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Supplemental information

Kir4.2 mediates proximal potassium effects on glutaminase activity and kidney injury

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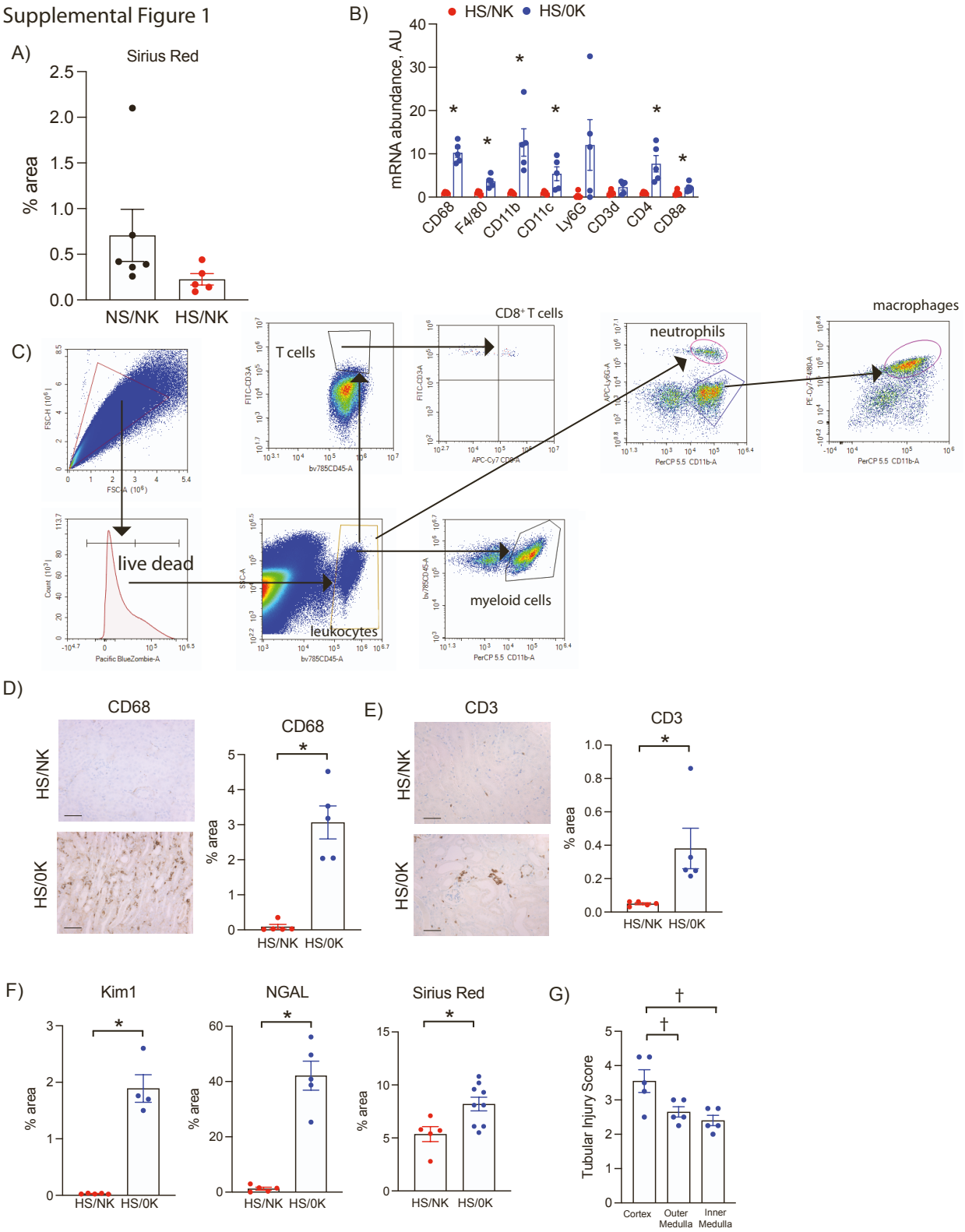
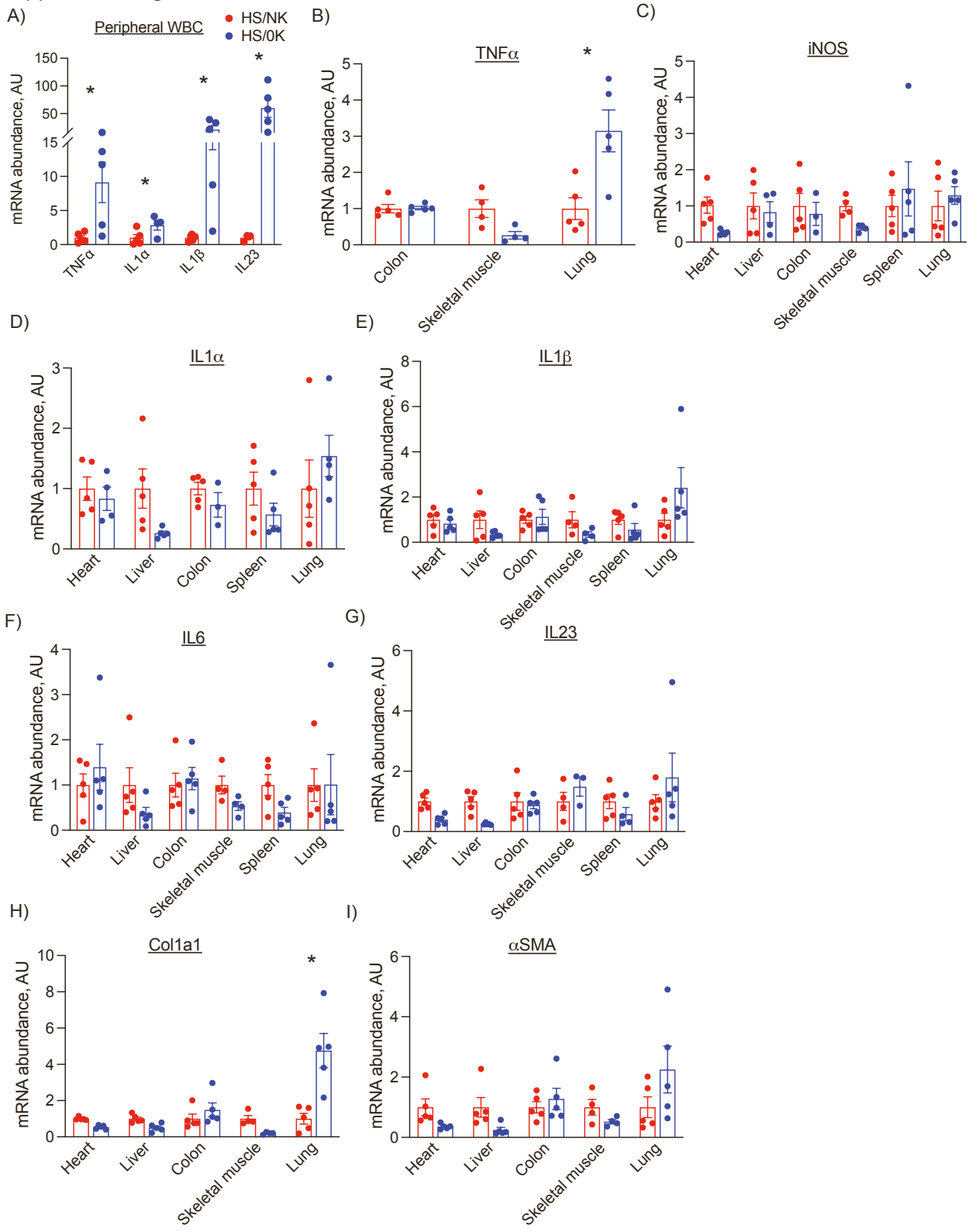
Supplemental Figure 1



