

Supplemental Information

Essential role of Kir5.1 channels in renal salt handling and blood pressure control

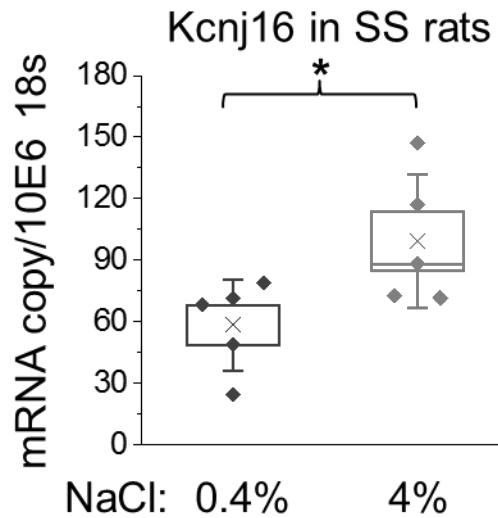
Oleg Palygin, Vladislav Levchenko, Daria V. Ilatovskaya, Tengis S. Pavlov, Oleh M. Pochynyuk, Howard J. Jacob, Aron M. Geurts, Matthew R. Hodges, and Alexander Staruschenko

Key Resources Table

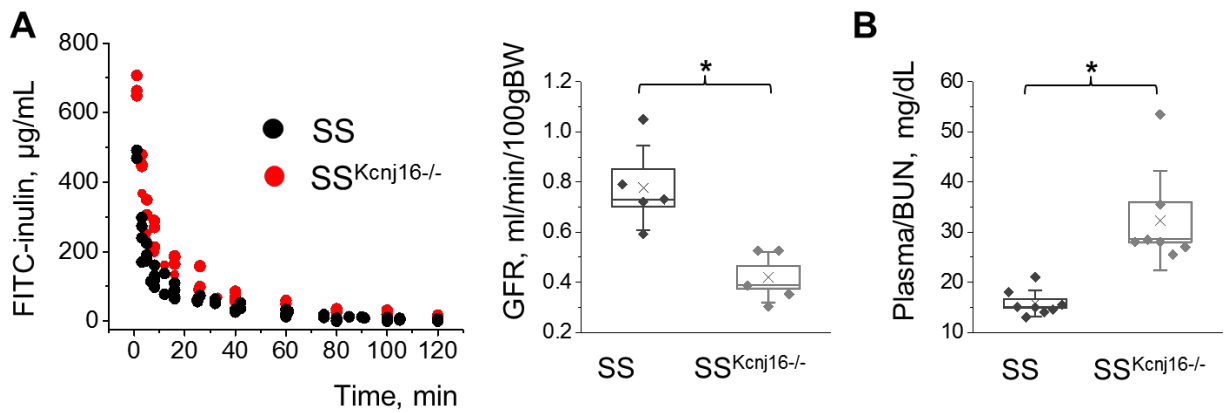
REAGENT or RESOURCE	SOURCE	IDENTIFIER
K _{ir} 4.1	Alomone Labs	APC-035 lot# apc035an1102
K _{ir} 5.1 C-terminal	Sigma Aldrich	SAB4501636 lot# 211083
NCC	Dr. David H. Ellison (Oregon University, Portland)	N/A
p-NCC	Dr. David H. Ellison (Oregon University, Portland) PhosphoSolutions	N/A p1311-53
NKCC2	StressMarq	SPC-401D; lot# 1202 (used for figure 5)

p-NKCC2	Dr. Pablo Ortiz (Henry Ford Hospital, Detroit)	N/A
NKCC2	Dr. Pablo Ortiz (Henry Ford Hospital, Detroit)	N/A (used for figure 8)
ENaC- α	Alomone Labs	ASC-030 lot# ASC030AN0402
ENaC- β	StressMarq	SPC-404-D lot# 1006
ENaC- γ	StressMarq	SPC-405-D lot# 1006
K _{ir} 5.1	Abcam	ab74130
K _{ir} 4.1	Abcam	ab105102
AQP2	Santa Cruz Biotechnology	sc-28629
Alexa Fluor 488	Molecular Probes	A-11034
Alexa Fluor 633	Molecular Probes	A-21071
Diets, Chemicals		
AIN-76A rodent 0.4% NaCl	Dyets, Inc; http://dyets.com/	#D113755
AIN-76A rodent 4% NaCl	Dyets, Inc; http://dyets.com/	#D113756
AIN-76A rodent 0.4% NaCl; 2% KCl	Dyets, Inc; http://dyets.com/	#D113521
AIN-76A rodent 4% NaCl; 2% KCl	Dyets, Inc; http://dyets.com/	#D113522
HCTZ	Sigma Aldrich	H4759
furosemide	Sigma Aldrich	PHR1057
benzamil	R&D Systems (Tocris)	3380

