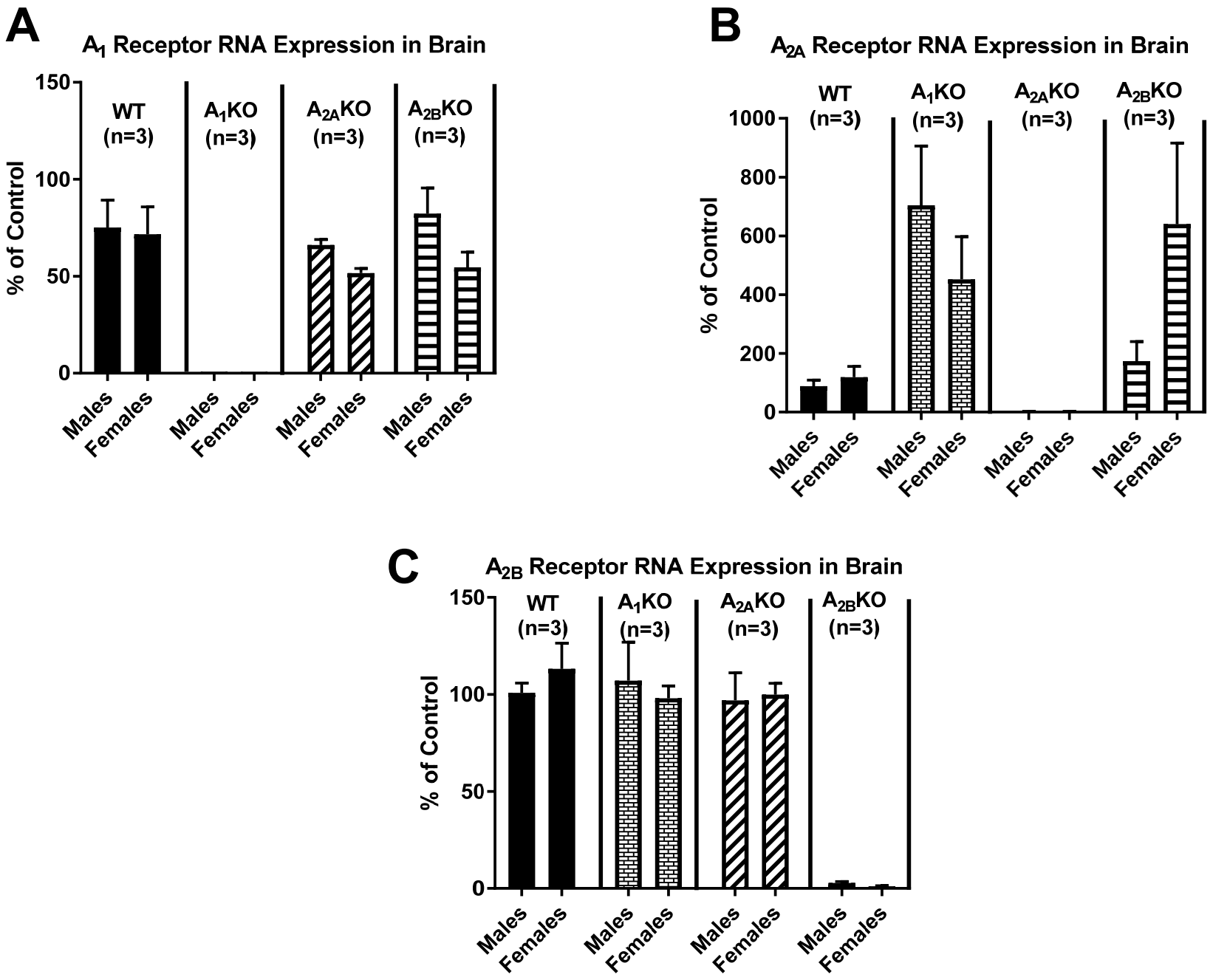

A
A1 Receptor RNA Expression in Brain
% of Control
150
100
50
0
WT (n=3)
A1KO (n=3)
A2AKO (n=3)
A2BKO (n=3)
Males
Females
B
A2A Receptor RNA Expression in Brain
% of Control
1000
800
600
400
200
0
WT (n=3)
A1KO (n=3)
A2AKO (n=3)
A2BKO (n=3)
Males
Females
C
A2B Receptor RNA Expression in Brain
% of Control
150
100
50
0
WT (n=3)
A1KO (n=3)
A2AKO (n=3)
A2BKO (n=3)
Males
Females

Figure S8. Figure summaries A_1 (A), A_{2A} (B), and A_{2B} (C) receptor mRNA expression in brain tissue from male and female wildtype (WT), A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats. Total RNA was isolated from brains of wildtype, A_1KO, A_{2A}KO, and A_{2B}KO Dahl SS rats (n=3 for each of the 8 sex/genotype combinations, total of 24 rats) using TRIZOL Reagent (Thermo Fisher Scientific; Waltham, MA) according to the manufacturer's instructions. The cDNA was synthesized using iScript^{TM} cDNA synthesis kit (Bio-Rad). qPCR analysis was performed using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific) in the Applied Biosystems QuantStudio 3 Real-Time PCR System (Thermo Fisher Scientific). Primers were: for *Adora1* 5'-tgctgtggatcgatacctcc-3' (forward) and 5'-tcttgctctaccacactcagg-3' (reverse); for *Adora2a* 5'-tgtacatcacggtggagctg-3' (forward) and 5'-gatggtgatagcgaagggga-3' (reverse); for *Adora2b* 5'-tactttctggtgtccctggc-3' (forward) and 5'-cttgctcgtgttccagtgac-3' (reverse); for β-actin 5'-actcttccagccttccttc-3' (forward) and 5'-atctccttctgcatcctgtc-3' (revserse). Threshold cycle (Ct) for β-actin was subtracted from Ct for target to calculate $2^{\Delta Ct}$, and results were expressed as % of control with control being the $2^{\Delta Ct}$ for an arbitrarily chosen wildtype rat. Values are means ± SEM.

HEART RATE

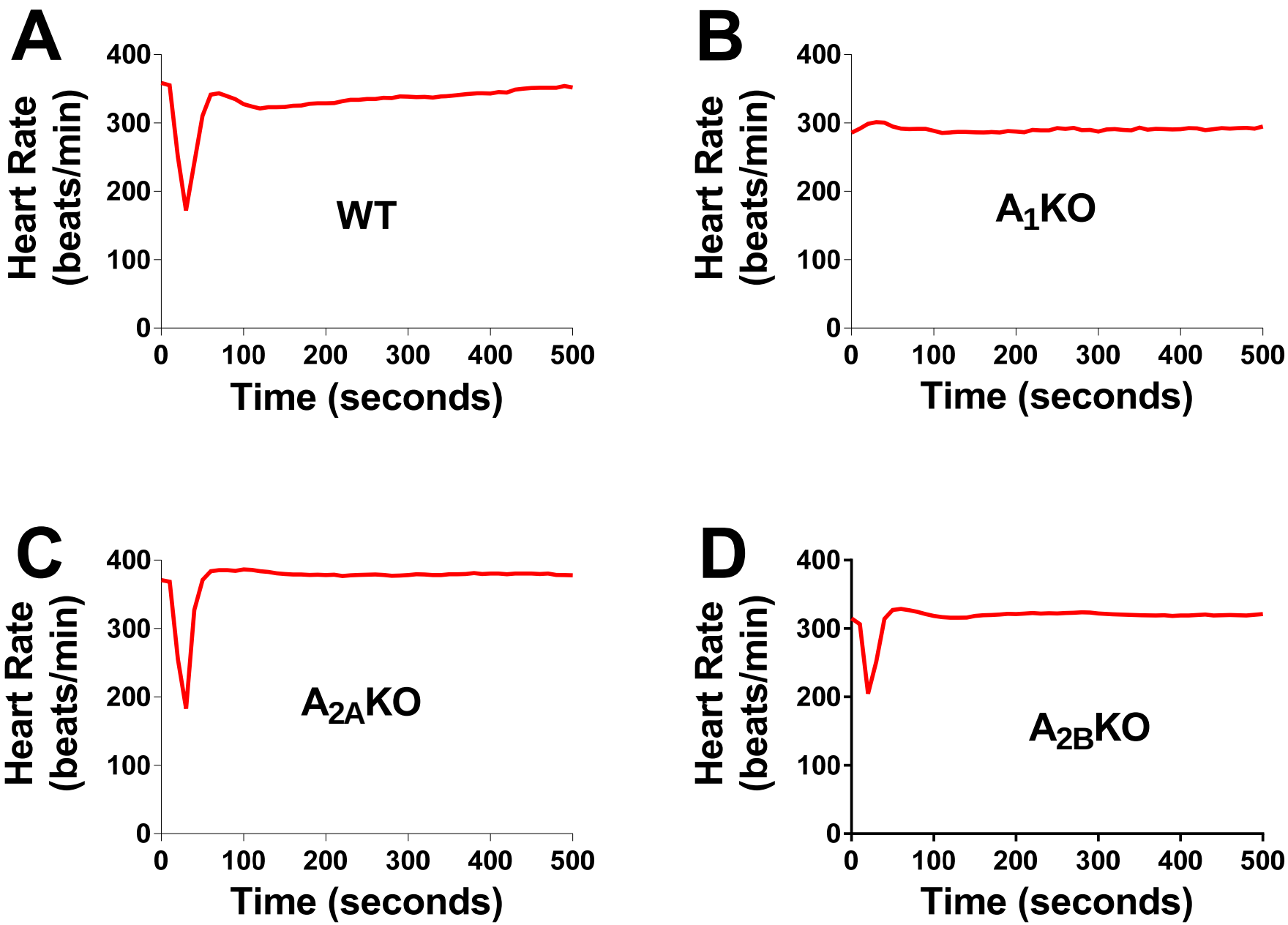
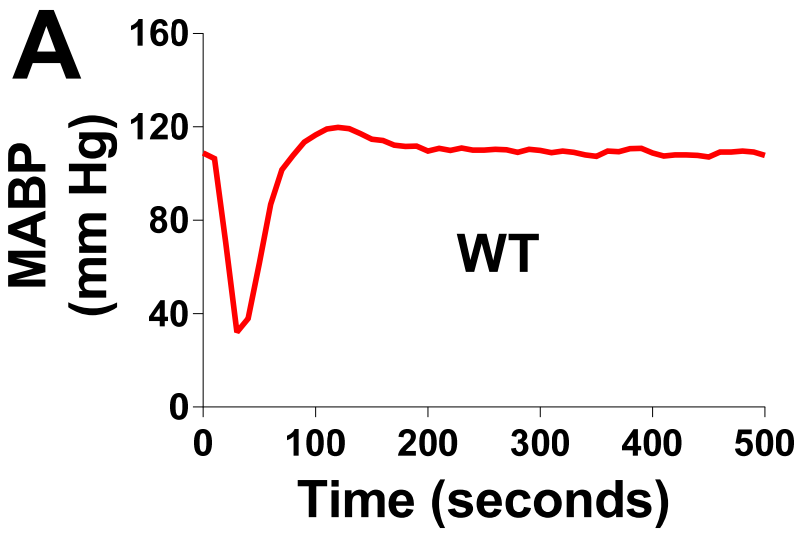
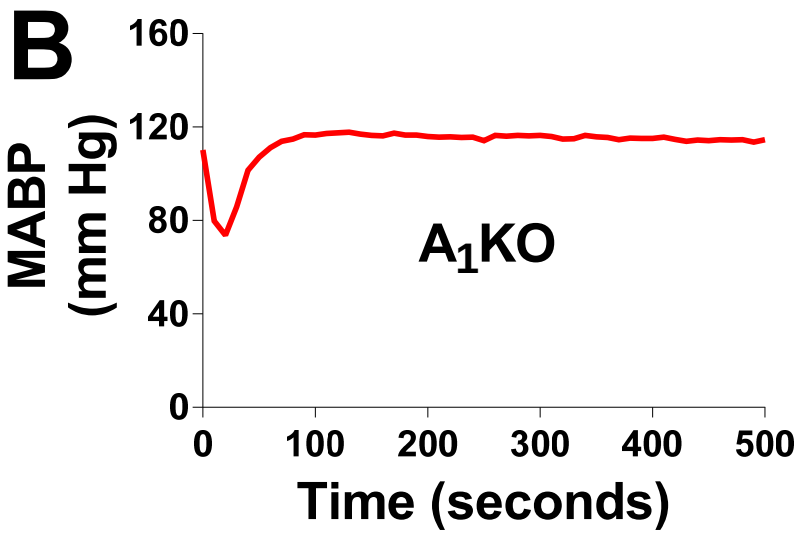
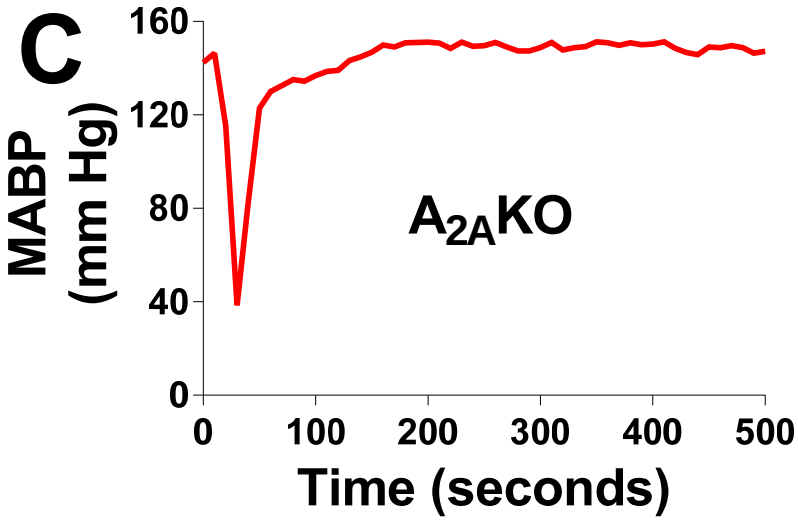

A
Heart Rate (beats/min)
400
300
200
100
0
WT
0
100
200
300
400
500
Time (seconds)
B
Heart Rate (beats/min)
400
300
200
100
0
A1KO
0
100
200
300
400
500
Time (seconds)
C
Heart Rate (beats/min)
400
300
200
100
0
A2AKO
0
100
200
300
400
500
Time (seconds)
D
Heart Rate (beats/min)
400
300
200
100
0
A2BKO
0
100
200
300
400
500
Time (seconds)

Figure S9. At time = 0, adenosine (3 μmoles/kg) was injected intravenously into wildtype (A, WT), A_1 knockout (B, A_1KO), A_{2A} knockout (C, A_{2A}KO), and A_{2B} knockout (D, A_{2B}KO) Dahl SS rats while recording heart rate. The effect of adenosine on heart rate was abolished in A_1KO rats.

MEAN ARTERIAL BLOOD PRESSURE







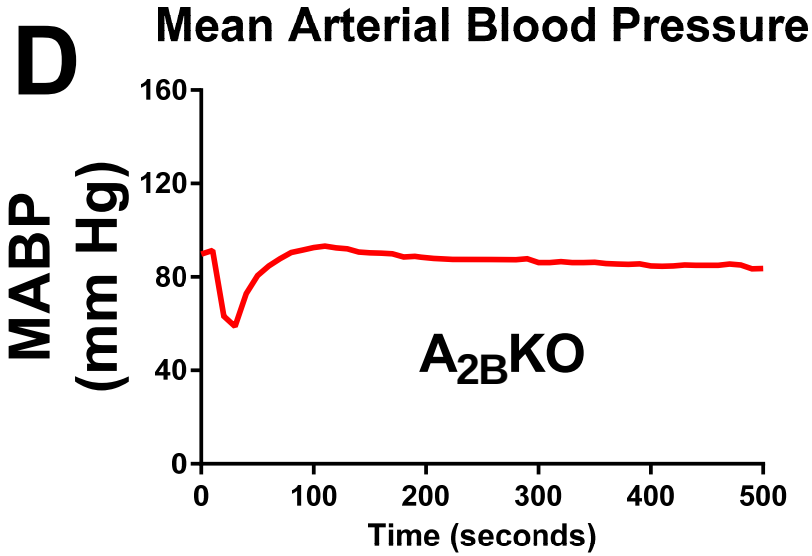


Figure S10. At time = 0, adenosine (3 μmoles/kg) was injected intravenously into wildtype (A, WT), A_1 knockout (B, A_1KO), A_{2A} knockout (C, A_{2A}KO), and A_{2B} knockout (D, A_{2B}KO) Dahl SS rats while recording mean arterial blood pressure (MABP). Adenosine reduced blood pressure in all four strains.