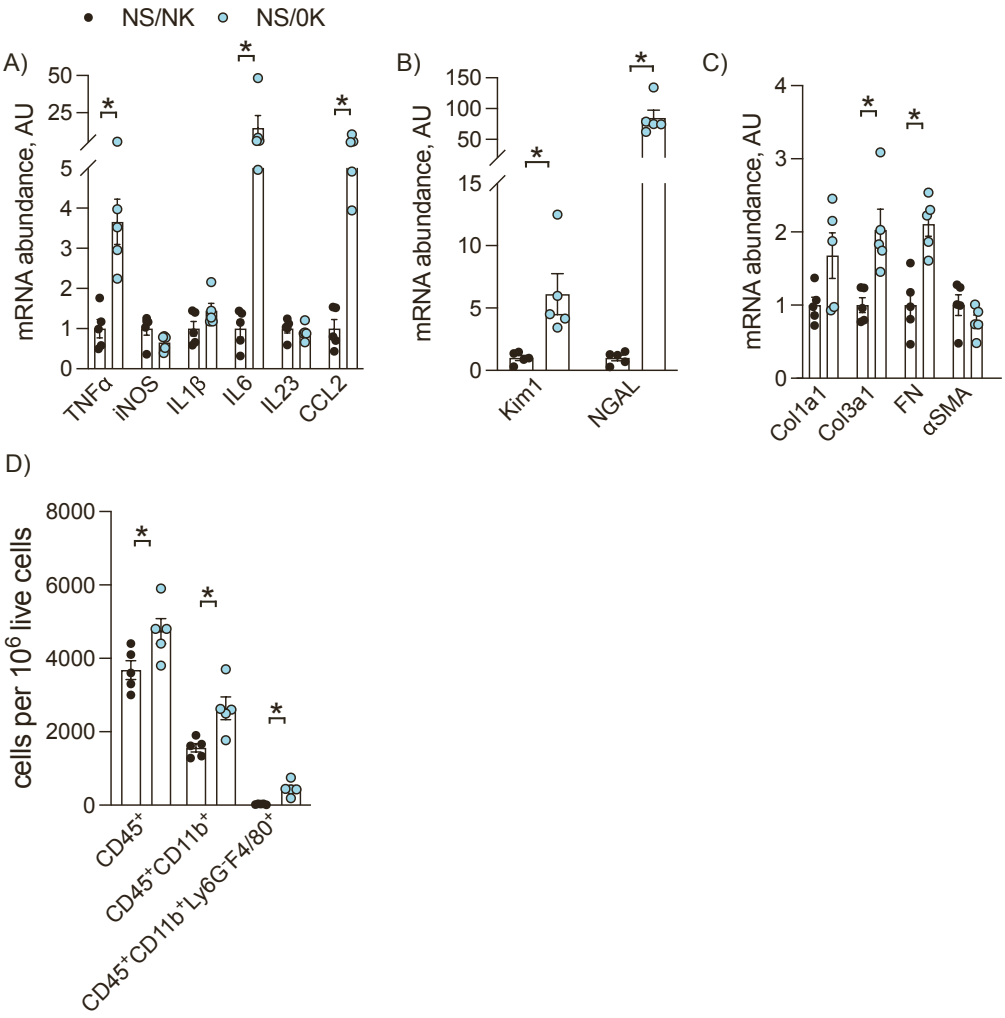
Supplemental Figure 1 Reduced dietary K^+ causes inflammation. A) Quantification of kidney Sirius red staining from animals that consumed NS/NK and HS/NK as shown in figure 1b. B) Total kidney transcript abundance of immune cell genes in animals that consumed a HS/NK and HS/0K diet for three weeks. C) Gating strategy used for flow cytometry. D, E) Representative immunohistochemical images of kidneys from animals that consumed diets as in A. Sections were stained for D) CD68 and E) CD3. F) Quantification of Kim1, NGAL, and Sirius red staining for data presented in figure 1G, H. G) Tubular injury score calculated for indicated kidney anatomical region of HS/0K fed mice. $N \geq 5$ for all panels. *, $P<0.05$ by unpaired t-test or Mann-Whitney. †, $P<0.05$ by one-way ANOVA with Dunnett's multiple comparison. Scale bar = 50 μm. Normality was determined by Shapiro-Wilk test.