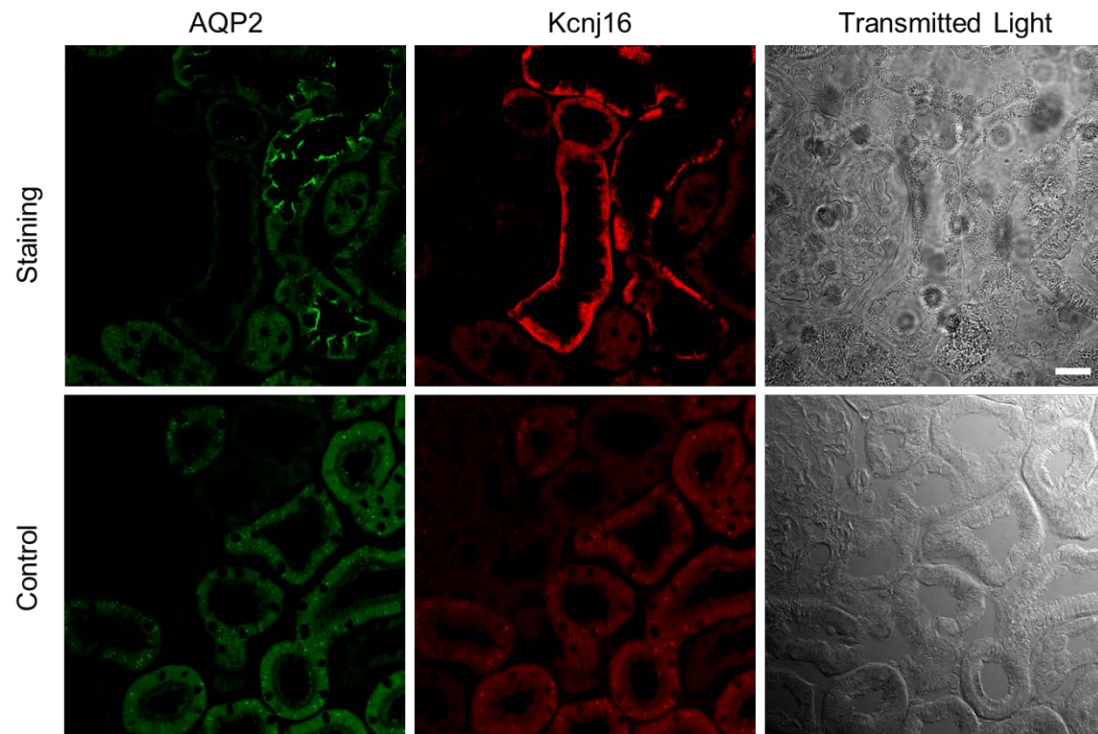
FITC-inulin	TdB Consultancy AB	http://tdbcons.com/fitc-inulin-3
Experimental Models		
SS-Kcnj16 ^{em1Mcowi}	http://rgd.mcw.edu/	6893423
SS/JrHsdMcowi	http://rgd.mcw.edu/	61499
Primers for mRNA analysis		
KCNJ16-1F	TGAGACCCAAACCACCATCG	
KCNJ16-1R	GTGCGAAATAGCTGAAGCGG	
Software and Algorithms		
MetaMorph	Molecular Devices	www.moleculardevices.com
pClamp 10.2	Molecular Devices	www.moleculardevices.com
OriginPro 7.0	OriginLab	http://originlab.com/



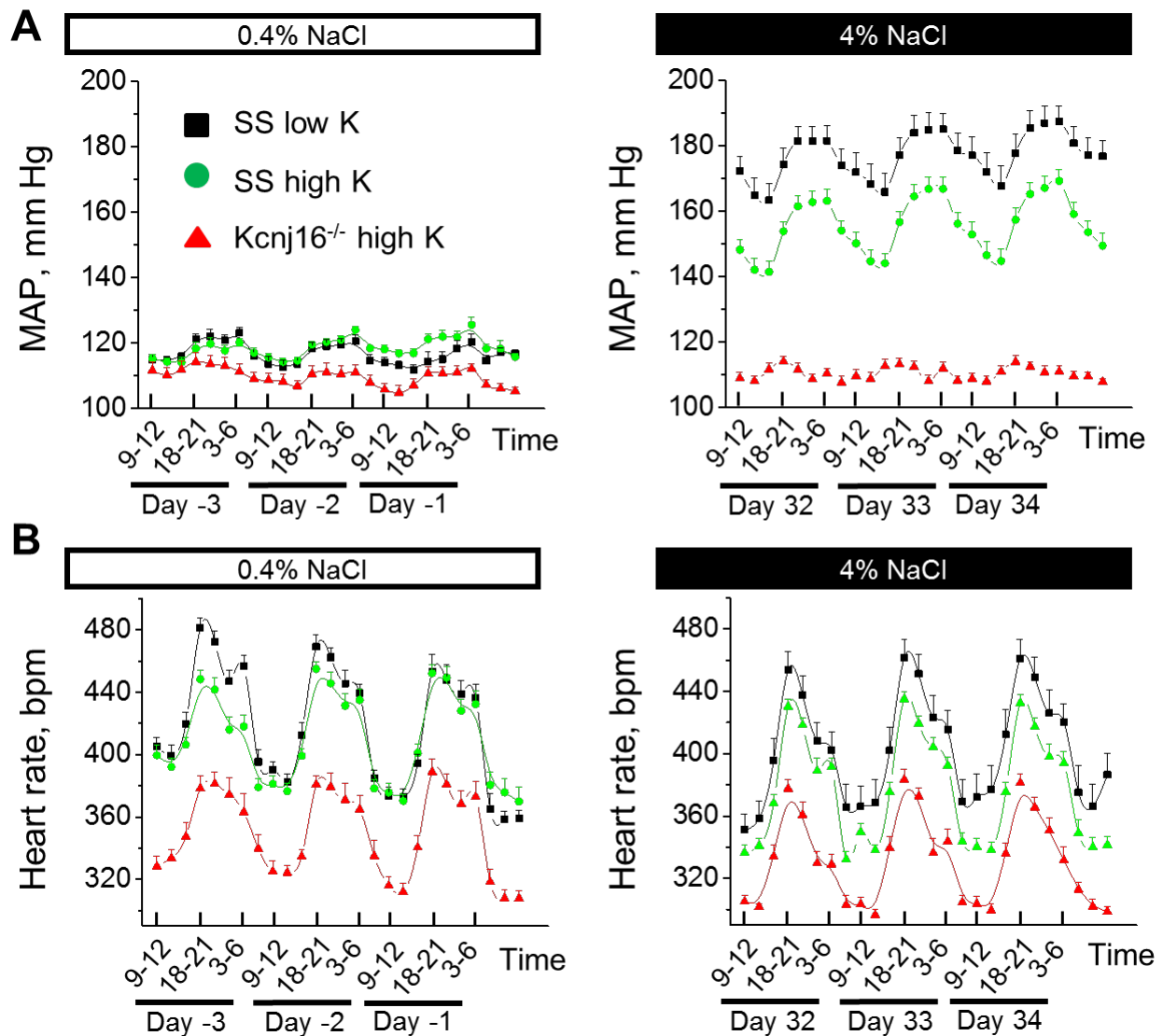
Supplementary Figure 1. mRNA expression of Kcnj16 in SS rats fed a low and high salt diets. mRNA expression was determined by quantitative polymerase chain reaction (qPCR) from RNA extracted from homogenates of renal cortical tissue collected from SS rats maintained on 0.4% or 4% (3 weeks) NaCl diet. Comparisons between groups were made using one-way ANOVA. A probability value of $P < 0.05$ was considered statistically significant.