RENAL VASCULAR RESISTANCE

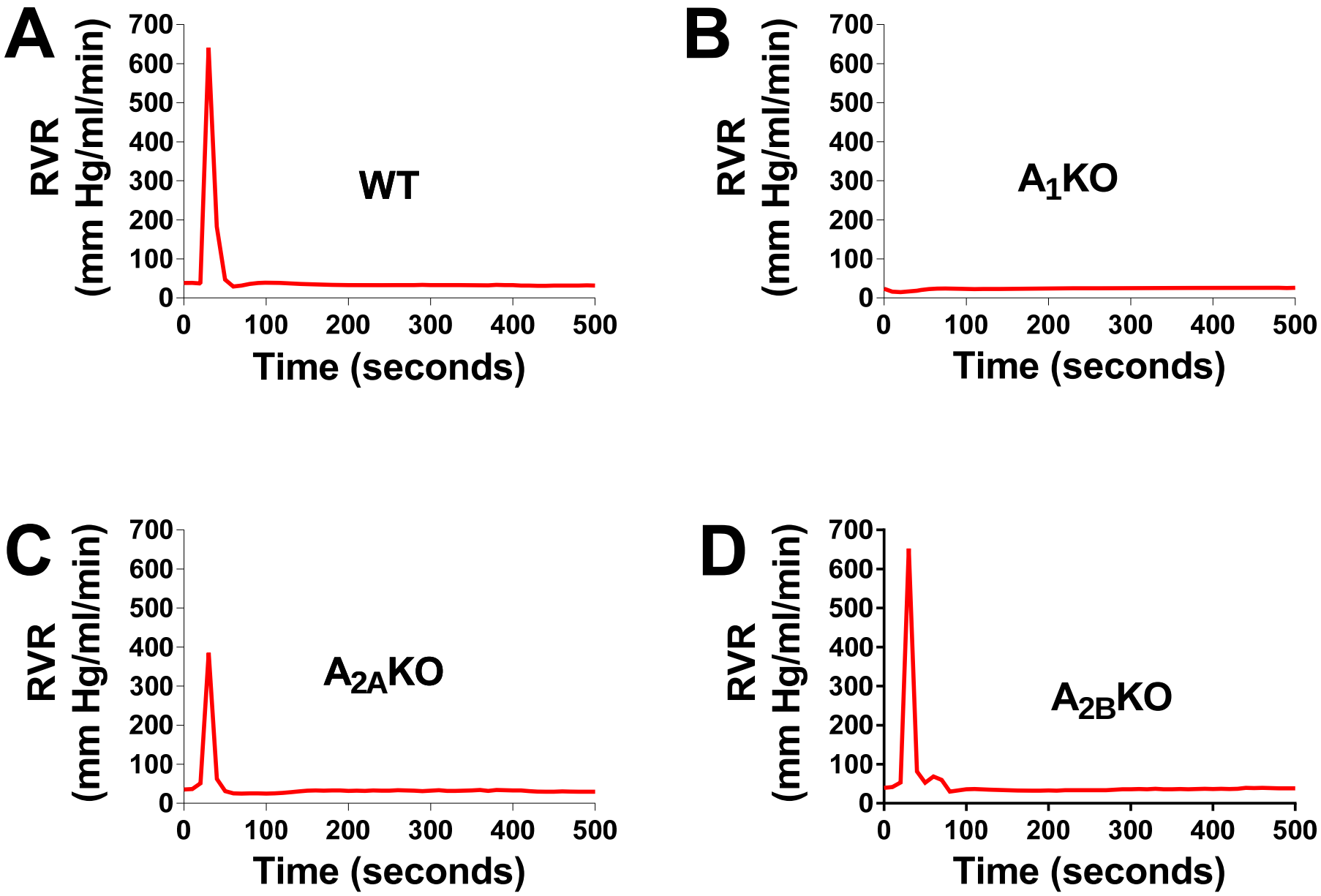


Figure S11. At time = 0, adenosine (3 μmoles/kg) was injected intravenously into wildtype (A, WT), A_1 knockout (B, A_1KO), A_{2A} knockout (C, A_{2A}KO), and A_{2B} knockout (D, A_{2B}KO) Dahl SS rats while recording renal vascular resistance (RVR = mean arterial blood pressure divided by renal blood flow). The ability of adenosine to spike RVR was abolished in A_1KO rats.

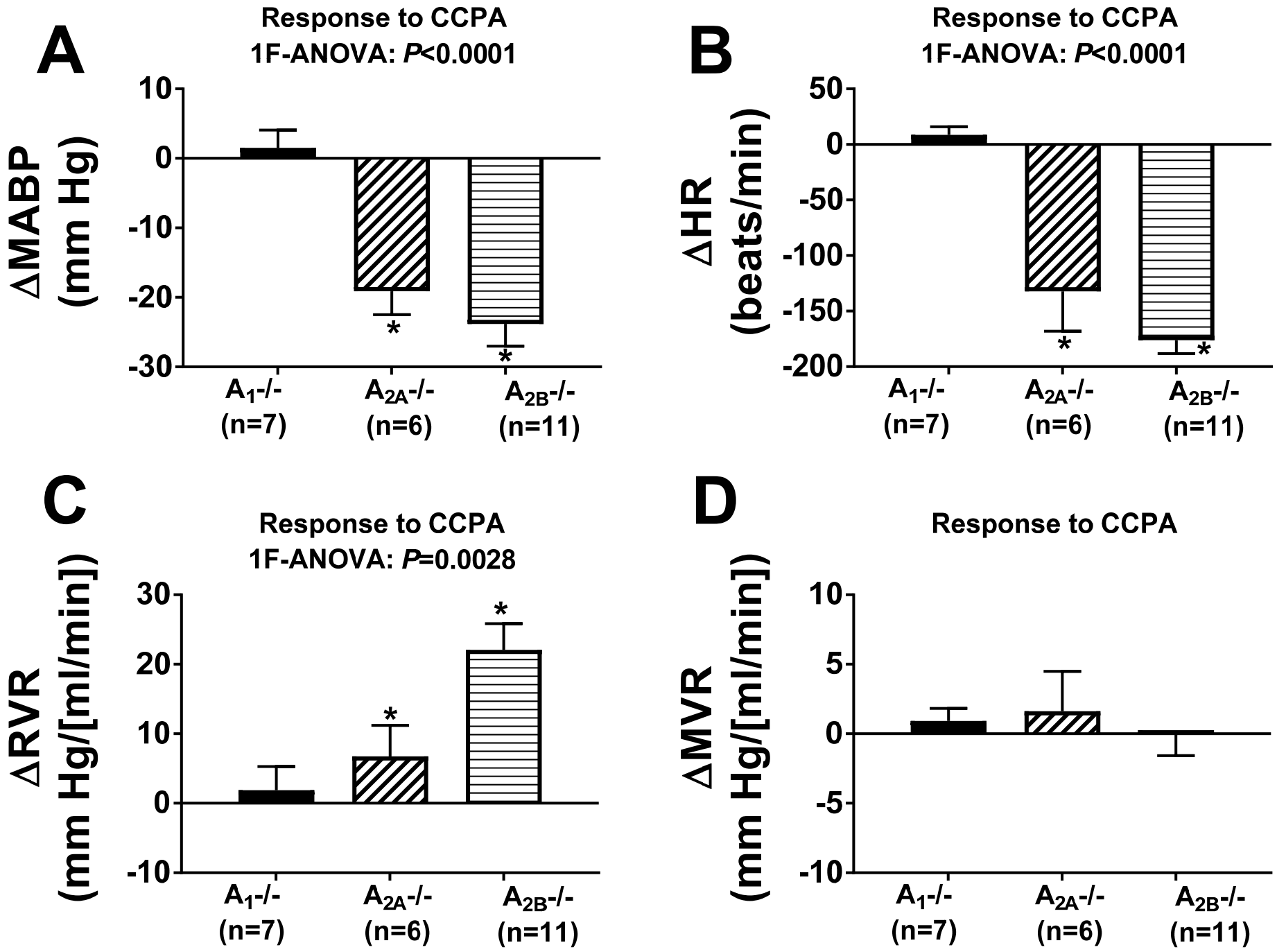

A
Response to CCPA
1F-ANOVA: P<0.0001
ΔMABP (mm Hg)
10
0
-10
-20
-30
*
*
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)
B
Response to CCPA
1F-ANOVA: P<0.0001
ΔHR (beats/min)
50
0
-50
-100
-150
-200
*
*
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)
C
Response to CCPA
1F-ANOVA: P=0.0028
ΔRVR (mm Hg/[ml/min])
30
20
10
0
-10
*
*
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)
D
Response to CCPA
ΔMVR (mm Hg/[ml/min])
10
5
0
-5
-10
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)

Figure S12. To further characterize the A_1, A_{2A}, and A_{2B} knockout (-/-) Dahl SS rats, we infused intravenously 2-chloro-N^6-cyclopentyladenosine (CCPA; selective A_1 receptor agonist; 1 μg/kg/min) while monitoring mean arterial blood pressure (MABP, A), heart rate (HR, B), renal vascular resistance (RVR = MABP/renal blood flow, C) and mesenteric vascular resistance (MVR = MABP/mesenteric blood flow, D). As show, in A_{2A} and A_{2B} knockout rats, CCPA reduced MABP and HR and increased RVR; and these responses were abolished in A_1 knockout rats. Values are means ± SEM.

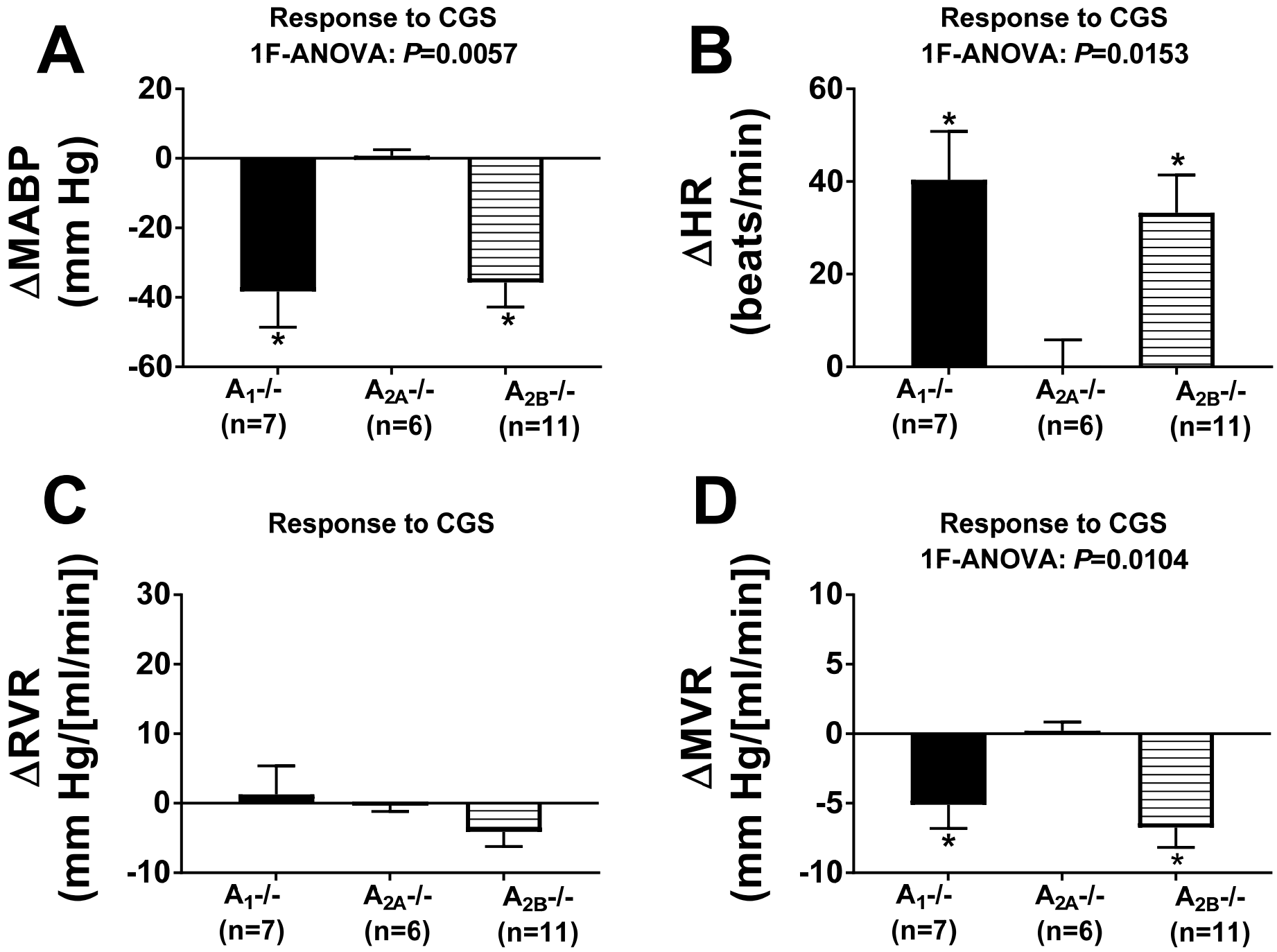

A
Response to CGS
1F-ANOVA: P=0.0057
ΔMABP (mm Hg)
20
0
-20
-40
-60
*
*
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)
B
Response to CGS
1F-ANOVA: P=0.0153
ΔHR (beats/min)
60
40
20
0
*
*
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)
C
Response to CGS
ΔRVR (mm Hg/[ml/min])
30
20
10
0
-10
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)
D
Response to CGS
1F-ANOVA: P=0.0104
ΔMVR (mm Hg/[ml/min])
10
5
0
-5
-10
*
*
A1-/- (n=7)
A2A-/- (n=6)
A2B-/- (n=11)

Figure S13. To further characterize the A_1, A_{2A}, and A_{2B} knockout (-/-) Dahl SS rats, we infused intravenously CGS21680 (selective A_{2A} receptor agonist; 1 μg/kg/min) while monitoring mean arterial blood pressure (MABP, A), heart rate (HR, B), renal vascular resistance (RVR = MABP/renal blood flow, C) and mesenteric vascular resistance (MVR = MABP/mesenteric blood flow, D). As show, in A_1 knockout and A_{2B} knockout rats, CGS21680 reduced MABP and HR and decreased MVR; and these responses were abolished in A_1 knockout rats. Values are means ± SEM.