Supplemental Figure 2

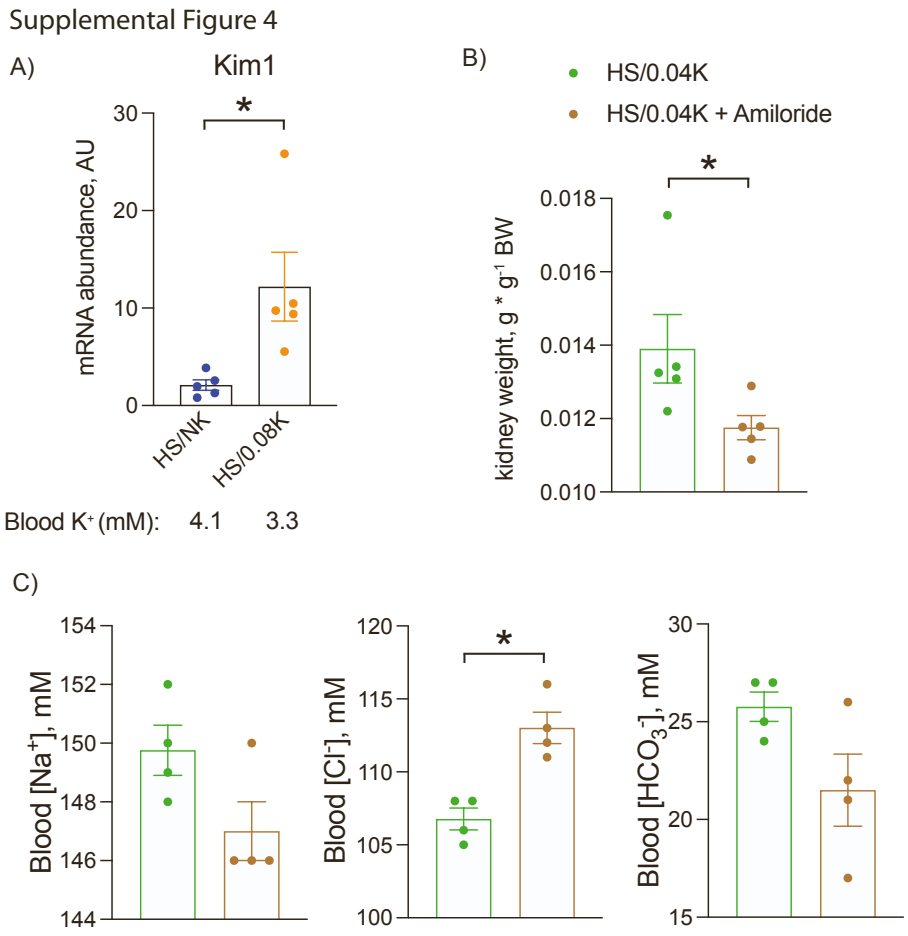


Supplemental Figure 2 Reduced dietary K^+ does not affect inflammatory or fibrotic transcript abundance in non-renal solid organs. A) Quantitative PCR data from peripheral leukocyte pellets obtain from animals treated with HS/NK and HS/0K. B-I) Whole organ transcript abundance of B) TNFα , C) iNOS, D) IL1α, E) IL1β, F) IL6, G) IL23, H) Col1a1, and I) α SMA in heart, liver, colon, skeletal muscle, spleen, and lung from animals that consumed a HS/NK and HS/0K diet for three weeks. N=5 per group. *, $P<0.05$ by unpaired t-test. Normality was determined by Shapiro-Wilk test.

Supplemental Figure 3

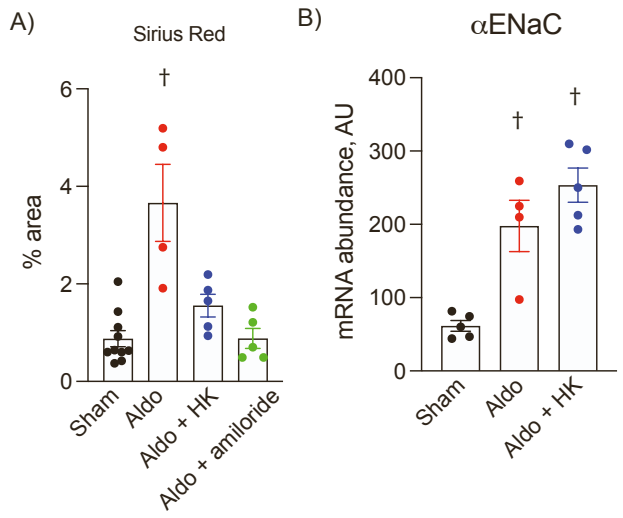


Supplemental Figure 3 Reduced dietary K^+, in the absence of high salt intake, causes kidney injury. Total kidney transcript abundance of A) inflammatory, B) injury, and C) fibrosis genes in animals that consumed a NS/NK diet compared with those that consumed a NS/0K diet for three weeks. D) Effects of the same dietary treatment as A-C on kidney abundance of immune cell subsets. *, $P<0.05$ by unpaired t-test or Mann-Whitney. Normality was determined by Shapiro-Wilk test. Additional physiological data presented in supplemental table 1.