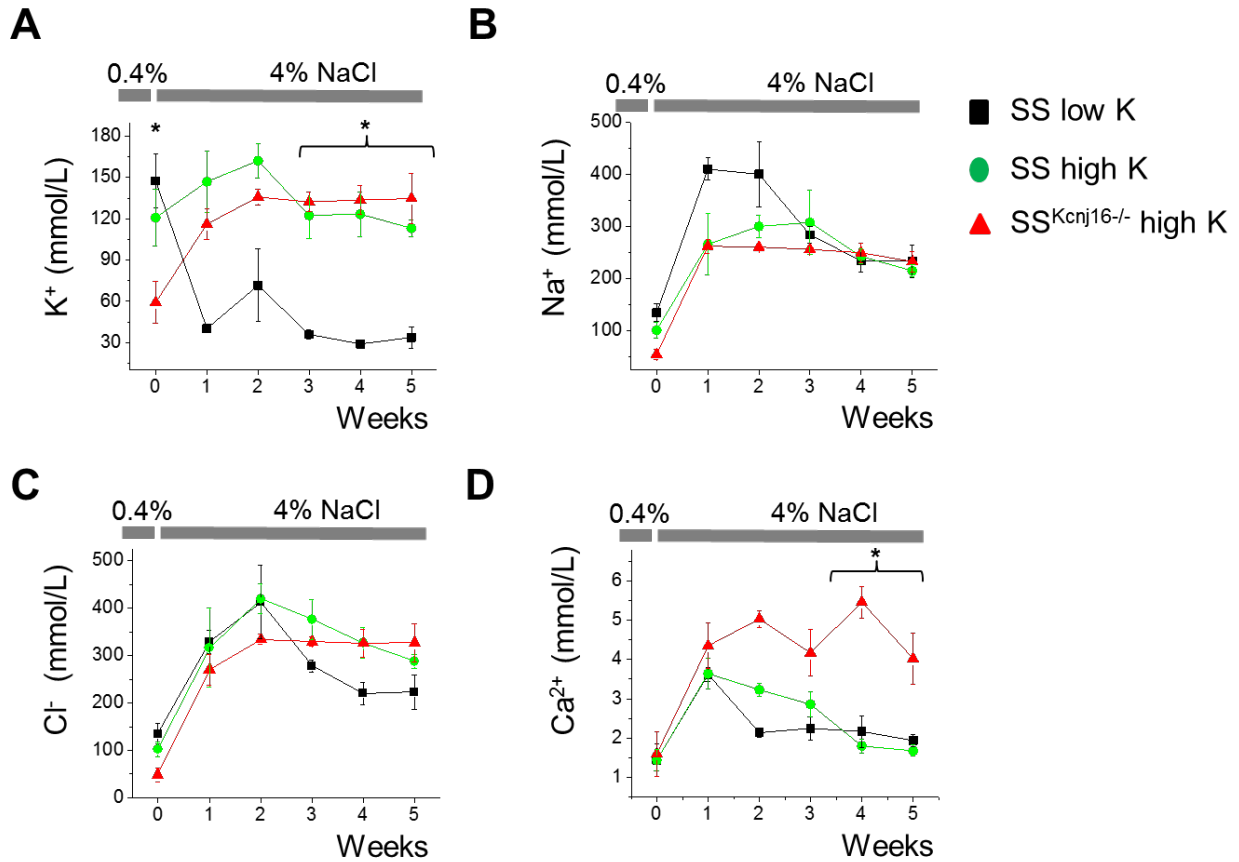
Supplementary Figure 2. Renal function of $\text{SS}^{\text{Kcnj16-/-}}$ rats. (A) Difference in glomerular filtration rate (GFR) between SS and $\text{SS}^{\text{Kcnj16-/-}}$ rats (N=5 rats). Shown are representative FITC-labeled inulin plasma distribution and elimination curves obtained after a single tail injection of FITC-inulin, and corresponding summary graph of GFR values normalized to 100 g of body weight (BW). (B) The blood urea nitrogen (BUN) level is higher in $\text{SS}^{\text{Kcnj16-/-}}$ rats (N $\geq$ 6). Comparisons between groups were made using one-way ANOVA. A probability value of $P < 0.05$ was considered statistically significant.



