

Supplemental Data

Preservation of Intracellular Renin Expression Is Insufficient to Compensate for Genetic Loss of Secreted Renin

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Running Title: Hypotension and Lethality in Secreted Renin Null

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Supplemental Methods

Targeting Vector Construction: The gene targeting vector was constructed by PCR-cloning the floxed region, and the upstream and downstream homologous regions into two intermediate vectors pBYloxP and pBY49a.¹ Cloning primers are shown in Table S1. We employed BAC RP23-41A16 which originated from a C57BL/6J mouse chromosome 1 genomic library as the PCR template (Children's Hospital Oakland Research Institution).

The targeting vector was electroporated into SCR mouse embryonic stem cells, which are derived from a tyrosinase mutant line of C57BL/6J-Tyr(c-2J) with a white coat color. The transfected ES cells were then subjected to positive selection for neomycin and negative selection by HSV-thymidine kinase. Germ-line transmission was screened in the offspring from the chimeras bred with C57BL/6J. Unless otherwise stated, all experimental mice were provided access to standard mouse chow and water ad libitum.

Saline Injection: Daily subcutaneous saline injection was performed on newborn mice from day 0 to day 14. The mother was separated before taking the pups out of their cages. A tent was made on the back of a pup and a 30G needle was used for injection. Injection dose started with 30 μ l and increased up to 100 μ l as the mice grew. A small amount of peanut oil was applied to the pups right before returning them to the cages. The mother was returned to the cage until all the pups are done with injections.

Reference

- (1) Shi PP, Cao XR, Sweezer EM, Kinney TS, Williams NR, Husted RF, Nair R, Weiss RM, Williamson RA, Sigmund CD, Snyder PM, Staub O, Stokes JB, Yang B. Salt-sensitive hypertension and cardiac hypertrophy in mice deficient in the ubiquitin ligase Nedd4-2. *Am J Physiol Renal Physiol.* 2008;295:F462-F470.

Supplemental Figures

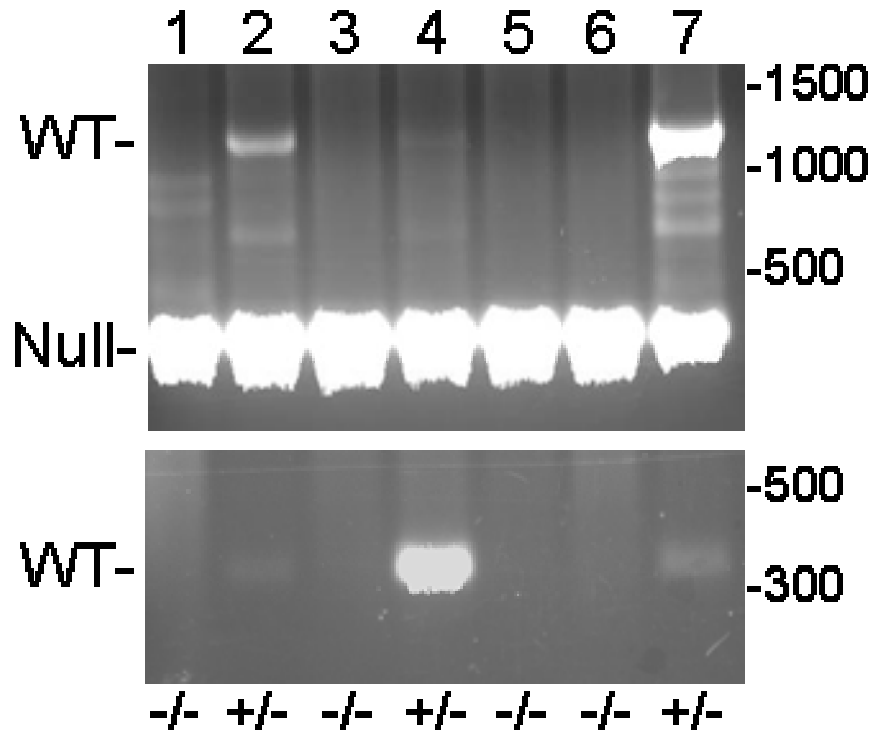


Figure S1: Genotyping of sRen^{-/-} Newborn Mice. Seven newborn mice were obtained from a breeding of sRen^{+/-} X sRen^{+/-} parents. The mice were genotyped using two different screens which detect either the wildtype allele only (bottom) or both the wildtype and null alleles (top).