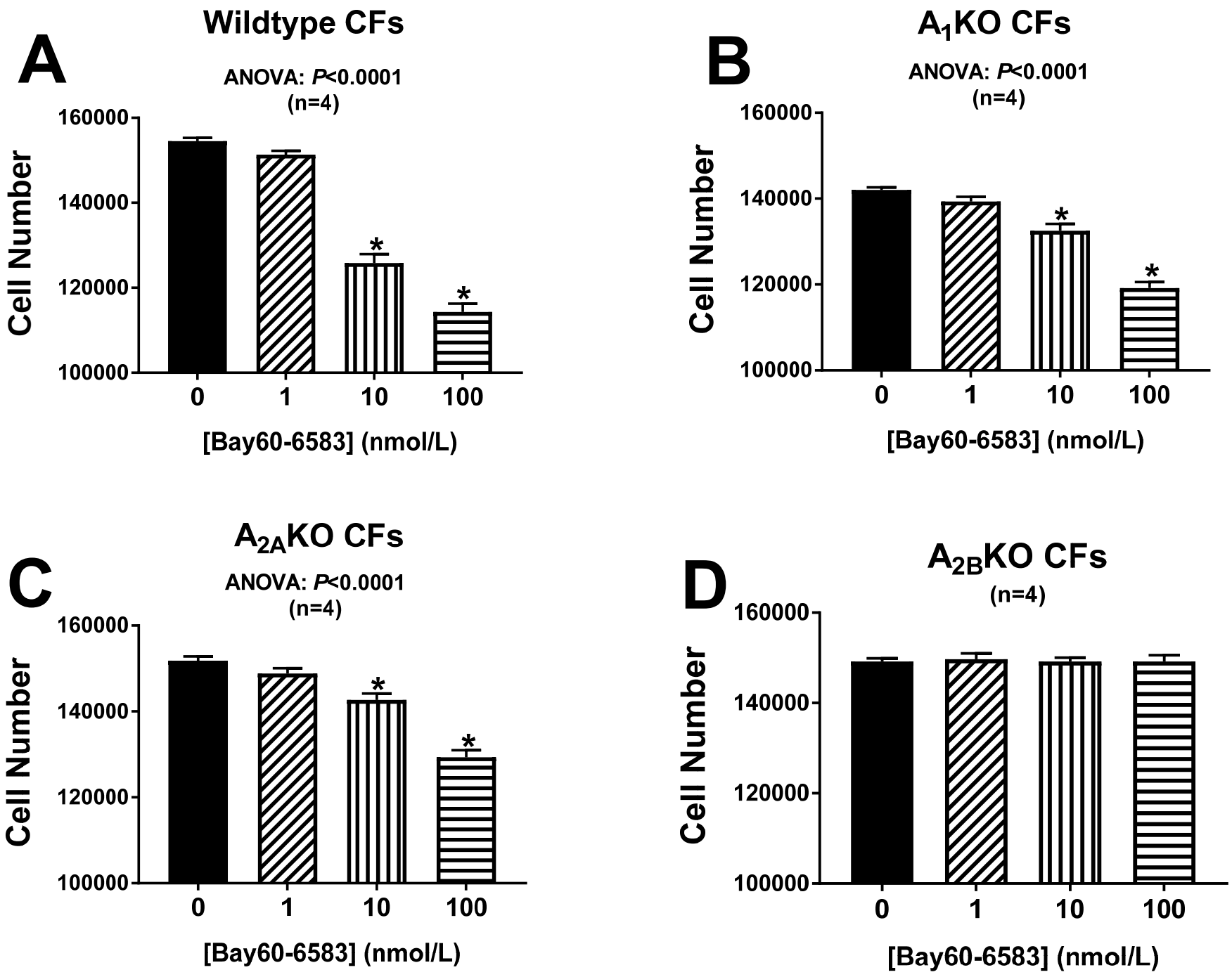
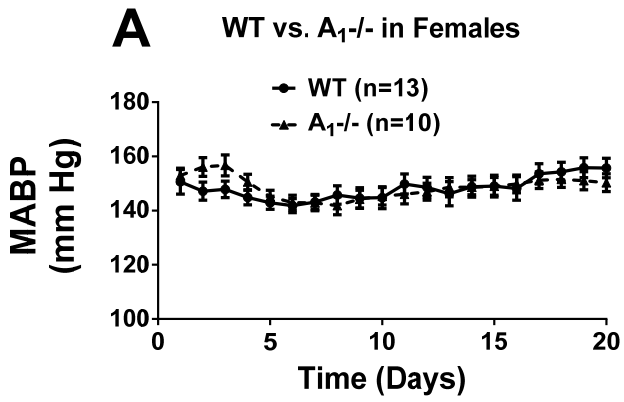
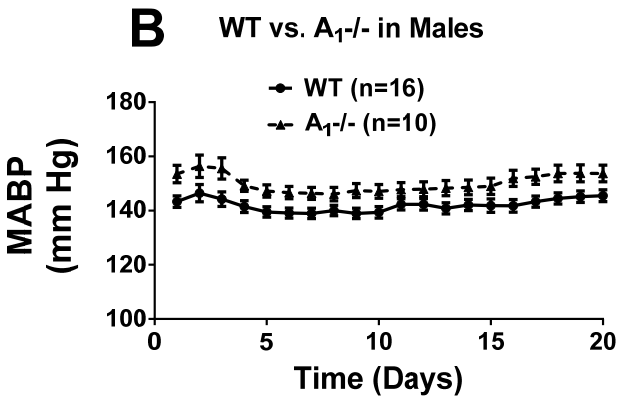
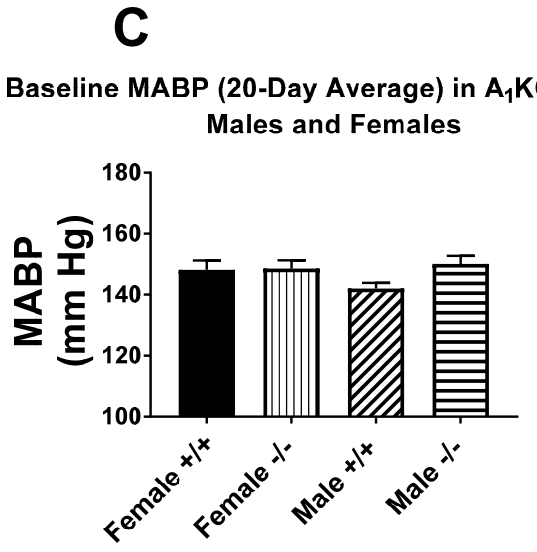
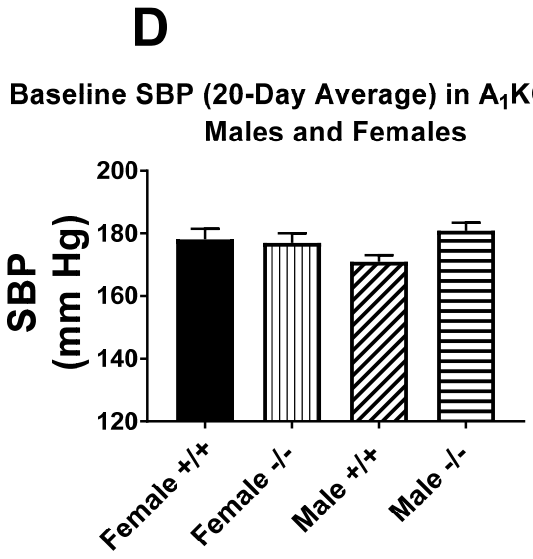

A
Wildtype CFs
ANOVA: P<0.0001
(n=4)
Cell Number
160000
140000
120000
100000
0
1
10
100
[Bay60-6583] (nmol/L)
B
A1KO CFs
ANOVA: P<0.0001
(n=4)
Cell Number
160000
140000
120000
100000
0
1
10
100
[Bay60-6583] (nmol/L)
C
A2AKO CFs
ANOVA: P<0.0001
(n=4)
Cell Number
160000
140000
120000
100000
0
1
10
100
[Bay60-6583] (nmol/L)
D
A2BKO CFs
(n=4)
Cell Number
160000
140000
120000
100000
0
1
10
100
[Bay60-6583] (nmol/L)

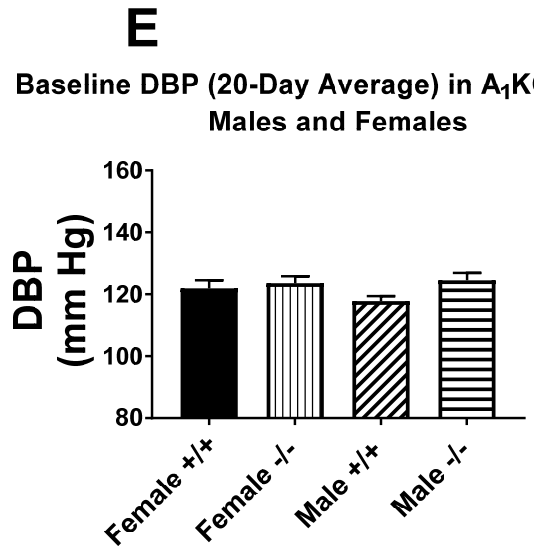
Figure S14. Bar graphs illustrate the effects of the selective A_{2B} receptor agonist (Bay60-6583) on proliferation of cardiac fibroblasts (CFs) cultured from the hearts of wildtype (A), A_1 receptor knockout (B), A_{2A} receptor knockout (C), and A_{2B} receptor knockout (D) rats. Data were analyzed by 1-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "0" concentration of Bay60-6583). Values are means ± SEM.


A
WT vs. A1-/- in Females
WT (n=13)
A1-/- (n=10)
MABP (mm Hg)
180
160
140
120
100
0
5
10
15
20
Time (Days)


B
WT vs. A1-/- in Males
WT (n=16)
A1-/- (n=10)
MABP (mm Hg)
180
160
140
120
100
0
5
10
15
20
Time (Days)


C
Baseline MABP (20-Day Average) in A1KOs vs WT Males and Females
MABP (mm Hg)
180
160
140
120
100
Female +/+
Female -/-
Male +/+
Male -/-


D
Baseline SBP (20-Day Average) in A1KOs vs WT Males and Females
SBP (mm Hg)
200
180
160
140
120
Female +/+
Female -/-
Male +/+
Male -/-


E
Baseline DBP (20-Day Average) in A1KOs vs WT Males and Females
DBP (mm Hg)
160
140
120
100
80
Female +/+
Female -/-
Male +/+
Male -/-

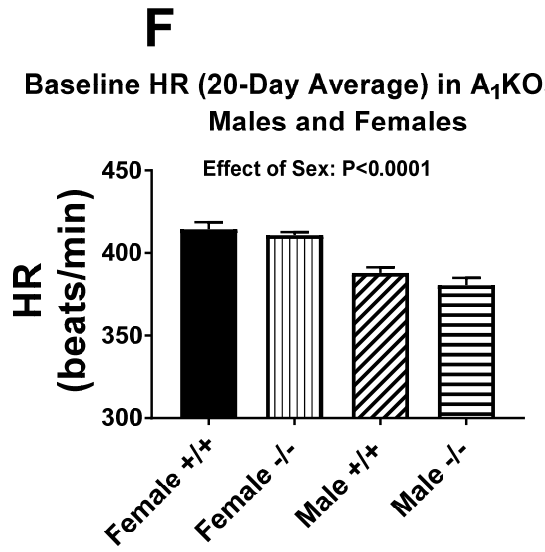
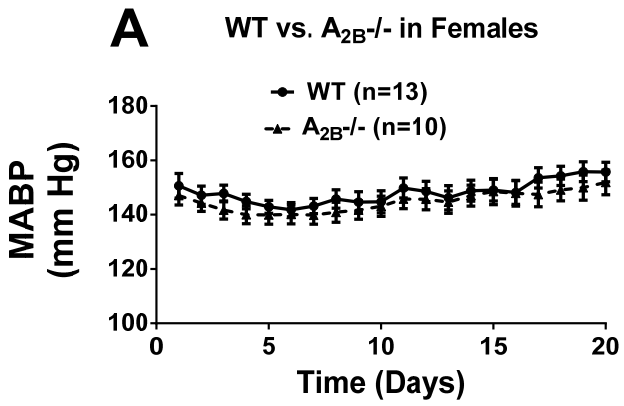
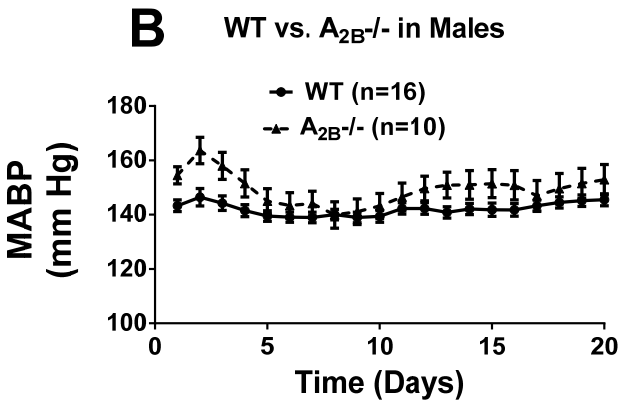
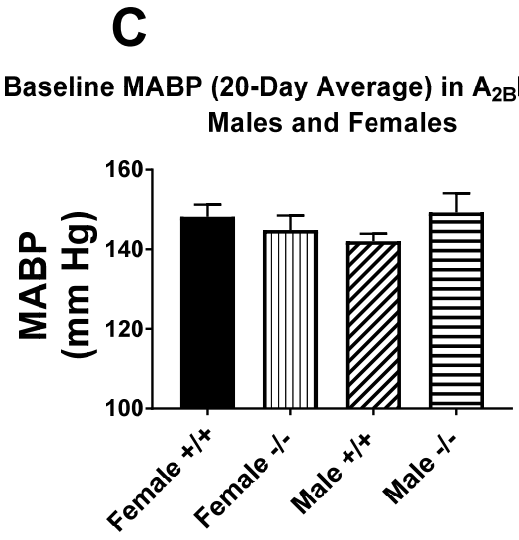
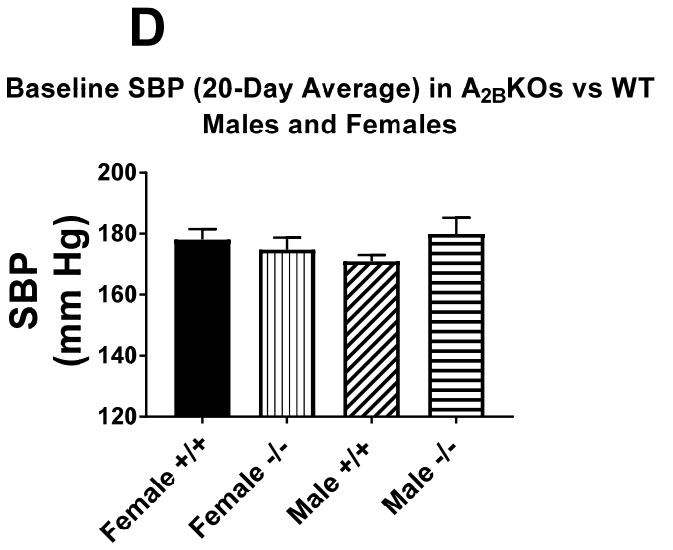

F
Baseline HR (20-Day Average) in A1KOs vs WT Males and Females
Effect of Sex: P<0.0001
HR (beats/min)
450
400
350
300
Female +/+
Female -/-
Male +/+
Male -/-

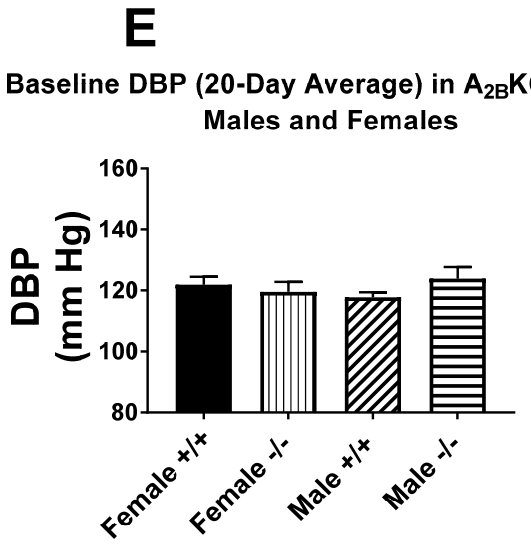
Figure S15. Panels A and B illustrate the day-to-day profile for 20 days of baseline (i.e., on 0.3% salt diet) mean arterial blood pressure (MABP) in wildtype Dahl SS rats (WT) versus A_1 knockout Dahl SS rats (A_1-/-). Panels A, B, C, and D summarize the 20-day average MABP (C), systolic blood pressure (SBP, D), diastolic blood pressure (DBP, E), and heart rate (HR, F) in female and male wildtype (+/+) versus A_1 knockout (-/-) Dahl SS rats. Values are means ± SEM.