Supplementary Figure 3. Control staining for image shown on Figure 3A. Immunostaining images of Aqp2 (marker of collecting duct principal cells) and Kcnj16. Images taken with transmitted light and control images (stained without primary abs) are also shown. Scale bar is 20 μm .



Supplementary Figure 4. Circadian variations in blood pressure and heart rates in SS and SS^{Kcnj16^{-/-}} rats. (A) Mean arterial pressure (MAP) for SS and SS^{Kcnj16^{-/-}} rats in control (0.4% NaCl) and after 30 days on a high salt diet (4% NaCl, with or without potassium supplement (2% KCl)). (B) Changes in the heart rates for the same conditions as in (A) (N≥8).



Supplementary Figure 5. Changes in urinary electrolytes during high sodium / high potassium diet challenge. Urine concentration of K^+ (A), Na^+ (B), Cl^- (C) and Ca^{2+} (D) measured by radiometer gas analyzer in control (0.4% NaCl) and weekly after the addition of high salt (4% NaCl, with or without potassium supplement (2% KCl)) for SS and SS^{Kcnj16-/-} rats ($N \geq 8$). Comparisons between groups were made using repeated measures ANOVA. A probability value of $P < 0.05$ was considered statistically significant.

Figure 1

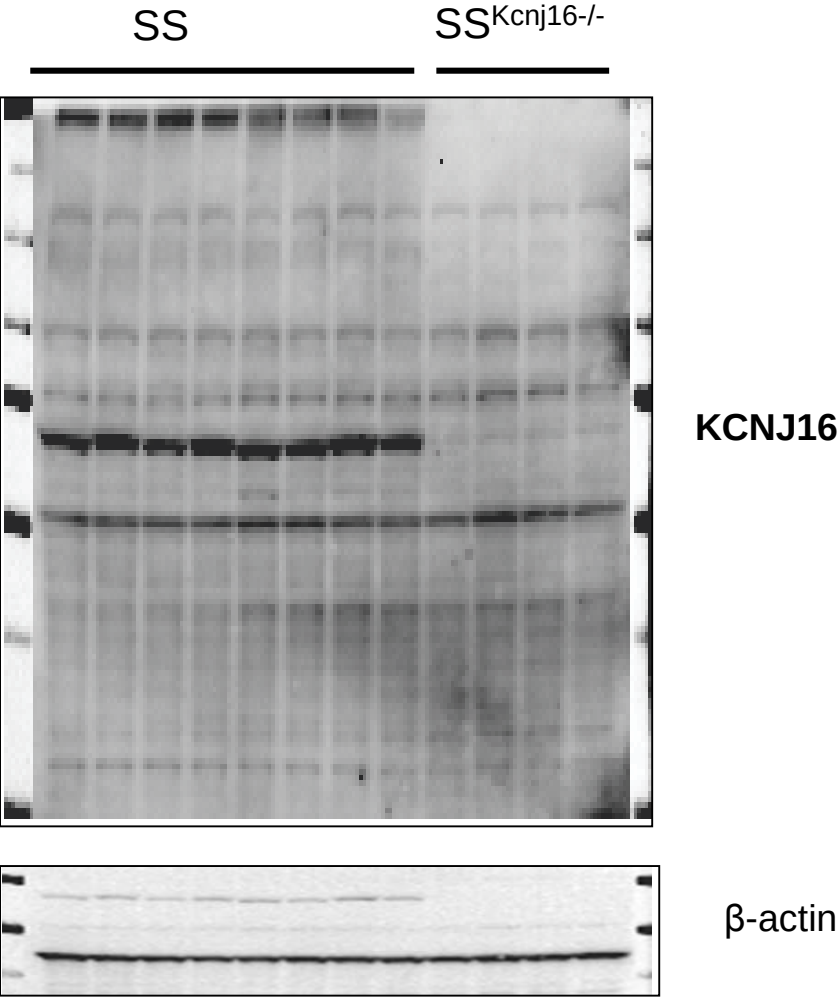
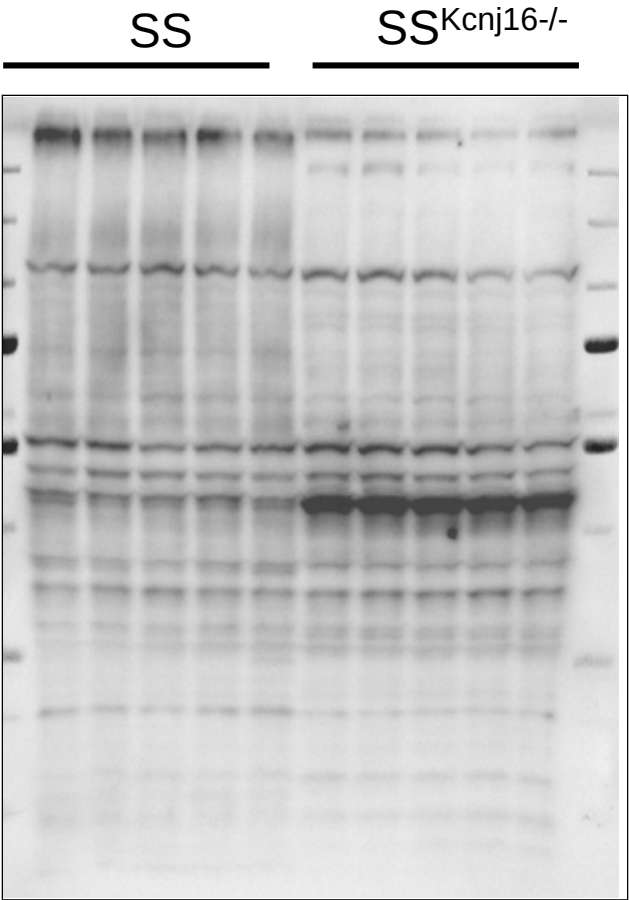
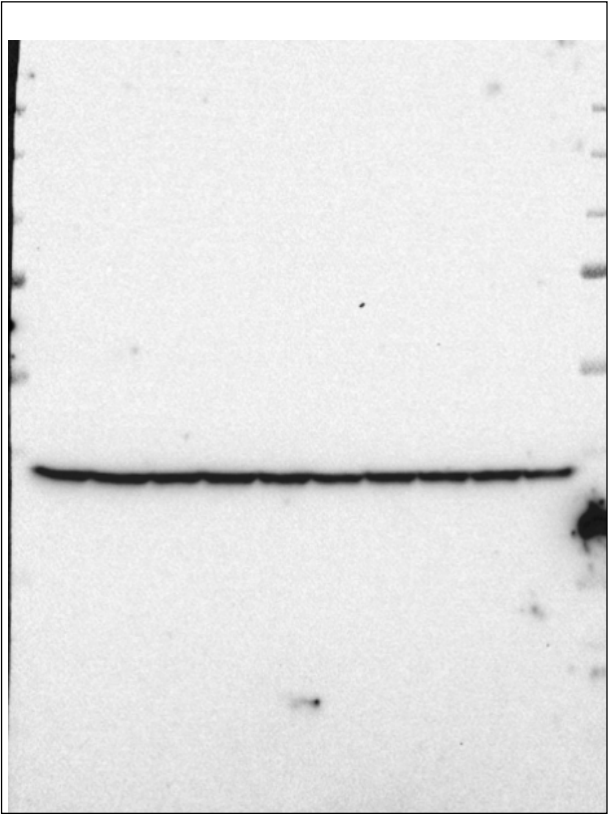