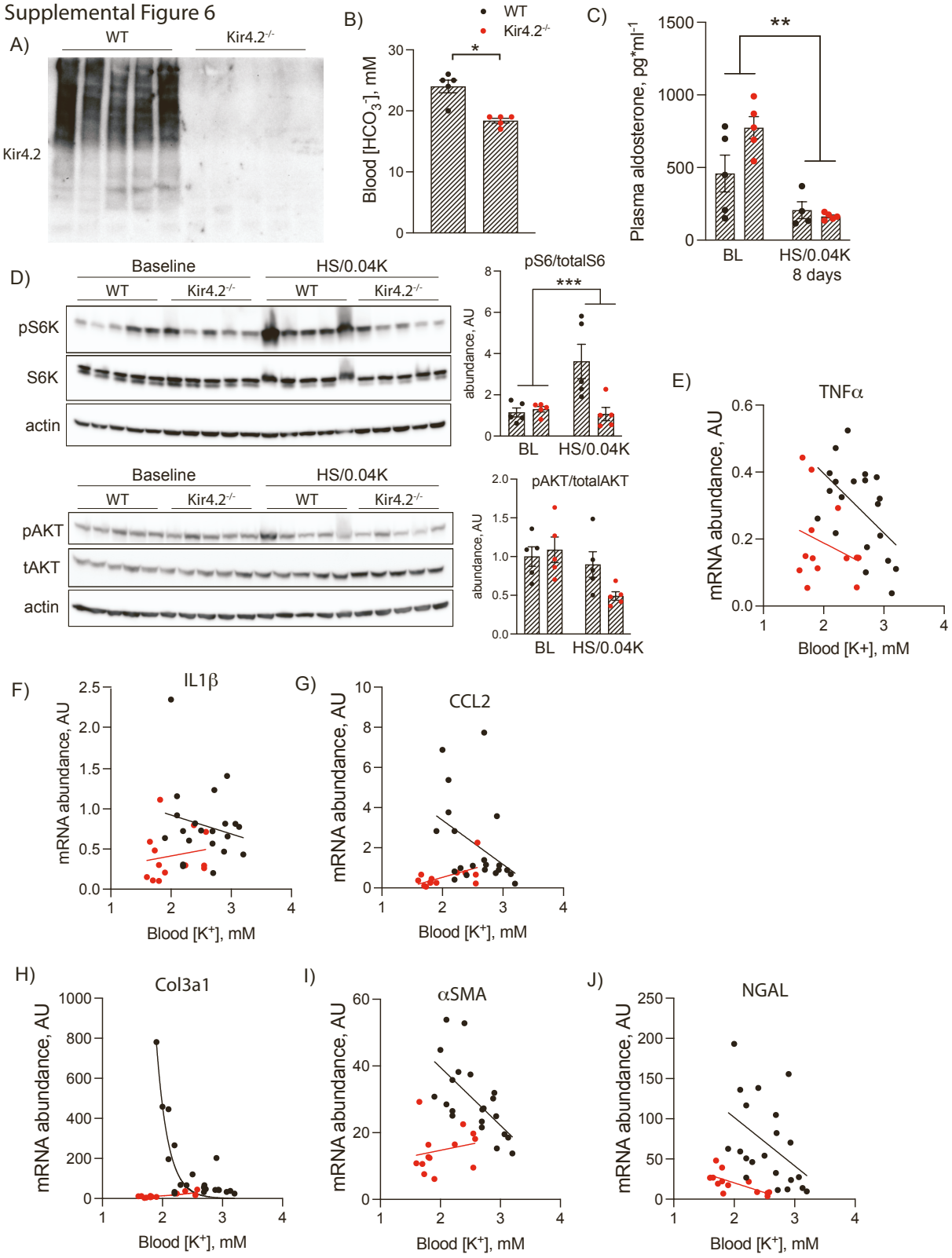
Supplemental Figure 4 Effects of amiloride on kidney weight and blood electrolytes from mice on a HS/0.04K diet. A) Total kidney Kim1 transcript abundance from animals that were fed either HS/NK or HS with 0.08% K^+ (HS/0.08K). Average blood K^+ are indicated below corresponding groups. B) Kidney weight from animals that consumed HS/0.04K for three weeks on normal drinking water and amiloride-supplemented water. C) Blood electrolytes from animals treated as in B. N=5 per group for A and B and N=4 per group for C. *, $p<0.05$ by unpaired t-test or Mann-Whitney. Normality was determined by Shapiro-Wilk test.