A
WT vs. A2B-/- in Females
WT (n=13)
A2B-/- (n=10)
MABP (mm Hg)
180
160
140
120
100
0
5
10
15
20
Time (Days)


B
WT vs. A2B-/- in Males
WT (n=16)
A2B-/- (n=10)
MABP (mm Hg)
180
160
140
120
100
0
5
10
15
20
Time (Days)


C
Baseline MABP (20-Day Average) in A2BKOs vs WT Males and Females
MABP (mm Hg)
160
140
120
100
Female +/+
Female -/-
Male +/+
Male -/-


D
Baseline SBP (20-Day Average) in A2BKOs vs WT Males and Females
SBP (mm Hg)
200
180
160
140
120
Female +/+
Female -/-
Male +/+
Male -/-


E
Baseline DBP (20-Day Average) in A2BKOs vs WT Males and Females
DBP (mm Hg)
160
140
120
100
80
Female +/+
Female -/-
Male +/+
Male -/-

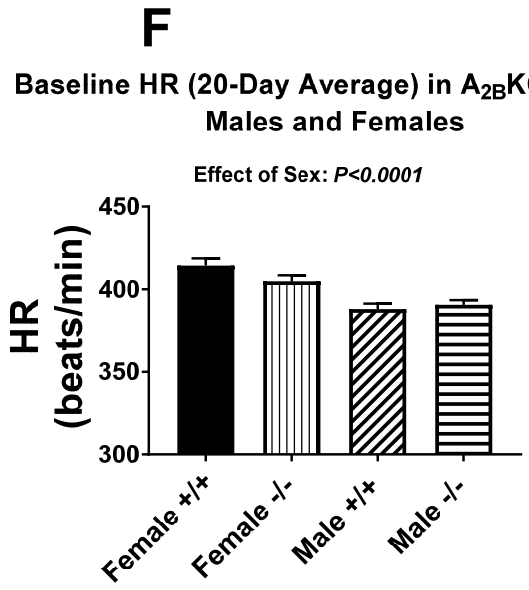
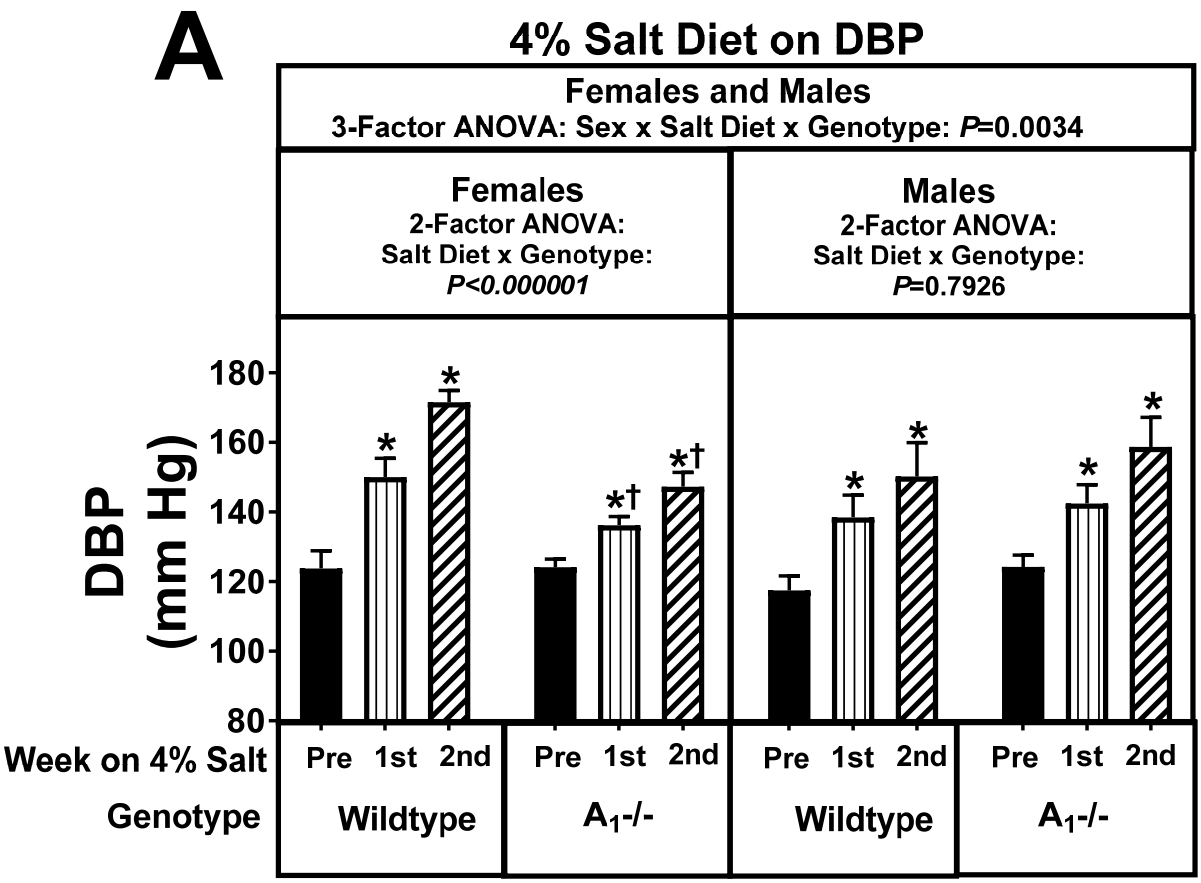

F
Baseline HR (20-Day Average) in A2BKOs vs WT Males and Females
Effect of Sex: P<0.0001
HR (beats/min)
450
400
350
300
Female +/+
Female -/-
Male +/+
Male -/-

Figure S16. Panels A and B illustrate the day-to-day profile for 20 days of baseline (i.e., on 0.3% salt diet) mean arterial blood pressure (MABP) in wildtype Dahl SS rats (WT) versus A_{2B} knockout Dahl SS rats (A_1-/-). Panels A, B, C, and D summarize the 20-day average MABP (C), systolic blood pressure (SBP, D), diastolic blood pressure (DBP, E), and heart rate (HR, F) in female and male wildtype (+/+) versus A_{2B} knockout (-/-) Dahl SS rats. Values are means ± SEM.


A
4% Salt Diet on DBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.0034
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P<0.000001
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.7926
DBP (mm Hg)
180
160
140
120
100
80
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-

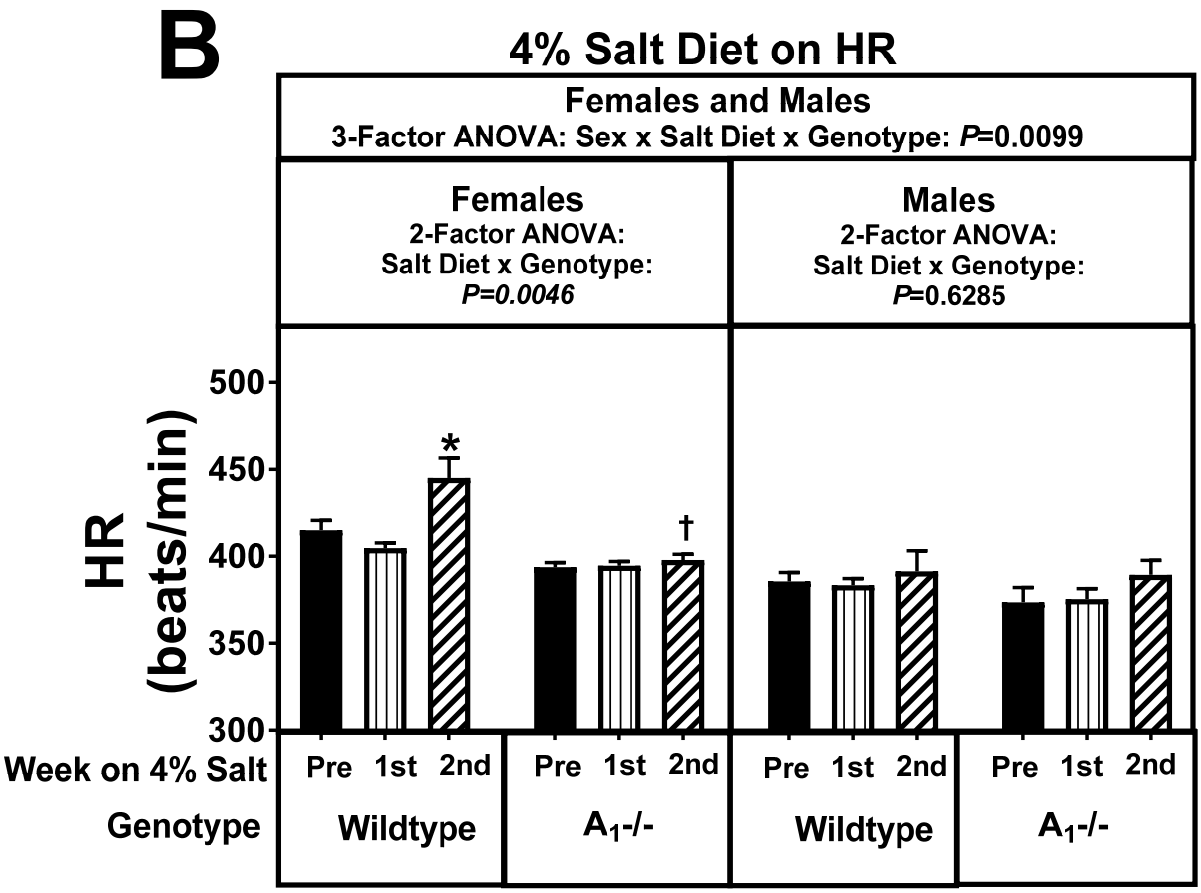

B
4% Salt Diet on HR
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.0099
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.0046
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.6285
HR (beats/min)
500
450
400
350
300
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-

Figure S17. Panel A summarizes for both female and male A_1 knockout (-/-) and wildtype Dahl SS rats the weekly average diastolic blood pressure (DBP) before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel B provides the same information and analysis as panel A, but for heart rate (HR). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.

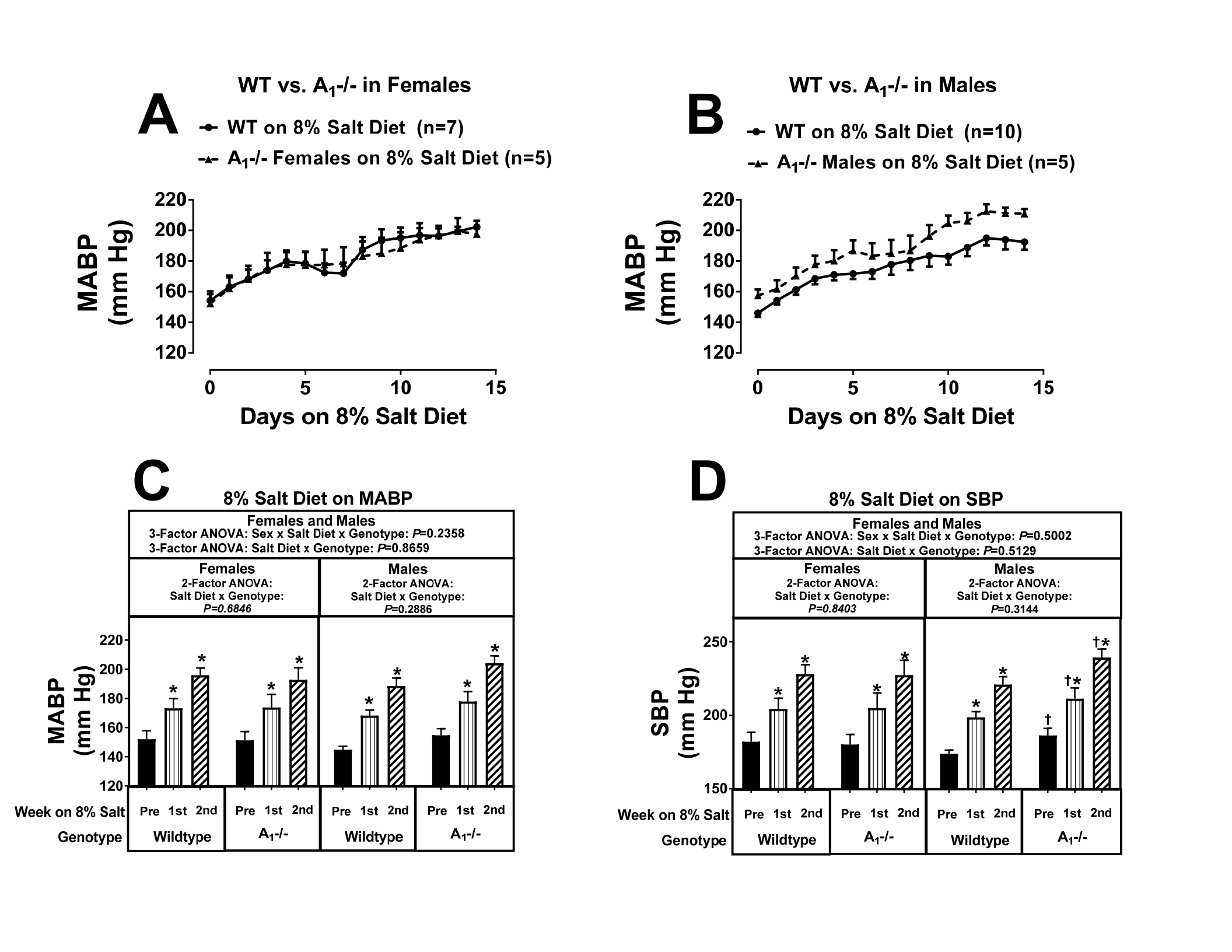

A
WT vs. A1-/- in Females
WT on 8% Salt Diet (n=7)
A1-/- Females on 8% Salt Diet (n=5)
MABP (mm Hg)
220
200
180
160
140
120
0
5
10
15
Days on 8% Salt Diet
B
WT vs. A1-/- in Males
WT on 8% Salt Diet (n=10)
A1-/- Males on 8% Salt Diet (n=5)
MABP (mm Hg)
220
200
180
160
140
120
0
5
10
15
Days on 8% Salt Diet
C
8% Salt Diet on MABP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.2358
3-Factor ANOVA: Salt Diet x Genotype: P=0.8659
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.6846
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.2886
MABP (mm Hg)
220
200
180
160
140
120
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-
D
8% Salt Diet on SBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.5002
3-Factor ANOVA: Salt Diet x Genotype: P=0.5129
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.8403
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.3144
SBP (mm Hg)
250
200
150
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-

Figure S18. Panel A illustrates the effects of a 8% salt diet on daily mean arterial blood pressure (MABP) in female wild-type Dahl SS rats (WT) versus female A_1 knockout Dahl SS rats (A_1-/-); panel B reports the effects of 8% salt diet on daily MABP in male WT versus male A_1-/- Dahl SS rats. Panel C summarizes for both females and males the weekly average MABP before (pre) starting the 8% salt diet and then during the 1st and 2nd weeks of the 8% salt diet. Panel D provides the same information and analysis as panel C for systolic blood pressure (SBP). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.