Figure 4



Kcnj10



GAPDH

Figure 5

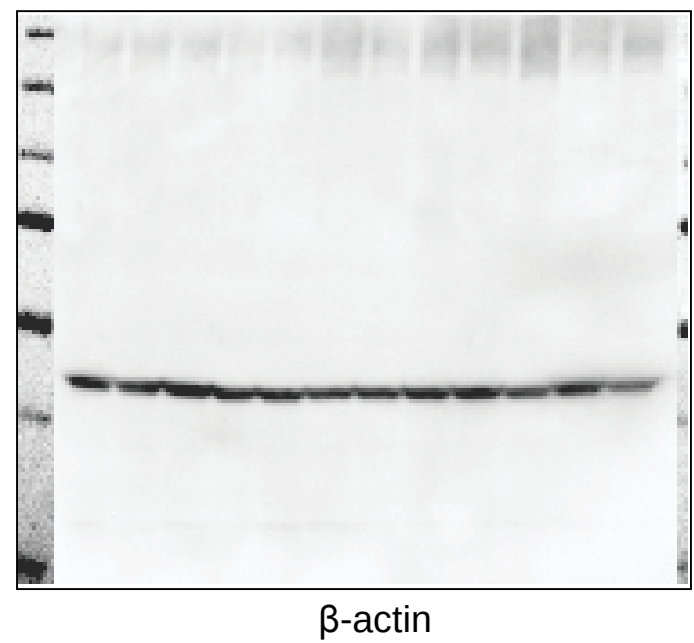
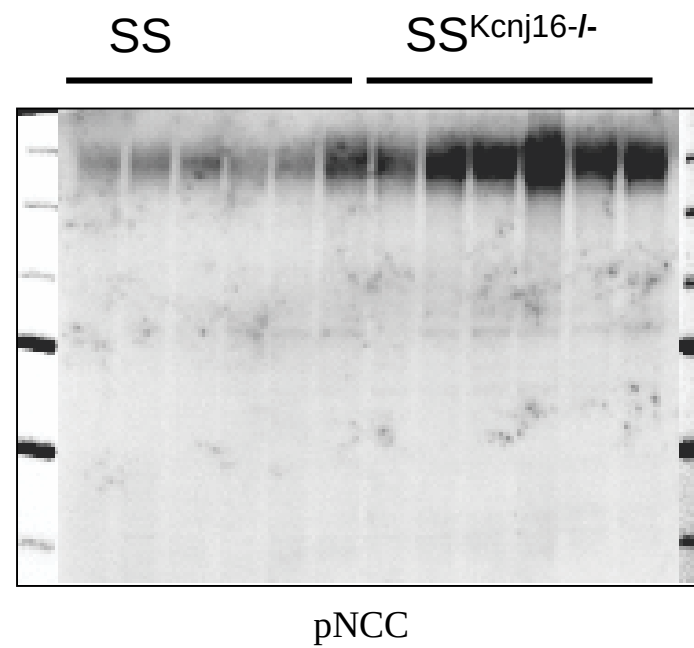
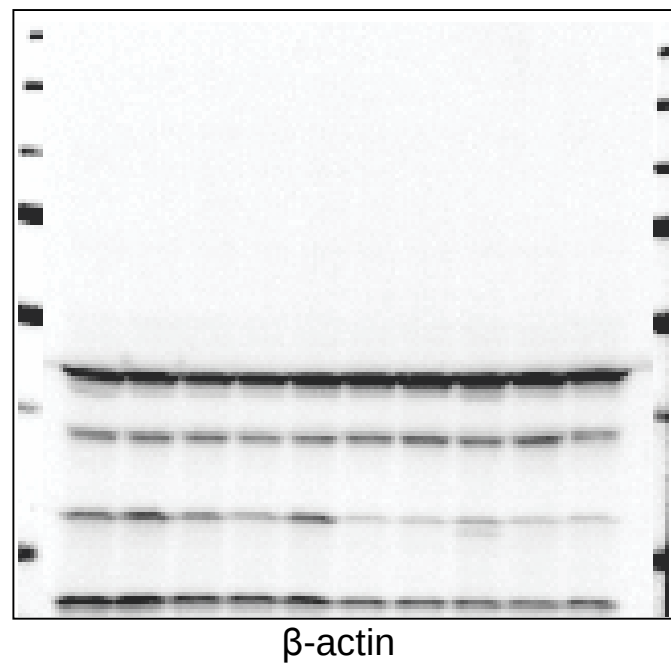