Supplemental Figure 5



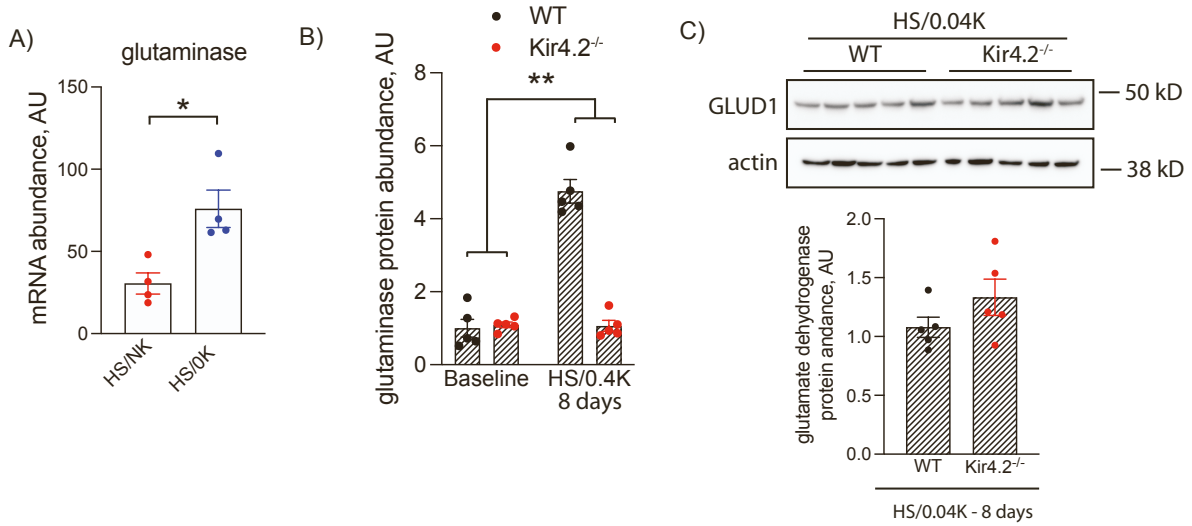
Supplemental Figure 5 Effects of aldosterone on fibrosis and αENAC mRNA abundance. A) Sirius red quantification from staining as presented in figure 3e B) Total kidney αENAC mRNA transcript abundance from animals that underwent a sham procedure, aldosterone infusion, and aldosterone infusion while also consuming a high K^+ diet. All animals were fed a high salt diet during the treatment period. N=5 for sham, aldo + HK, and aldo + amiloride groups and N=4 for the aldo group. †, $P<0.05$ by Kruskall-Wallis followed by Dunn's multiple comparison vs Sham for A and one-way ANOVA with Dunnett's multiple comparison vs Sham for B. Normality was determined by Shapiro-Wilk test.

Supplemental Figure 6



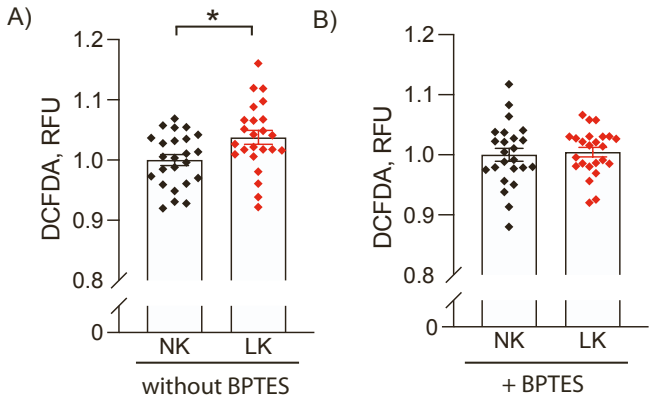
Supplemental Figure 6 Effects of dietary K^+ restriction on Kir4.2$^{-/-}$ animals. A) Kidney Western blot for Kir4.2 from WT and Kir4.2$^{-/-}$ animals. Actin is the same as in figure 7d. B) Blood $HCO3^-$ from WT and Kir4.2$^{-/-}$ mice following consumption of a HS/0.04K diet for eight days. C) Plasma aldosterone concentrations from WT and Kir4.2$^{-/-}$ mice at baseline and following consumption of a HS/0.04K diet for eight days. D) Kidney Western blot analysis of phospho-S6 kinase, total S6 kinase, phospho-AKT, and total AKT from animals treated as in C. E-J) Relationship between total kidney mRNA abundance of indicated transcript and blood K^+. Black represents WT mice and red represents Kir4.2 knockouts. *, $P<0.05$ by Mann-Whitney test. **, $P<0.05$ for HS/0.04K diet reducing aldosterone levels vs baseline without an effect of genotype as compared by two-way ANOVA. ***, $P<0.05$ for interaction by two-way ANOVA. For E-G and J, differences in slopes were not statistically different between genotypes, though transcript abundances for knockouts were lower than WT ($P<0.05$). For H and I, the differences in slopes are statistically significant ($P<0.05$). $N \geq 5$ per group. Normality was determined by Shapiro-Wilk test.

Supplemental Figure 7



Supplemental Figure 7 Quantification of kidney glutaminase and glutamate dehydrogenase abundances. A) Total kidney glutaminase mRNA abundance in WT animals that were treated with a HS/NK diet and a HS/0K diet for three weeks. B) Glutaminase protein abundance quantification from figure 6b. C) Glutamate dehydrogenase protein abundance and quantification from WT and Kir4.2$^{-/-}$ animals fed a HS/0.04K diet for 8 days. N=4 per group for A and N=5 per group for B and C. *, P<0.05 by unpaired t-test; **P<0.05 for interaction by two-way ANOVA. Normality was determined by Shapiro-Wilk test.