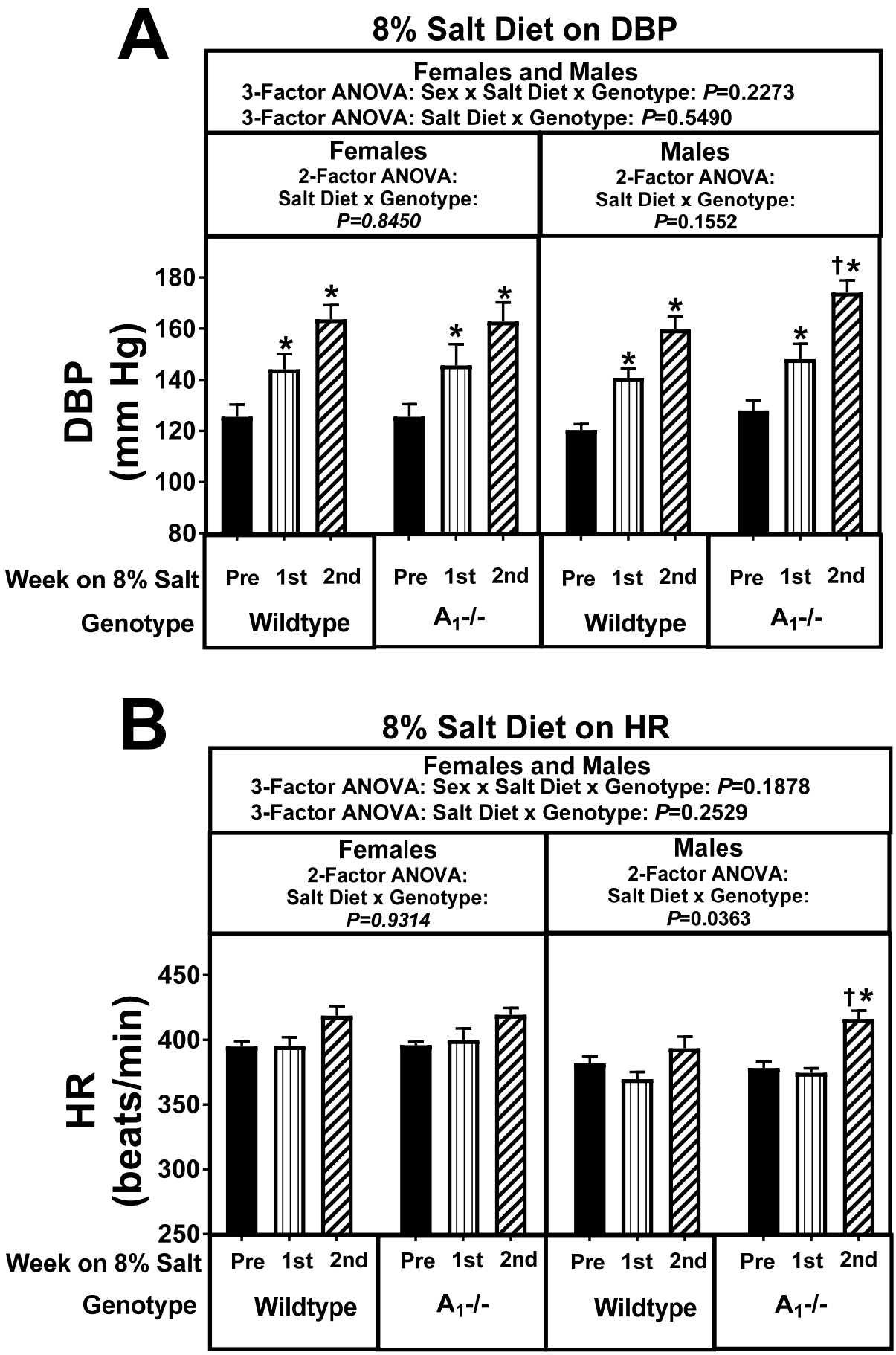
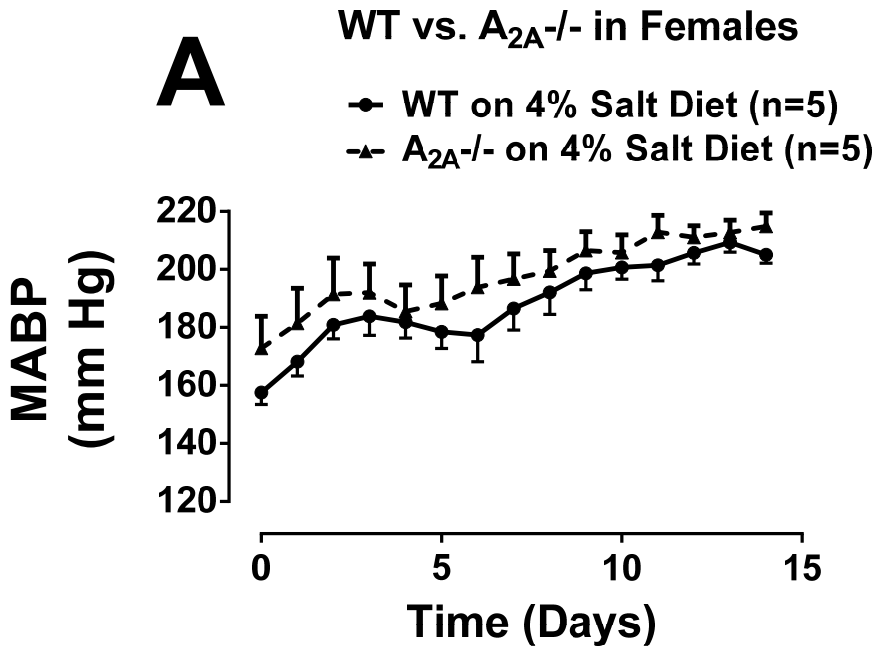
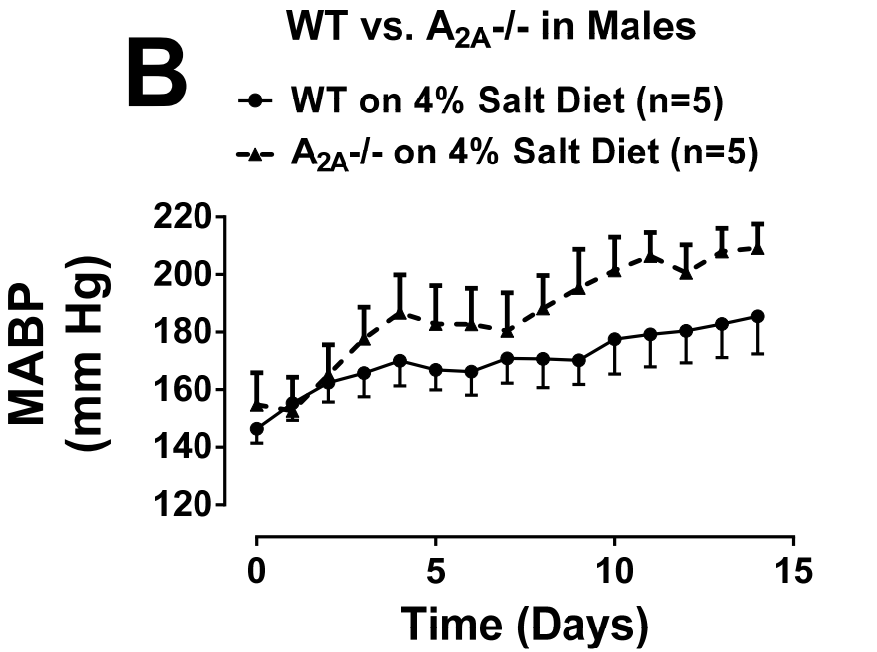
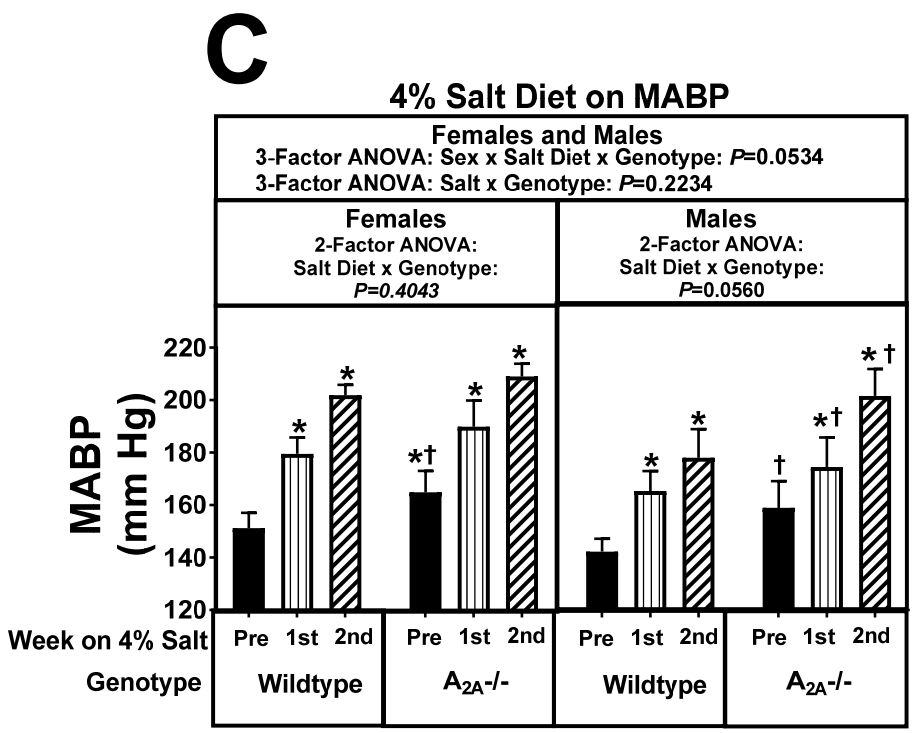

A
8% Salt Diet on DBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.2273
3-Factor ANOVA: Salt Diet x Genotype: P=0.5490
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.8450
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.1552
DBP (mm Hg)
180
160
140
120
100
80
*
†*
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-
B
8% Salt Diet on HR
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.1878
3-Factor ANOVA: Salt Diet x Genotype: P=0.2529
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.9314
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.0363
HR (beats/min)
450
400
350
300
250
†*
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-

Figure S19. Panel A summarizes for both female and male A_1 knockout (-/-) and wildtype Dahl SS rats the weekly average diastolic blood pressure (DBP) before (pre) starting the 8% salt diet and then during the 1st and 2nd weeks of the 8% salt diet. Panel B provides the same information and analysis as panel A, but for heart rate (HR). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.


A
WT vs. A2A-/- in Females
WT on 4% Salt Diet (n=5)
A2A-/- on 4% Salt Diet (n=5)
MABP (mm Hg)
220
200
180
160
140
120
0
5
10
15
Time (Days)


B
WT vs. A2A-/- in Males
WT on 4% Salt Diet (n=5)
A2A-/- on 4% Salt Diet (n=5)
MABP (mm Hg)
220
200
180
160
140
120
0
5
10
15
Time (Days)


C
4% Salt Diet on MABP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.0534
3-Factor ANOVA: Salt x Genotype: P=0.2234
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.4043
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.0560
MABP (mm Hg)
220
200
180
160
140
120
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2A-/-
Wildtype
A2A-/-

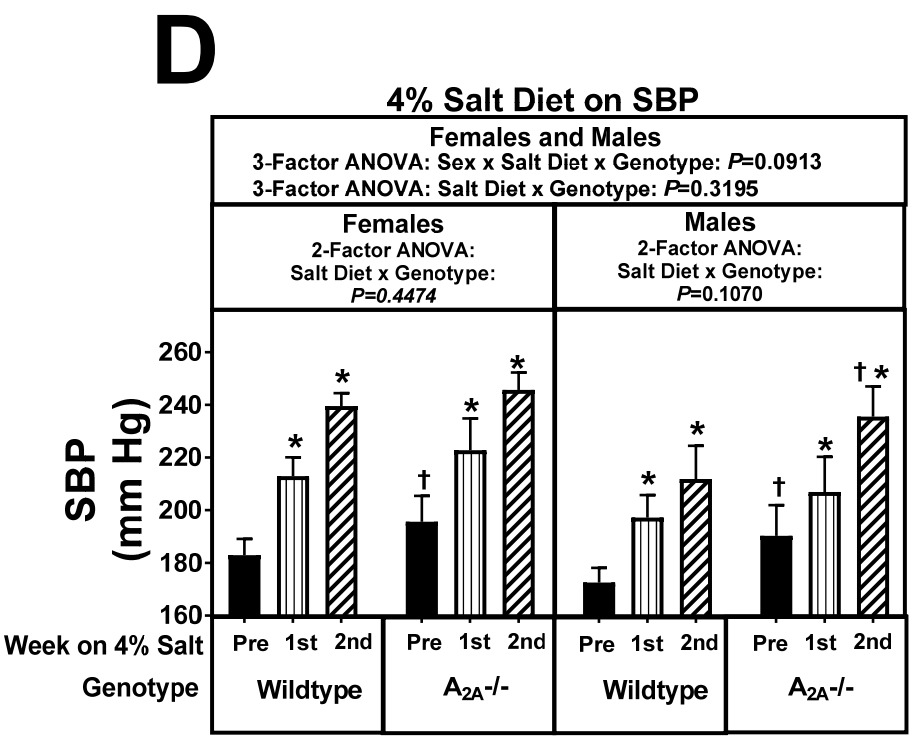

D
4% Salt Diet on SBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.0913
3-Factor ANOVA: Salt Diet x Genotype: P=0.3195
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.4474
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.1070
SBP (mm Hg)
260
240
220
200
180
160
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2A-/-
Wildtype
A2A-/-

Figure S20. Panel A illustrates the effects of a 4% salt diet on daily mean arterial blood pressure (MABP) in female wildtype Dahl SS rats (WT) versus female A_{2A} knockout Dahl SS rats (A_{2A}-/-); panel B reports the effects of 4% salt diet on daily MABP in male WT versus male A_{2A}-/- Dahl SS rats. Panel C summarizes for both females and males the weekly average MABP before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel D provides the same information and analysis as panel C for systolic blood pressure (SBP). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.

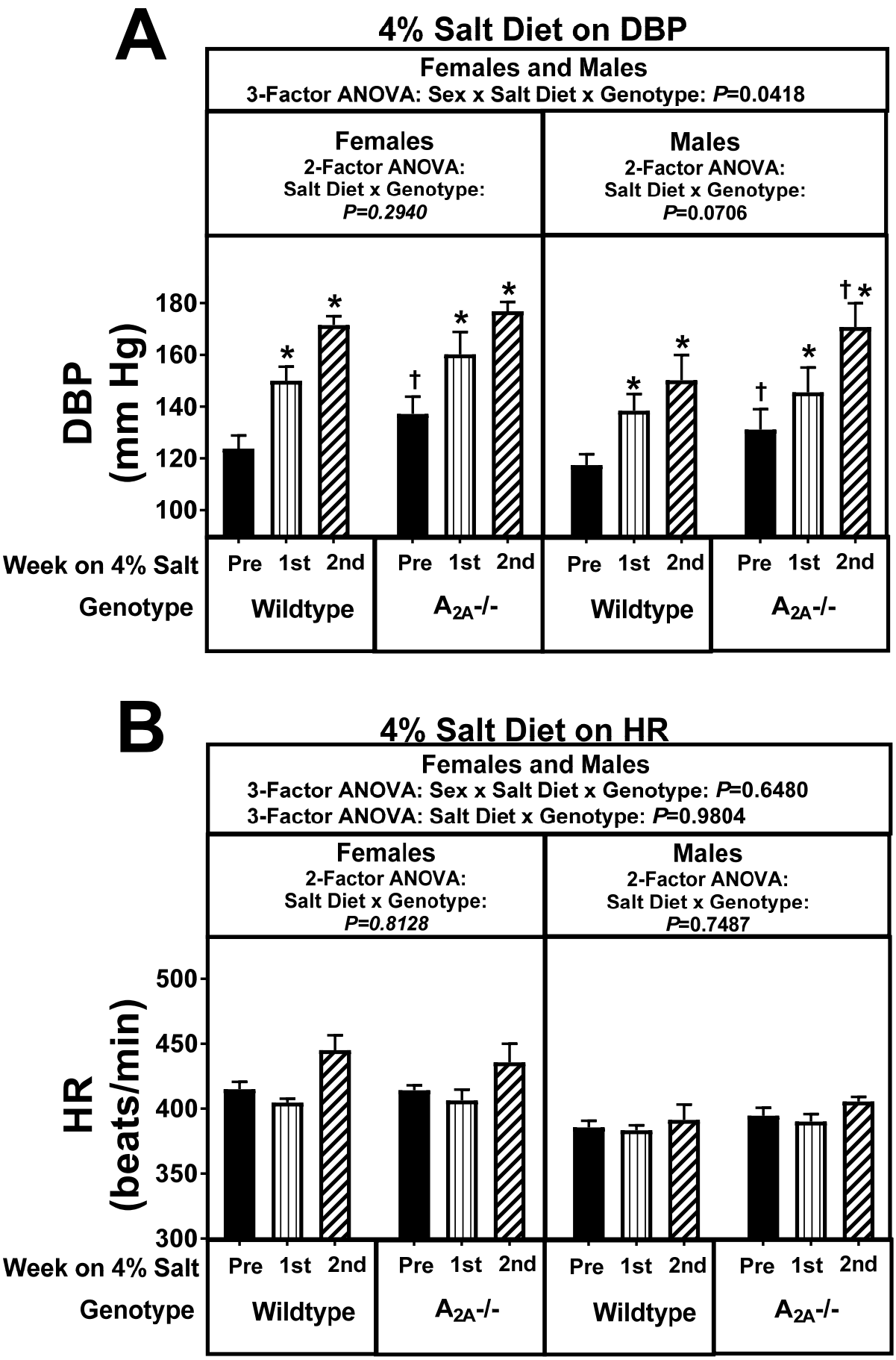

A
4% Salt Diet on DBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.0418
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.2940
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.0706
DBP (mm Hg)
180
160
140
120
100
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2A-/-
Wildtype
A2A-/-
B
4% Salt Diet on HR
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.6480
3-Factor ANOVA: Salt Diet x Genotype: P=0.9804
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.8128
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.7487
HR (beats/min)
500
450
400
350
300
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2A-/-
Wildtype
A2A-/-

Figure S21. Panel A summarizes for both female and male A_{2A} knockout (-/-) and wildtype Dahl SS rats the weekly average diastolic blood pressure (DBP) before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel B provides the same information and analysis as panel A, but for heart rate (HR). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.