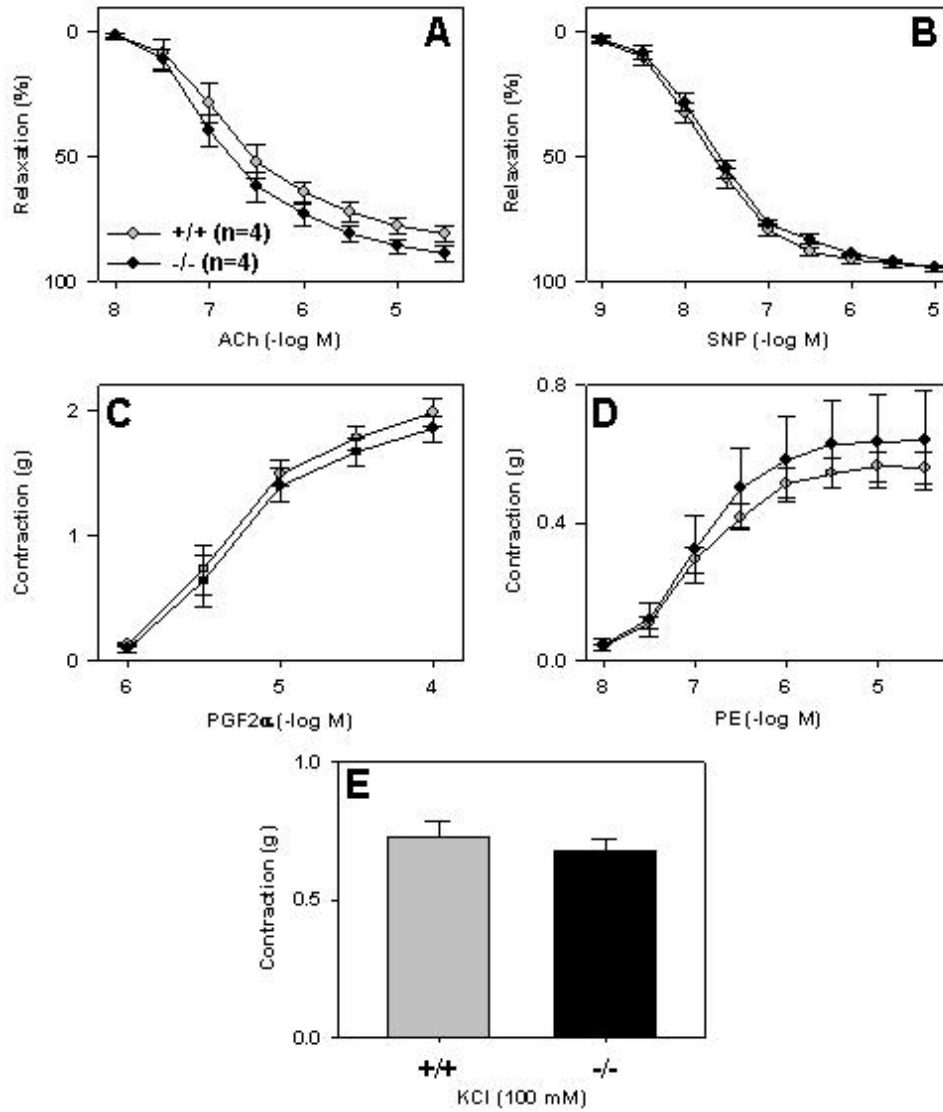


Figure S2: *Aortic Function*

A-B. Dose response to ACh (A) and SNP (B) after pre-constriction with PGF_{2α}. C-E. Dose response to PGF_{2α} (C) and phenylephrine (D). E. Contraction to 100 mM KCl is shown. *+/+*, gray lines (n=4); *-/-*, black lines (n=4).

Table S1: Oligonucleotides and Primers**Gene Targeting Vector Cloning Primers**

Floxed Region Cloning Primers

Forward: 5'-ATCGCAGAGCTCGGCCATGCAGGCCTTGGGGGAGATTAGATAAAGC-3'

Reverse: 5'-ATCGCACTGCAGTCCAGCTACAGATGAACTC-3'

Upstream Homologous Region Cloning Primers

Forward: 5'-ATCGCAGCGGCCGCGGGCTTTCCTGTGCTCATC-3'

Reverse: 5'-ATCGCAGTTAACAAGGCCTCCAGCATTTTC-3'

Downstream Homologous Region Cloning Primers

Forward: 5'-ATCGCAGTCGACGACCTCAAGTCTGTCCTCTGTG-3'

Reverse: 5'-ATCGCACTCGAGGCCTCAGTGGCCATCTTATGTGCTCGTGCTTCTG-3'

Note: The sequences in **bold** represent genomic sequences in the renin locus.**RT-PCR Primers**

sRen Forward: 5'-ACCTTCAGTCTCCCAACACGCACC-3'

sRen Reverse: 5'-GGGAGGTAAGATTGGTCAAGGAAGG-3'

icRen Forward: 5'-TTTGATGAGAGGATACGCATAGCACTTC-3'

icRen Reverse: 5'-GGGAGGTAAGATTGGTCAAGGAAGG-3'

Total Ren Forward: 5'-GTCCGACTTCACCATCCACTAC-3'

Total Ren Reverse: 5'-AGAACACTTCCTCCTTTAGCAC-3'

Q-PCR Primers and Probes

sRen Forward: 5'-GCACCTTCAGTCTCCCAACAC-3'

sRen Reverse: 5'-TCCCGGACAGAAGGCATTTTC-3'

sRen Probe: 5'-CCTTTGAACGAATCCC-3'

icRen Forward: 5'-CCGGCTGCTTTGAAGATTTGAT-3'

(CYBER)

icRen Reverse: 5'-ATGCCAATCTCGCCGTAGTA-3'

(CYBER)

Total Ren:

Assay ID-Mm02342888_Gh (Applied Biosystems)

Probe Sequence: TCACGATGAAGGGGGTGTCTGTGGG