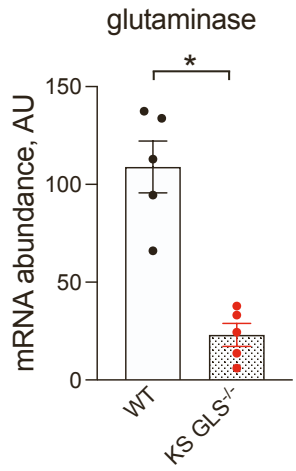
Supplemental Figure 8



Supplemental Figure 8 Effects of low K^+ culture and glutaminase inhibition on ROS production in hRPTECs.

A) Effects of normal or low K^+ culture on H_2DCFDA fluorescence in hRPTECs. B) Effects of normal or low K^+ culture on H_2DCFDA fluorescence in the presence of BPTES. RFU, relative fluorescence units. N=24 per group. *, $p<0.05$ by unpaired t-test. Normality was determined by Shapiro-Wilk test.

Supplemental Figure 9



Supplemental Figure 9 Total kidney mRNA transcript abundance of glutaminase from control and KS GLS$^{-/-}$ animals. N=5 per group. *, $p<0.05$ by unpaired t-test. Normality was determined by Shapiro-Wilk test.

Supplemental Table 1. Additional physiological data for NS/NK and NS/0K-treated mice (Supplemental Figure 3). Data are presented as mean ± SEM. * indicates $P<0.05$ by Mann-Whitney test.

	Treatment	
	NS/NK	NS/0K
BW, g	25.3 ± 0.69	23.3 ± 0.55
kidney weight, g/g BW	0.0119 ± 0.00043*	0.0152 ± 0.00025*
Na, mM	141.9 ± 0.32	141.5 ± 0.67
K, mM	3.6 ± 0.07*	2.4 ± 0.06*
Cl, mM	not measured	not measured
HCO3, mM	not measured	not measured
BUN, mg/dL	not measured	not measured

Supplemental Table 2a. Additional physiological data for dietary K^+ titration (Figure 2). Data are presented as mean ± SEM. ** indicates P<0.05 by Kruskal-Wallis followed by Dunn's multiple comparison test vs 0.8% group.

	Dietary K, % w/w						
	0	0.02	0.04	0.08	0.27	0.53	0.8
BW, g	22.4 ± 0.90**	23.5 ± 0.56**	24.3 ± 1.10	25.5 ± 0.55	27.4 ± 1.22	27.5 ± 0.54	28.3 ± 1.00
Na, mM	151.8 ± 1.11	148.3 ± 0.94	147.8 ± 0.63	148 ± 0.45	146 ± 0.71	144.2 ± 0.66	146.8 ± 0.2
Cl, mM	107.7 ± 0.33	103 ± 1.08	103.3 ± 1.3	108 ± 0.71	108.6 ± 1.03	108.8 ± 0.66	109.3 ± 0.95
HCO3, mM	23.3 ± 0.95	24.8 ± 1.31	26.8 ± 1.11	22.6 ± 0.68	18.8 ± 0.92	22.2 ± 1.77	21.2 ± 0.86
BUN, mg/dL	46 ± 1.78**	22.3 ± .63	23 ± 1.68	29.8 ± 2.67**	26.5 ± 5.92	21.8 ± 0.97	20.2 ± 0.58

Supplemental Table 2b. Additional physiological data for amiloride-treated animals (Figure 2). Data are presented as mean ± SEM. * indicates P<0.05 by Mann-Whitney test.

	Treatment	
	HS/0.04K	HS/0.04K + Amiloride
BW, g	26.4 ± 0.78	24.6 ± 0.82
BUN, mg/dL	18.8 ± 2.50	22.8 ± 3.07

Supplemental Table 3. Additional physiological data for aldosterone-infused animals (Figure 3). Data are presented as mean ± SEM. ** indicates P<0.05 by Kruskal-Wallis followed by Dunn's multiple comparison test vs Sham.

	Treatment			
	Sham	Aldo	Aldo + HK	Aldo + Amiloride
BW, g	32.3 ± 0.43	28.5 ± 0.24**	27.5 ± 0.70**	32.6 ± 0.84
Na, mM	144.4 ± 0.50	146.8 ± 0.48	153.6 ± 0.51**	147.0 ± 0.45**
Cl, mM	108.8 ± 0.74	92.3 ± 1.25**	111.2 ± 0.86	112.4 ± 0.51
HCO3, mM	23.7 ± 0.92	40.5 ± 1.76**	30.2 ± 1.02	22.8 ± 0.2
BUN, mg/dL	24.0 ± 0.60	16.0 ± 1.22**	14.6 ± 1.33**	15.4 ± 0.75**

Supplemental Table 4. Additional physiological data for HCO_3-reated animals (Figure 4). Data are presented as mean ± SEM. * indicates $P<0.05$ by Mann-Whitney test.