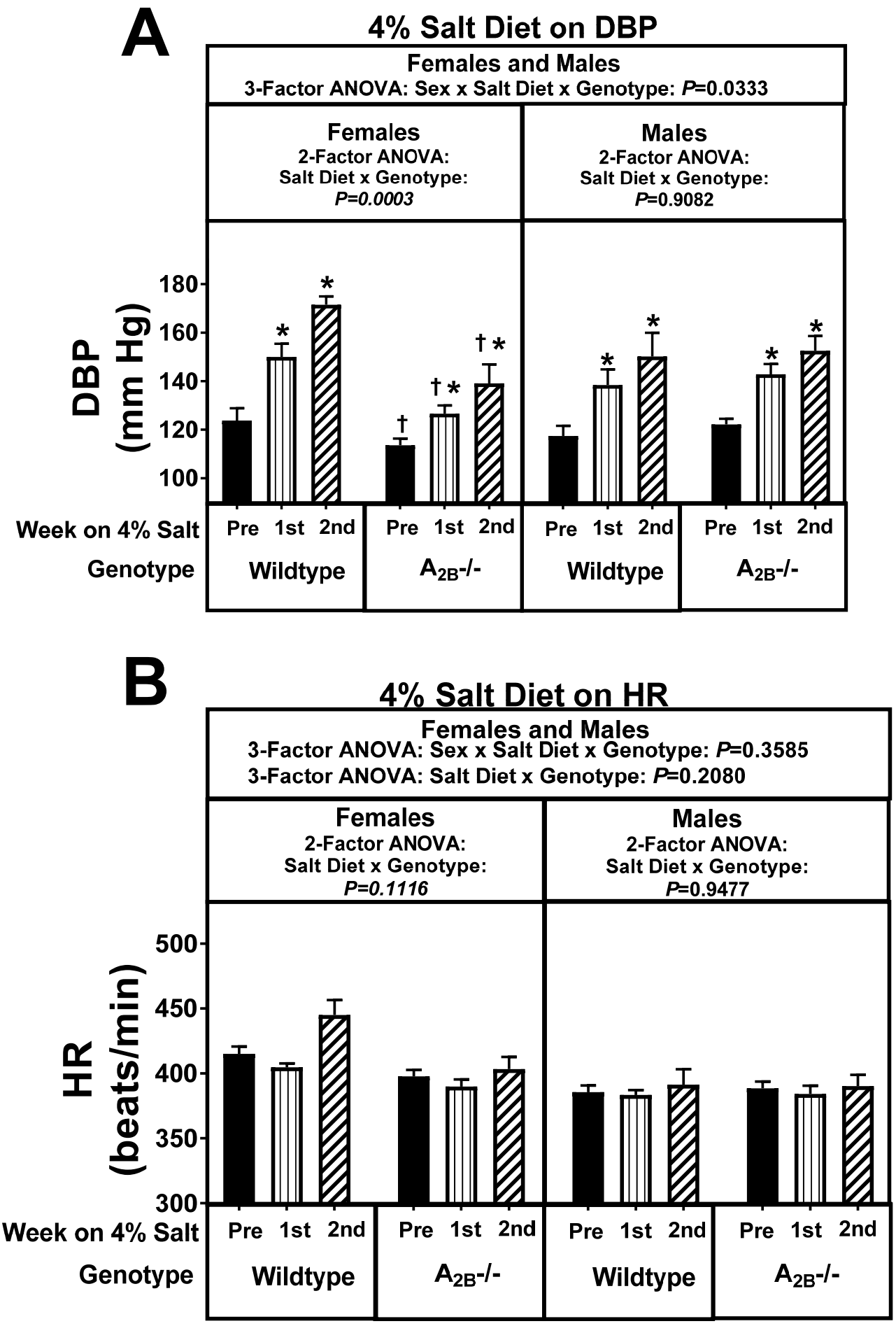
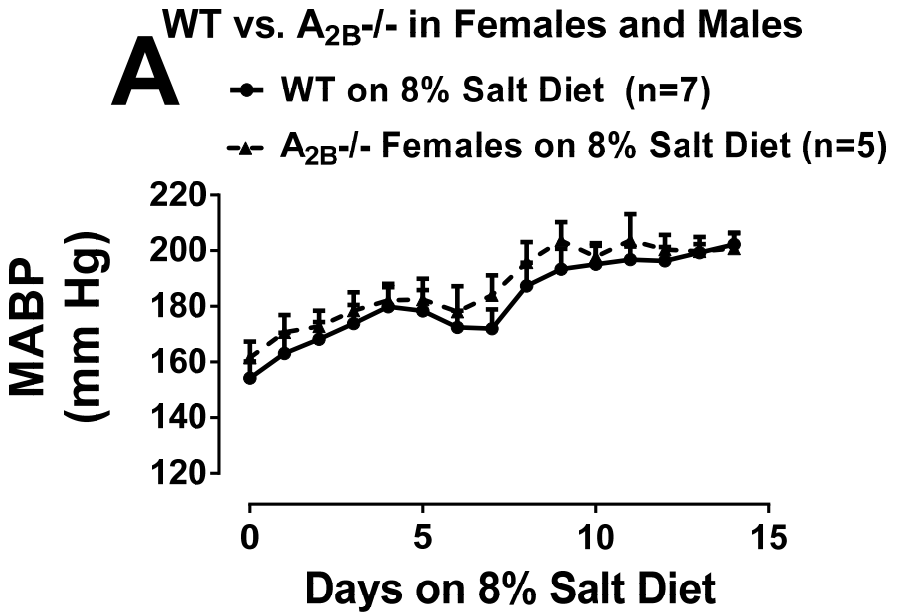
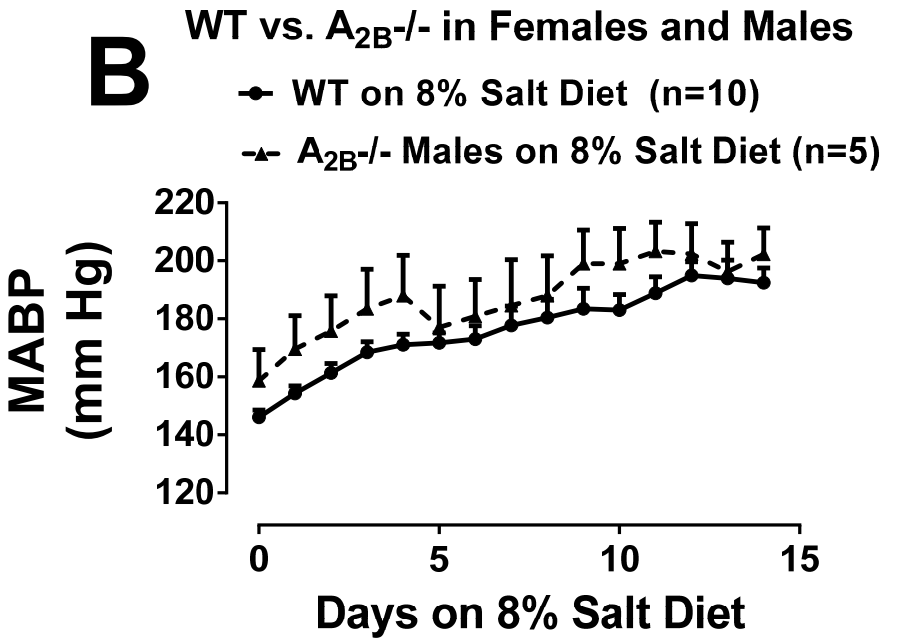
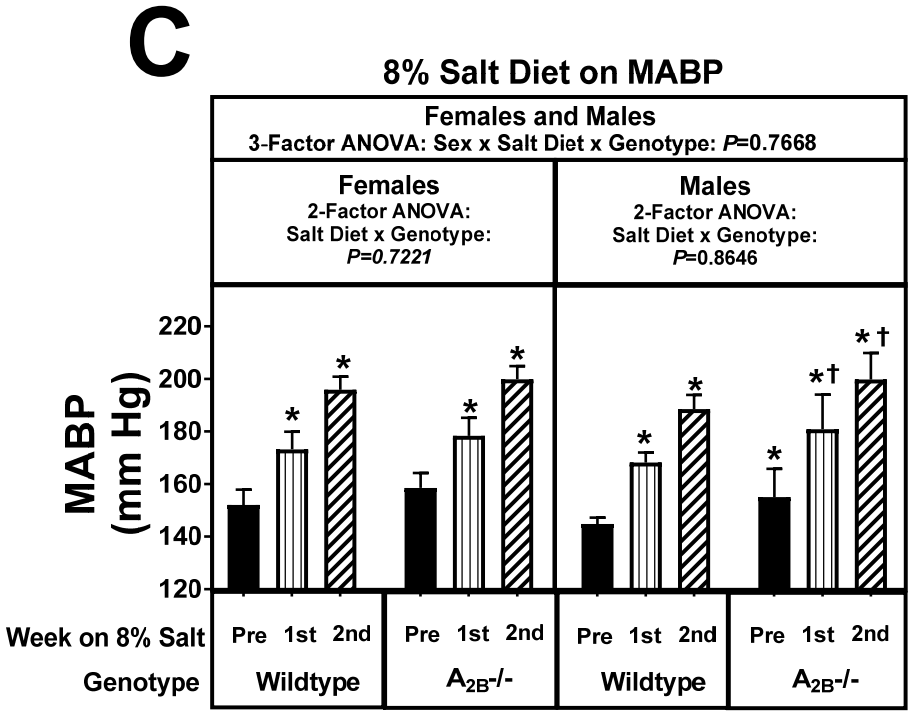

A
4% Salt Diet on DBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.0333
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.0003
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.9082
DBP (mm Hg)
180
160
140
120
100
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-
B
4% Salt Diet on HR
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.3585
3-Factor ANOVA: Salt Diet x Genotype: P=0.2080
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.1116
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.9477
HR (beats/min)
500
450
400
350
300
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-

Figure S22. Panel A summarizes for both female and male A_{2B} knockout (-/-) and wildtype Dahl SS rats the weekly average diastolic blood pressure (DBP) before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel B provides the same information and analysis as panel A, but for heart rate (HR). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.


A
WT vs. A2B-/- in Females and Males
WT on 8% Salt Diet (n=7)
A2B-/- Females on 8% Salt Diet (n=5)
MABP (mm Hg)
220
200
180
160
140
120
0
5
10
15
Days on 8% Salt Diet


B
WT vs. A2B-/- in Females and Males
WT on 8% Salt Diet (n=10)
A2B-/- Males on 8% Salt Diet (n=5)
MABP (mm Hg)
220
200
180
160
140
120
0
5
10
15
Days on 8% Salt Diet


C
8% Salt Diet on MABP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.7668
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.7221
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.8646
MABP (mm Hg)
220
200
180
160
140
120
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-

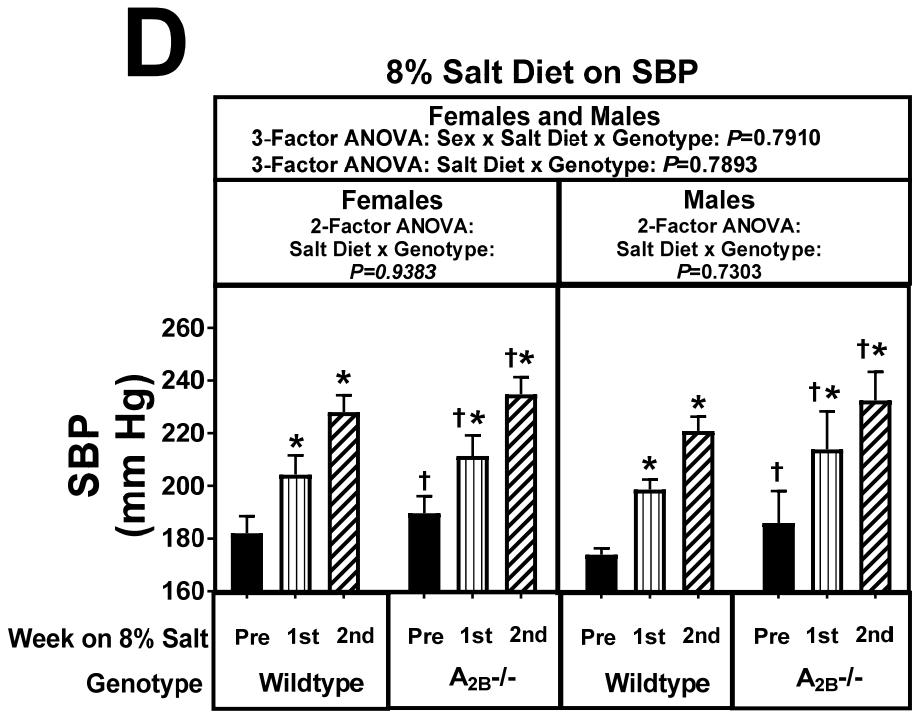

D
8% Salt Diet on SBP
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.7910
3-Factor ANOVA: Salt Diet x Genotype: P=0.7893
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.9383
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.7303
SBP (mm Hg)
260
240
220
200
180
160
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-

Figure S23. Panel A illustrates the effects of a 8% salt diet on daily mean arterial blood pressure (MABP) in female wild-type Dahl SS rats (WT) versus female A_{2B} knockout Dahl SS rats (A_{2B}-/-); panel B reports the effects of 8% salt diet on daily MABP in male WT versus male A_{2B}-/- Dahl SS rats. Panel C summarizes for both females and males the weekly average MABP before (pre) starting the 8% salt diet and then during the 1st and 2nd weeks of the 8% salt diet. Panel D provides the same information and analysis as panel C for systolic blood pressure (SBP). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.