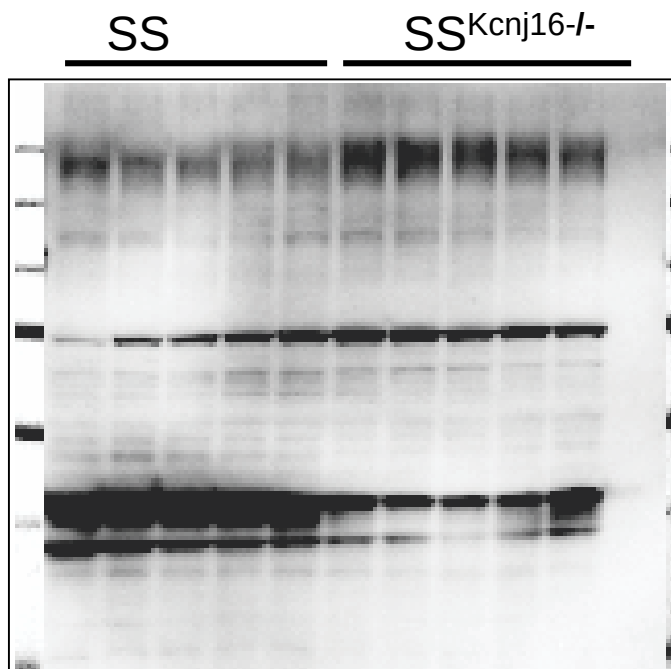
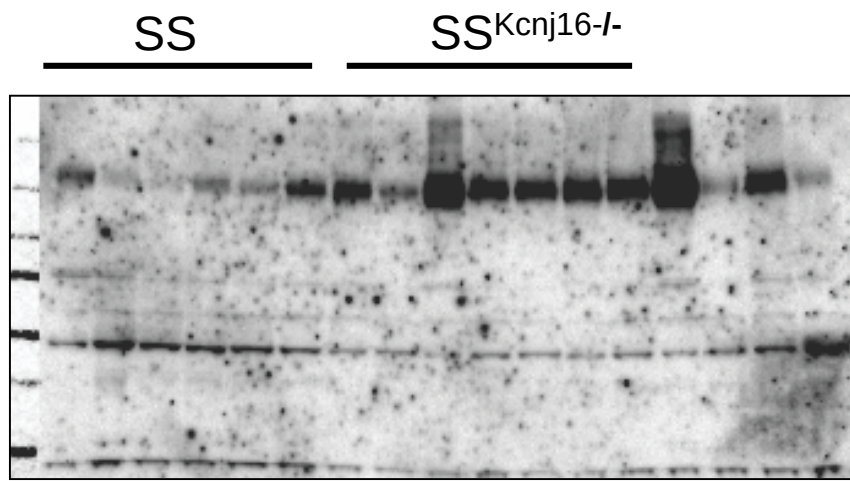
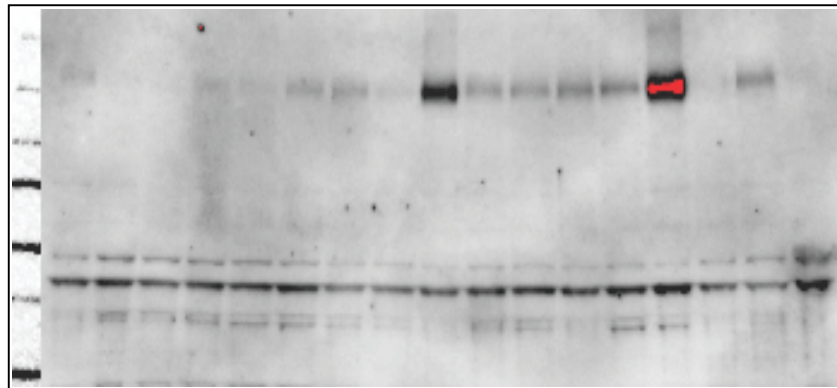