	Treatment	
	HS/0.04K + NaCl	HS/0.04K + NaHCO3
BW, g	18.7 ± 0.46	17.5 ± 1.22
Na, mM	152.8 ± 0.74	151.6 ± 1.86
BUN, mg/dL	19.8 ± 2.22	23.2 ± 2.52
UNH_4, mmol/mmol Cr	25.7 ± 9.92	0.84 ± 0.76

Supplemental Table 5. Additional physiological data for HS/0.04K-treated wild-type and Kir4.2 knockout animals (Figure 5a-g). Data are presented as mean ± SEM. * indicates P<0.05 by Mann-Whitney test vs WT at the indicated timepoint.

	Wild-type		Kir4.2 knockout	
	HS/0.04K - 3 days	HS/0.04K - 8 days	HS/0.04K - 3 days	HS/0.04K - 8 days
BW, g	21.1 ± 0.22	22.8 ± 0.75*	20.1 ± 0.60	19.8 ± 1.08*
Na, mM	147.0 ± 0.53	149.5 ± 0.50	147.2 ± 0.65	149.1 ± 0.84
Cl, mM	110.4 ± 1.45	108.0 ± 0.80*	112.6 ± 0.56	114.8 ± 1.07*
HCO3, mM	not measured	not measured	not measured	not measured
BUN, mg/dL	not measured	not measured	not measured	not measured

Supplemental Table 6. Additional physiological data for aldosterone-treated wild-type and Kir4.2 knockout animals (Figure 5h-k). Data are presented as mean ± SEM. * indicates $P<0.05$ by Mann-Whitney test.

	Wild-type	Kir4.2 knockout
BW, g	24.1 ± 0.46*	22.1 ± 0.59*
kidney weight, g/g BW	0.0148 ± 0.00027	0.0143 ± 0.00032
Na, mM	147.5 ± 0.55*	144.2 ± 0.80*
Cl, mM	102.7 ± 0.85*	107.3 ± 1.62*
HCO3, mM	not measured	not measured
BUN, mg/dL	not measured	not measured

Supplemental Table 7. Additional physiological data for HS/0.04K-treated wild-type and KS GLS knockout animals (Figure 6). Data are presented as mean ± SEM. * indicates $P<0.05$ by Mann-Whitney test.

	Wild-type	GLS knockout
BW, g	29.6 ± 0.56*	32.1 ± 0.62*
Na, mM	145.8 ± 0.92	146.0 ± 1.30
Cl, mM	104 ± 0.89	105.4 ± 0.40
BUN, mg/dL	23.4 ± 1.69	25.0 ± 0.89

Supplemental Table 8. TaqMan™ qPRC probes

Transcript	Source	Identifier
TNFa	Thermo Fisher	Mm99999068
iNOS	Thermo Fisher	Mm00440502
IL1a	Thermo Fisher	Mm00439621
IL1b	Thermo Fisher	Mm00434228
IL6	Thermo Fisher	Mm00446190
IL23	Thermo Fisher	Mm00518984
CCL2	Thermo Fisher	Mm00441242
Kim1	Thermo Fisher	Mm00506686
NGAL	Thermo Fisher	Mm01324470
Col1a1	Thermo Fisher	Mm00801666
Col3a1	Thermo Fisher	Mm01254476
FN	Thermo Fisher	Mm01256744
aSMA	Thermo Fisher	Mm01546133
TGFb1	Thermo Fisher	Mm01178820
RPS18	Thermo Fisher	Mm02601777
GLS1	Thermo Fisher	Mm01257297
CD68	Thermo Fisher	Mm03047343
F4/80	Thermo Fisher	Mm00802529
CD11b	Thermo Fisher	Mm00434455
CD11c	Thermo Fisher	Mm00498701
Ly6G	Thermo Fisher	Mm04934123
CD3d	Thermo Fisher	Mm00442746
CD4	Thermo Fisher	Mm00442754
CD8a	Thermo Fisher	Mm01182107

Supplemental Table 9. Genotyping Oligonucleotides

Sequence	Source	Identifier
GGTAGGAGATTAACACCATACTG	Sigma	Kir4.2 F
GAGAGTCCACTTTCATAATGCAG	Sigma	Kir4.2 R
GGCCTGCTTAATGTTTCCTG	Sigma	GLS floxed F
GGCATATCCCTGAGTTCGAG	Sigma	GLS floxed R
CCA TGT CTA GAC TGG ACA AGA	Sigma	Pax8 F
CTC CAG GCC ACA TAT GAT TAG	Sigma	Pax8 R
TGGGCGGCATGGTGCAAGTT	Sigma	LC1 F
CGGTGCTAACCAGCGTTTTC	Sigma	LC1 R