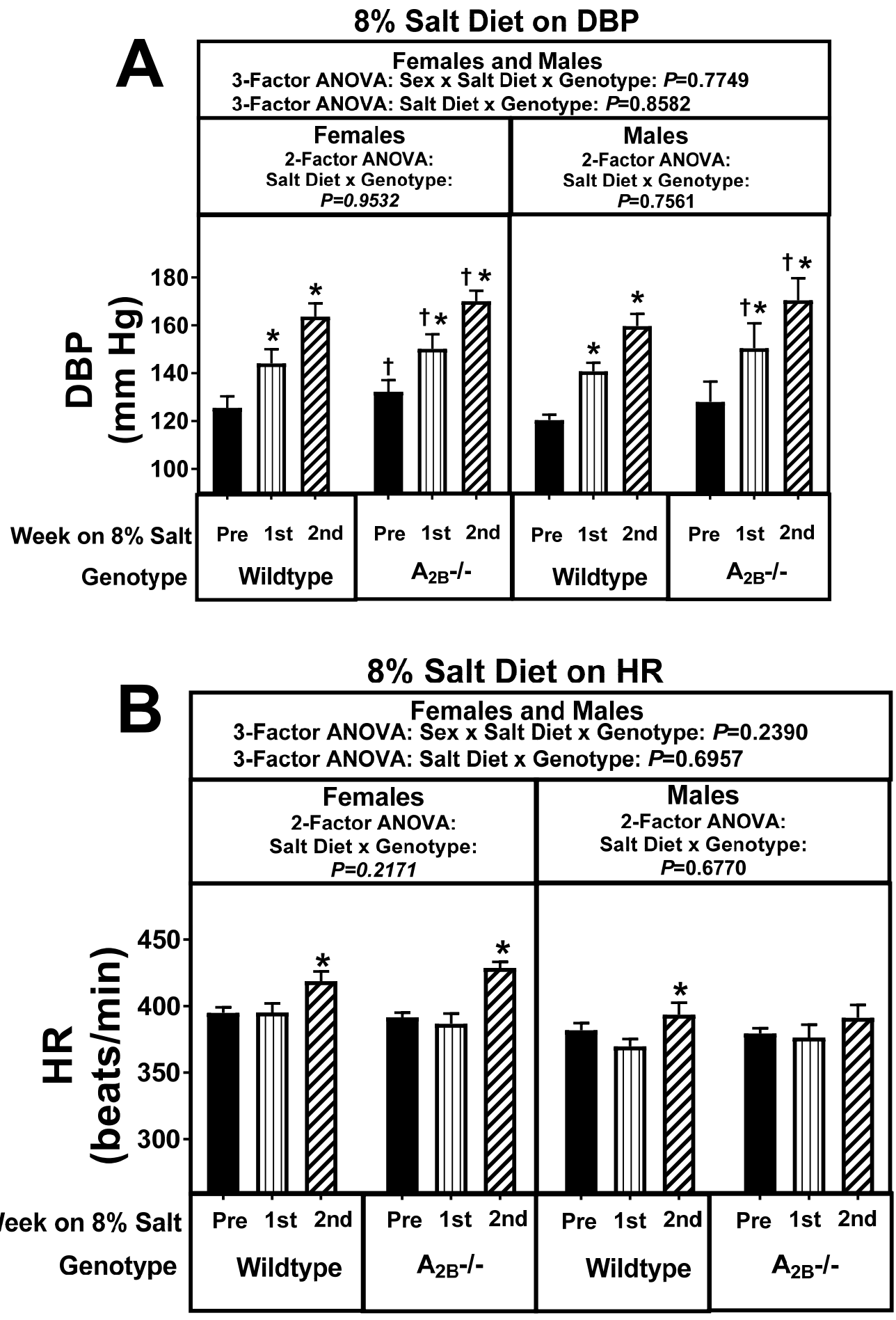

8% Salt Diet on DBP
A
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.7749
3-Factor ANOVA: Salt Diet x Genotype: P=0.8582
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.9532
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.7561
DBP (mm Hg)
180
160
140
120
100
Week on 8% Salt
Pre 1st 2nd
Pre 1st 2nd
Pre 1st 2nd
Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-
8% Salt Diet on HR
B
Females and Males
3-Factor ANOVA: Sex x Salt Diet x Genotype: P=0.2390
3-Factor ANOVA: Salt Diet x Genotype: P=0.6957
Females
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.2171
Males
2-Factor ANOVA:
Salt Diet x Genotype:
P=0.6770
HR (beats/min)
450
400
350
300
Week on 8% Salt
Pre 1st 2nd
Pre 1st 2nd
Pre 1st 2nd
Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-

Figure S24. Panel A summarizes for both female and male A_{2B} knockout (-/-) and wildtype Dahl SS rats the weekly average diastolic blood pressure (DBP) before (pre) starting the 8% salt diet and then during the 1st and 2nd weeks of the 8% salt diet. Panel B provides the same information and analysis as panel A, but for heart rate (HR). Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.

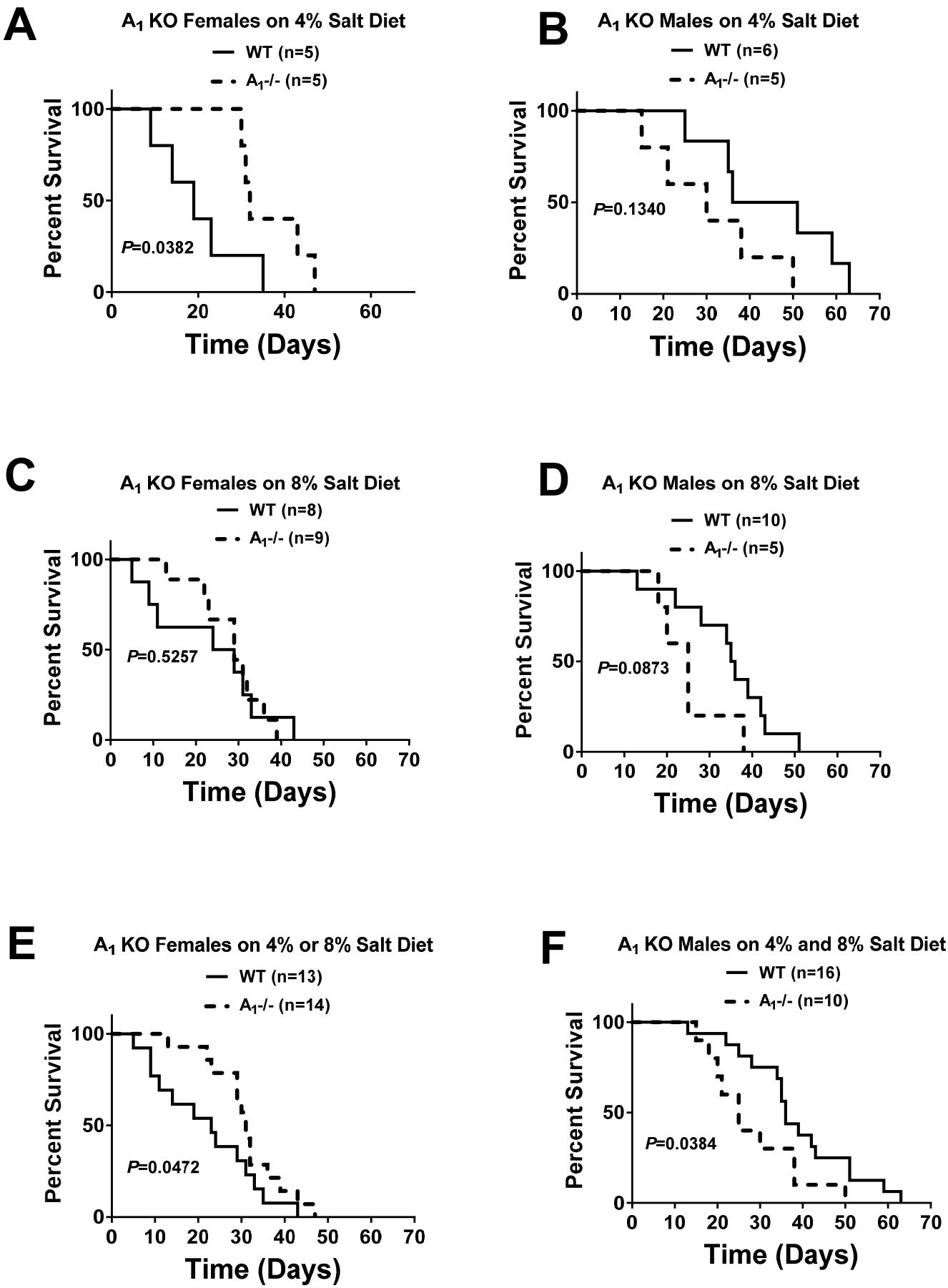
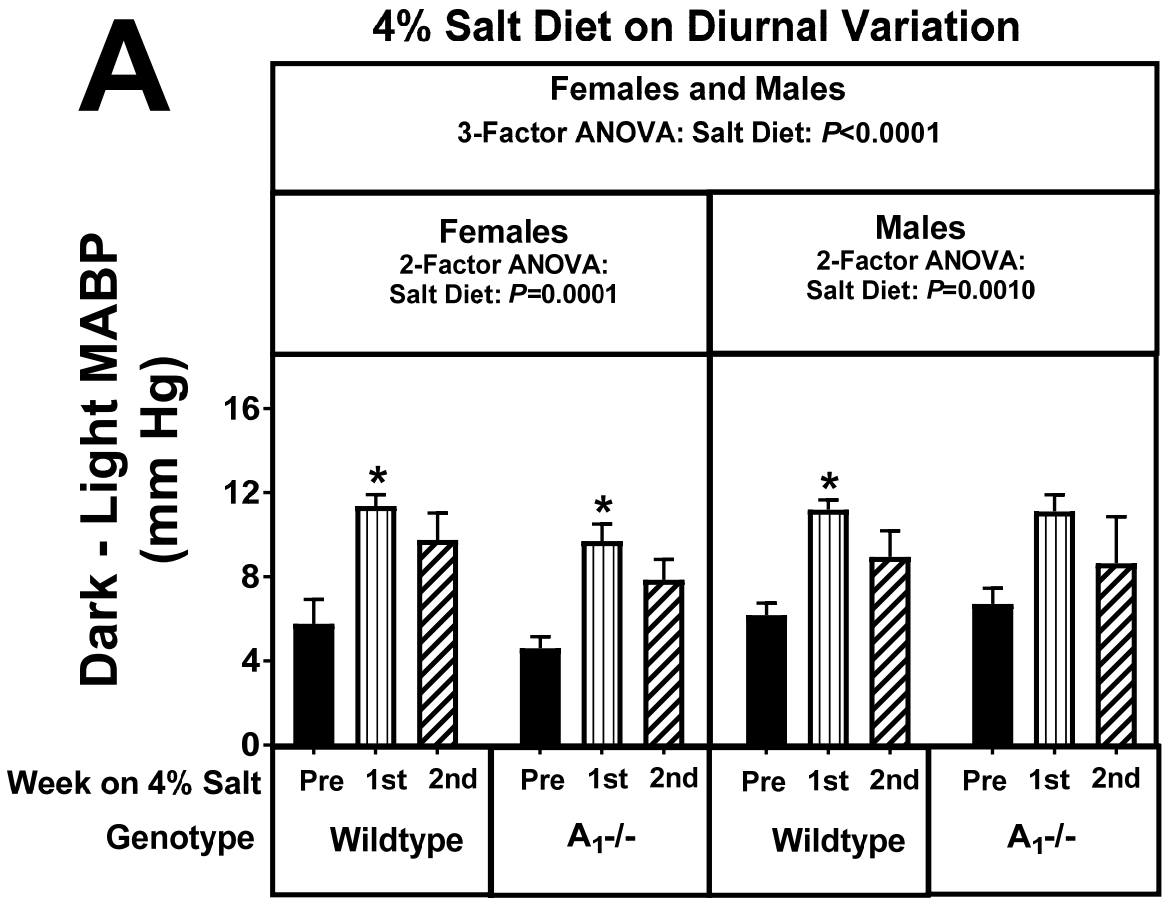

A
A1 KO Females on 4% Salt Diet
WT (n=5)
A1-/- (n=5)
Percent Survival
100
50
0
P=0.0382
0
20
40
60
Time (Days)
B
A1 KO Males on 4% Salt Diet
WT (n=6)
A1-/- (n=5)
Percent Survival
100
50
0
P=0.1340
0
10
20
30
40
50
60
70
Time (Days)
C
A1 KO Females on 8% Salt Diet
WT (n=8)
A1-/- (n=9)
Percent Survival
100
50
0
P=0.5257
0
10
20
30
40
50
60
70
Time (Days)
D
A1 KO Males on 8% Salt Diet
WT (n=10)
A1-/- (n=5)
Percent Survival
100
50
0
P=0.0873
0
10
20
30
40
50
60
70
Time (Days)
E
A1 KO Females on 4% or 8% Salt Diet
WT (n=13)
A1-/- (n=14)
Percent Survival
100
50
0
P=0.0472
0
10
20
30
40
50
60
70
Time (Days)
F
A1 KO Males on 4% and 8% Salt Diet
WT (n=16)
A1-/- (n=10)
Percent Survival
100
50
0
P=0.0384
0
10
20
30
40
50
60
70
Time (Days)

Figure S25. Figure summarizes survival curves for wildtype (WT) versus A_1 knockout (KO, A_1-/-) Dahl SS rats for females (A, C, and E) and males (B, D, and F) on either a 4% (A, B) or 8% (C, D) salt diet. Panels E and F show the survival curves for females and males when the data from all high salt diets (4% + 8%) were combined.


A
4% Salt Diet on Diurnal Variation
Females and Males
3-Factor ANOVA: Salt Diet: P<0.0001
Females
2-Factor ANOVA:
Salt Diet: P=0.0001
Males
2-Factor ANOVA:
Salt Diet: P=0.0010
Dark - Light MABP (mm Hg)
16
12
8
4
0
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-

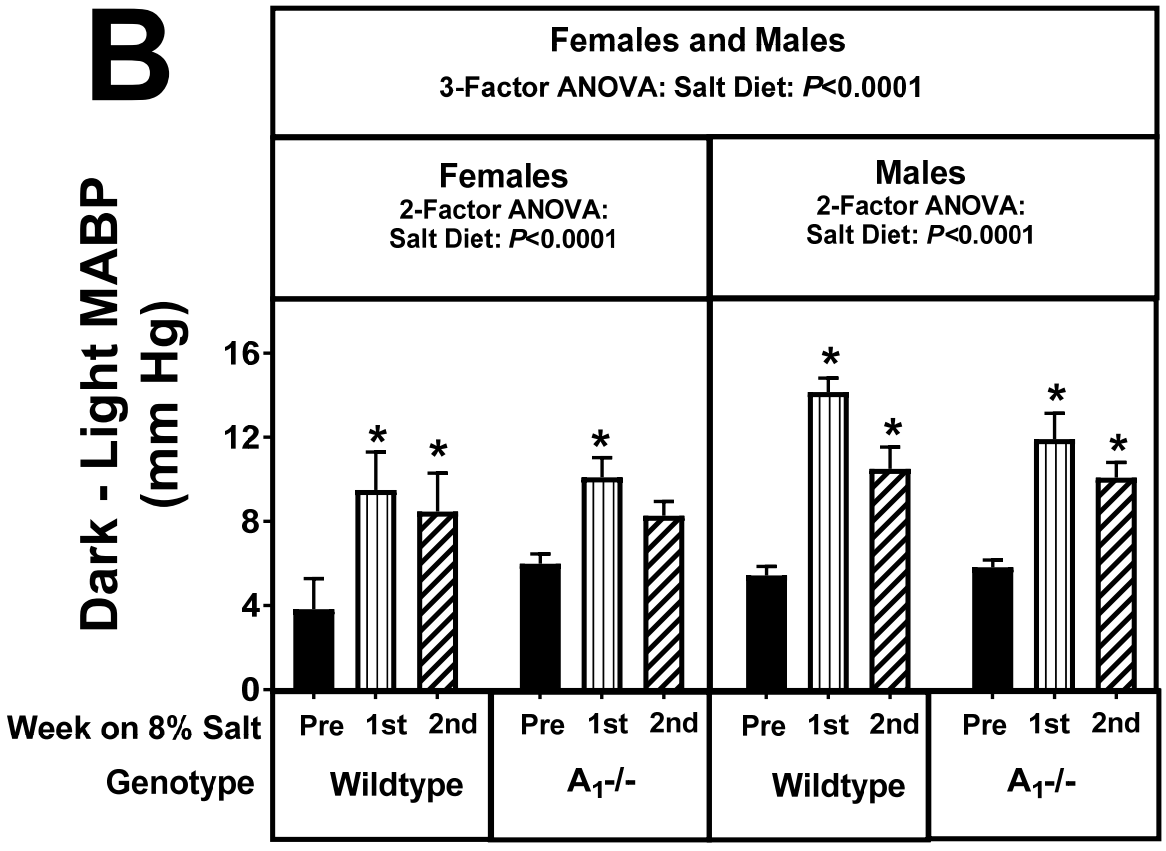
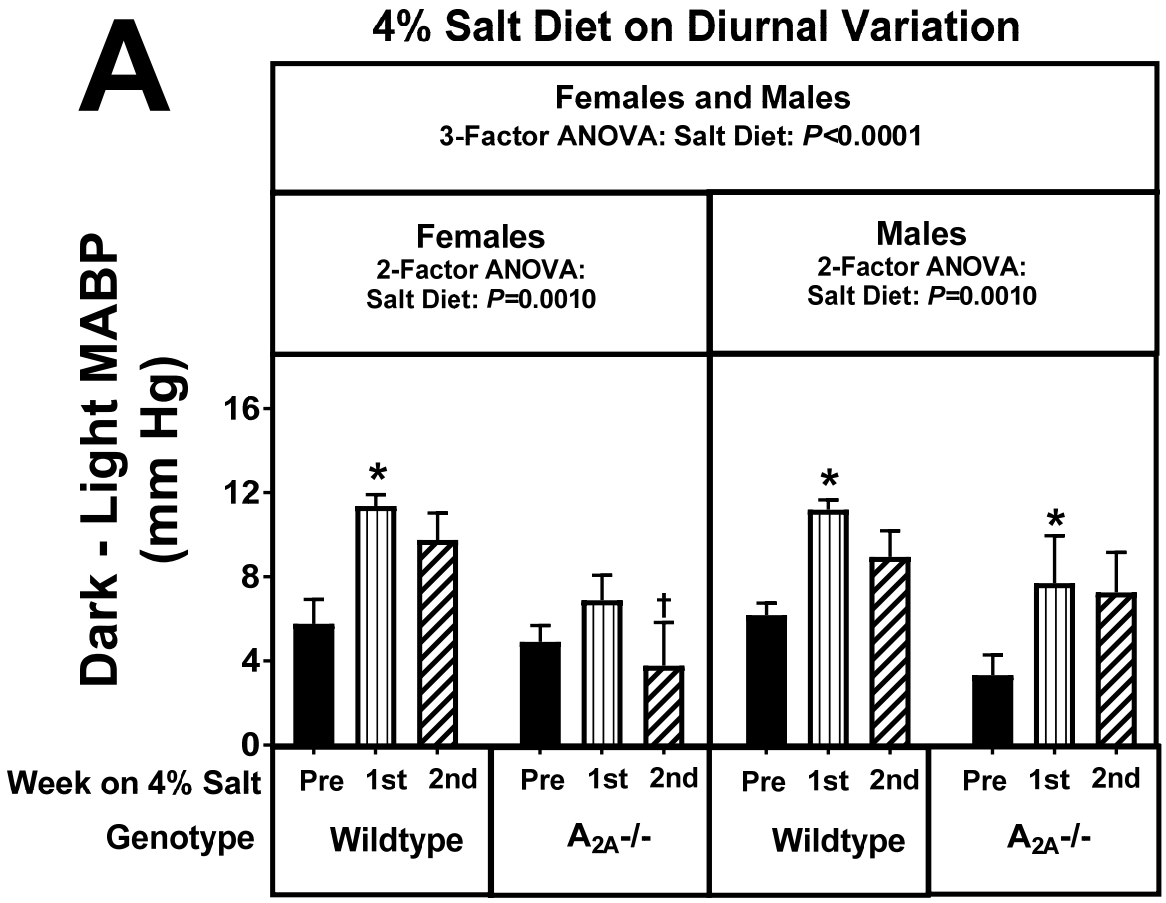