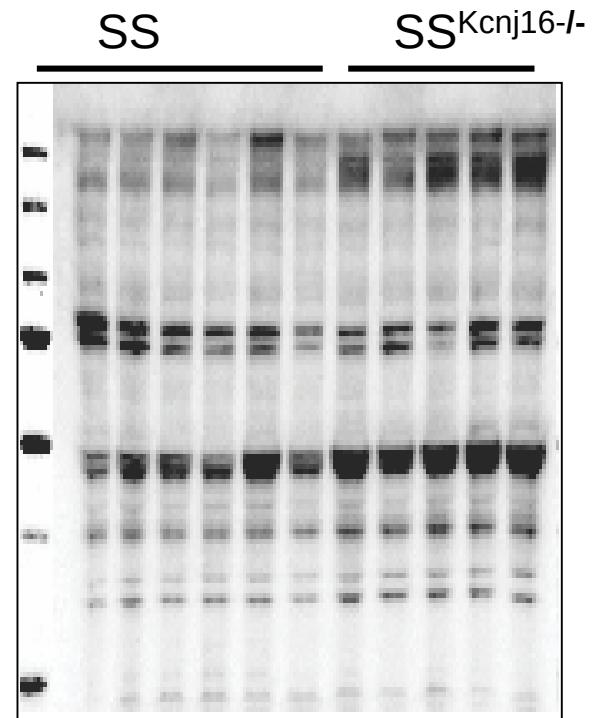
Figure 5



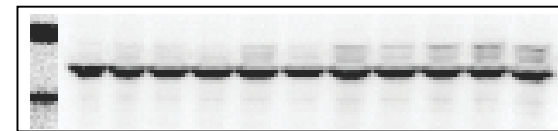
NKCC2



β-actin



pNKCC2



β-actin

Figure 8A