B
8% Salt Diet on Diurnal Variation
Females and Males
3-Factor ANOVA: Salt Diet: P<0.0001
Females
2-Factor ANOVA:
Salt Diet: P<0.0001
Males
2-Factor ANOVA:
Salt Diet: P<0.0001
Dark - Light MABP (mm Hg)
16
12
8
4
0
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A1-/-
Wildtype
A1-/-

Figure S26. Panel A summarizes for both female and male A_1 knockout (-/-) and wildtype Dahl SS rats the weekly diurnal variation of mean arterial blood pressure (MABP) before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel B provides the same information and analysis as panel A, but for rats on the 8% salt diet. Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period). Values are means ± SEM.


A
4% Salt Diet on Diurnal Variation
Females and Males
3-Factor ANOVA: Salt Diet: P<0.0001
Females
2-Factor ANOVA:
Salt Diet: P=0.0010
Males
2-Factor ANOVA:
Salt Diet: P=0.0010
Dark - Light MABP (mm Hg)
16
12
8
4
0
*
*
*
†
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2A-/-
Wildtype
A2A-/-

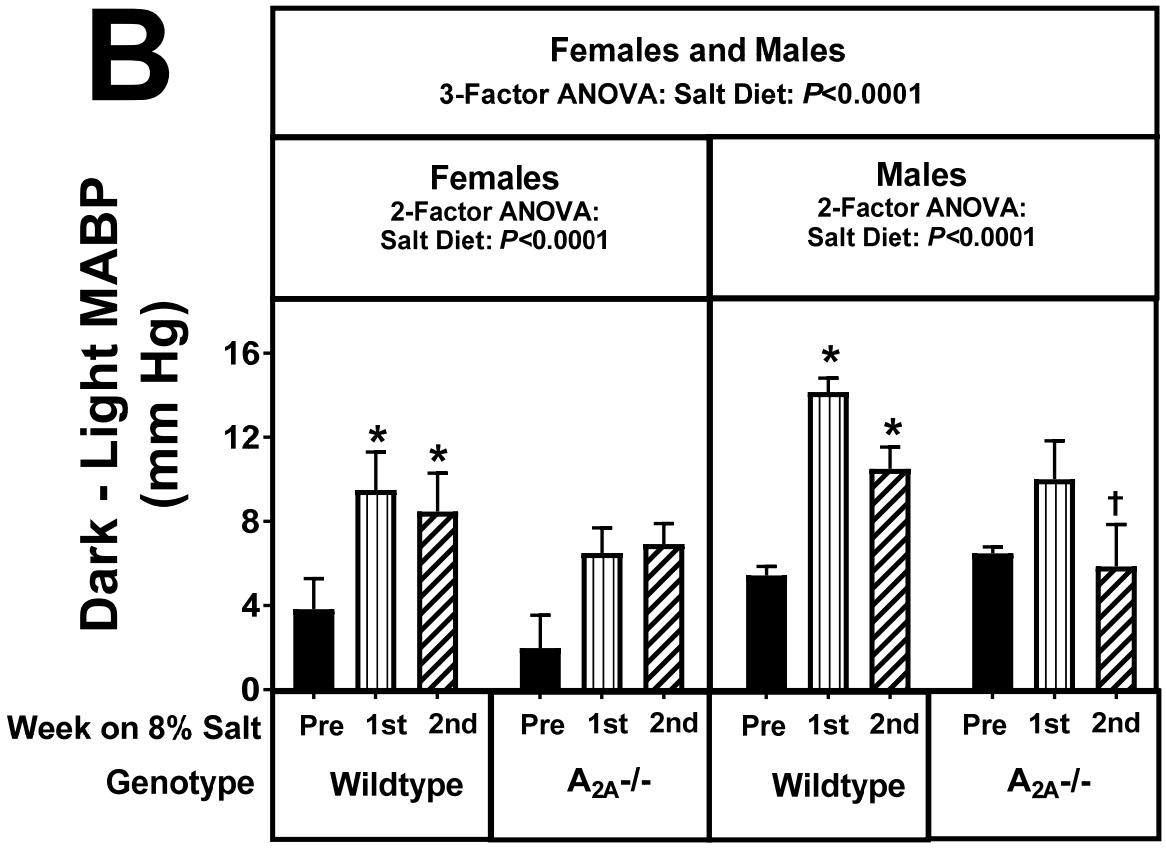
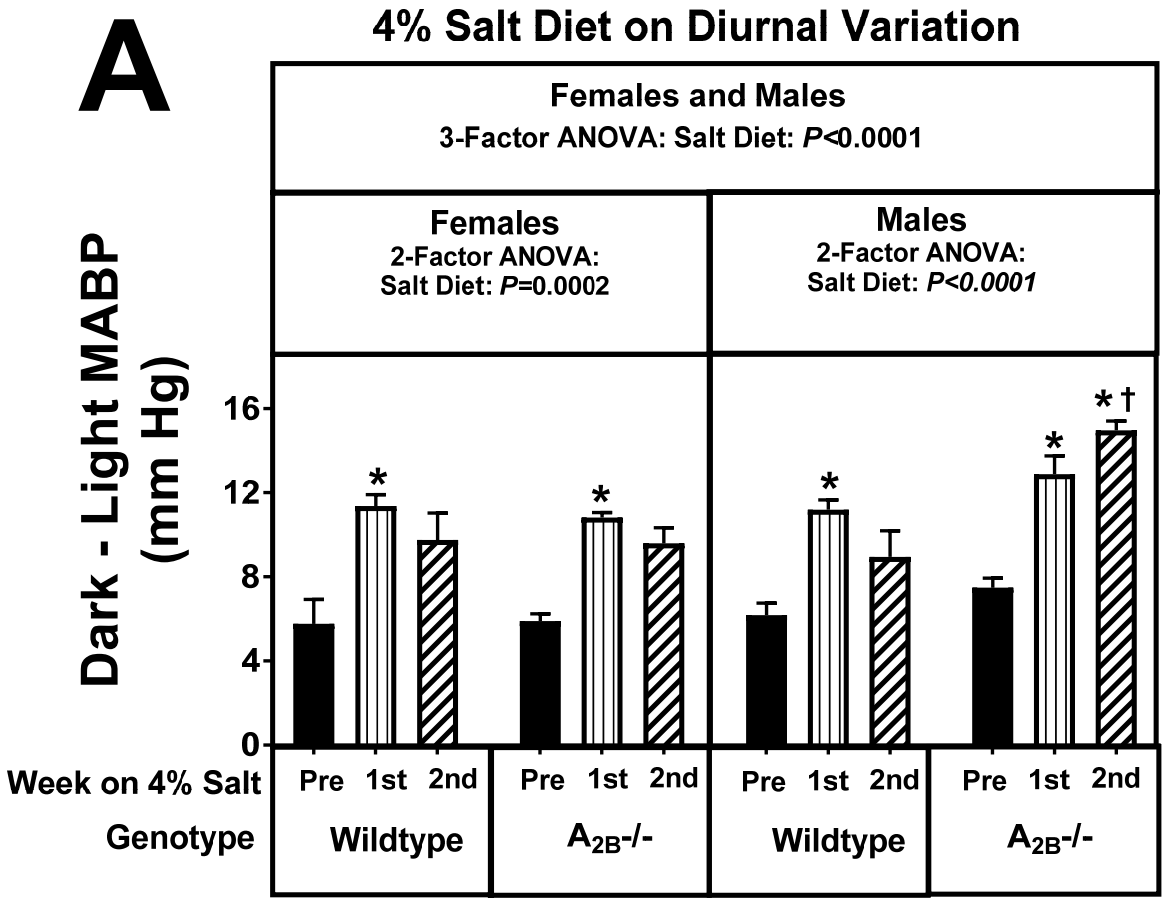

B
8% Salt Diet on Diurnal Variation
Females and Males
3-Factor ANOVA: Salt Diet: P<0.0001
Females
2-Factor ANOVA:
Salt Diet: P<0.0001
Males
2-Factor ANOVA:
Salt Diet: P<0.0001
Dark - Light MABP (mm Hg)
16
12
8
4
0
*
*
*
*
†
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2A-/-
Wildtype
A2A-/-

Figure S27. Panel A summarizes for both female and male A_{2A} knockout (-/-) and wildtype Dahl SS rats the weekly diurnal variation of mean arterial blood pressure (MABP) before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel B provides the same information and analysis as panel A, but for rats on the 8% salt diet. Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.


A
4% Salt Diet on Diurnal Variation
Females and Males
3-Factor ANOVA: Salt Diet: P<0.0001
Females
2-Factor ANOVA:
Salt Diet: P=0.0002
Males
2-Factor ANOVA:
Salt Diet: P<0.0001
Dark - Light MABP (mm Hg)
16
12
8
4
0
*
*
*
*
*†
Week on 4% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-

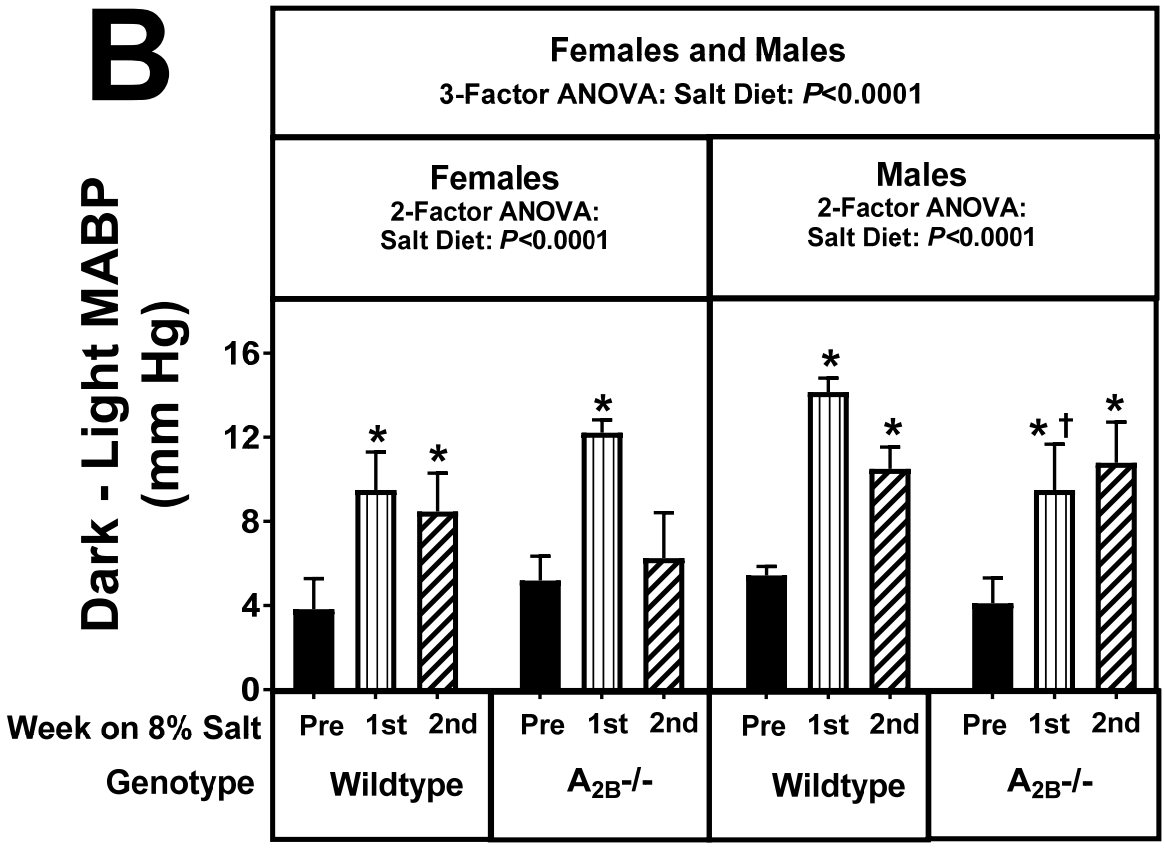

B
8% Salt Diet on Diurnal Variation
Females and Males
3-Factor ANOVA: Salt Diet: P<0.0001
Females
2-Factor ANOVA:
Salt Diet: P<0.0001
Males
2-Factor ANOVA:
Salt Diet: P<0.0001
Dark - Light MABP (mm Hg)
16
12
8
4
0
*
*
*
*
*
*†
*
Week on 8% Salt
Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd Pre 1st 2nd
Genotype
Wildtype
A2B-/-
Wildtype
A2B-/-

Figure S28. Panel A summarizes for both female and male A_{2B} knockout (-/-) and wildtype Dahl SS rats the weekly diurnal variation of mean arterial blood pressure (MABP) before (pre) starting the 4% salt diet and then during the 1st and 2nd weeks of the 4% salt diet. Panel B provides the same information and analysis as panel A, but for rats on the 8% salt diet. Data were analyzed by 2-factor and 3-factor analysis of variance (ANOVA), with specific contrasts using Bonferroni tests (*indicates $P<0.05$ versus corresponding "pre" period; †indicates $P<0.05$ versus corresponding period in wildtype). Values are means ± SEM.