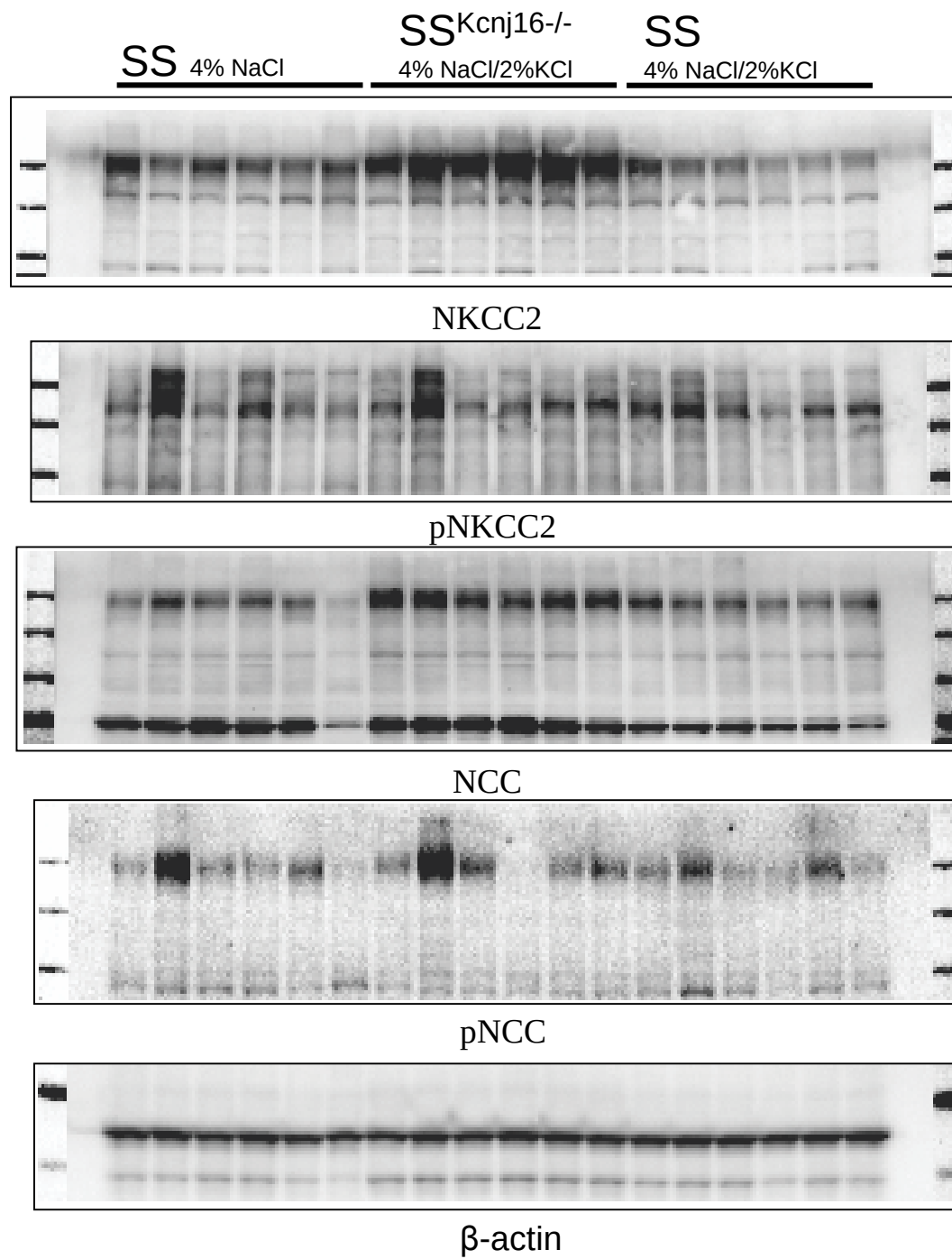


Figure 8B

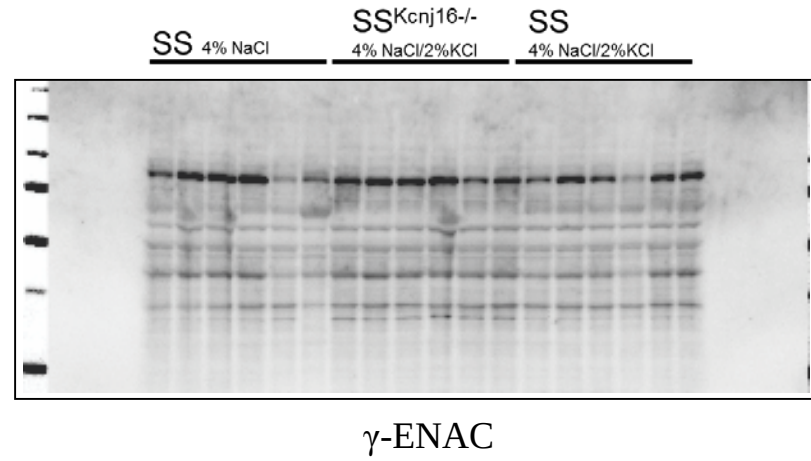
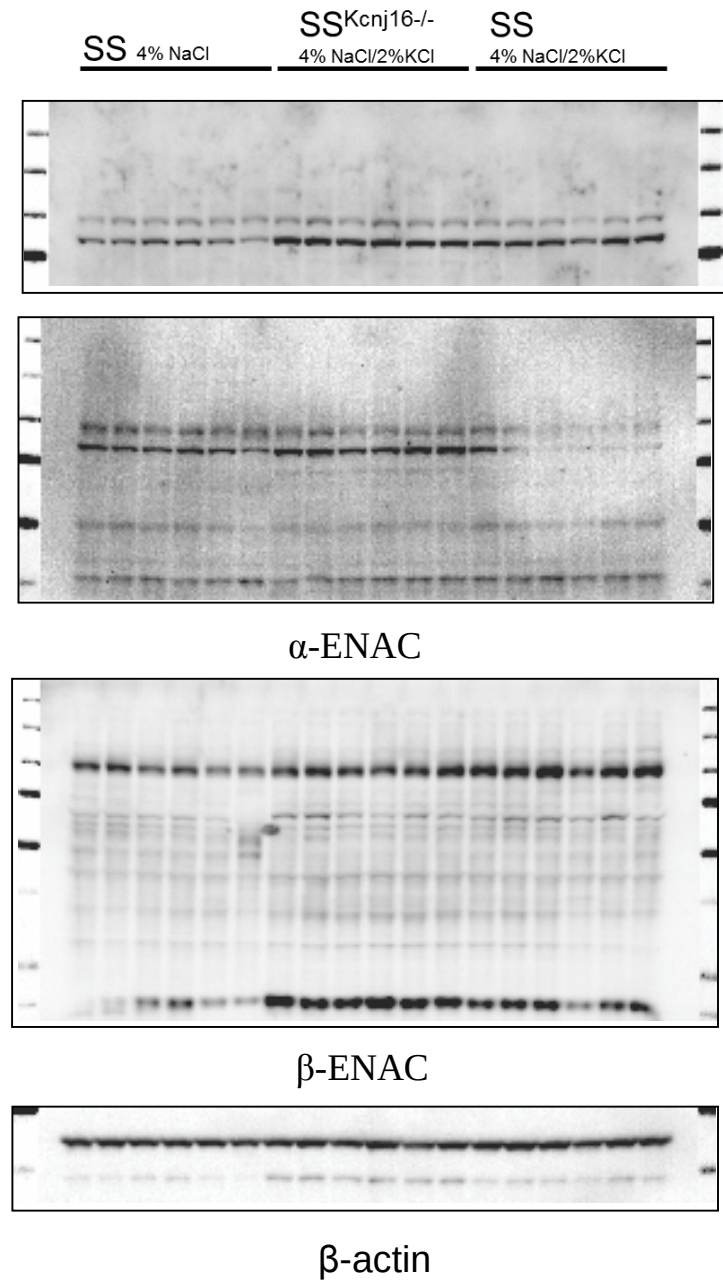


Figure 8

