Online Data Supplement

Anti-aging Gene *Klotho* Regulates Adrenal CYP11B2 Expression and Aldosterone Synthesis

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Running title: Klotho and Hypertension

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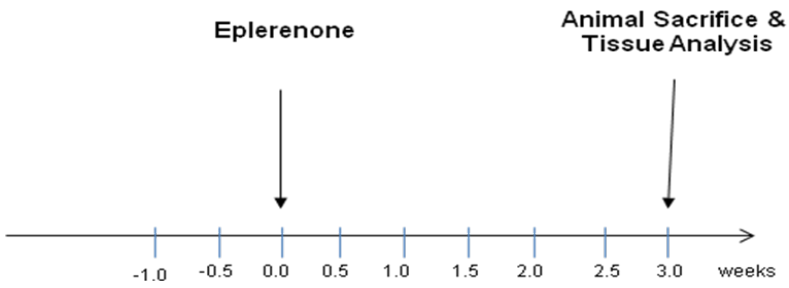
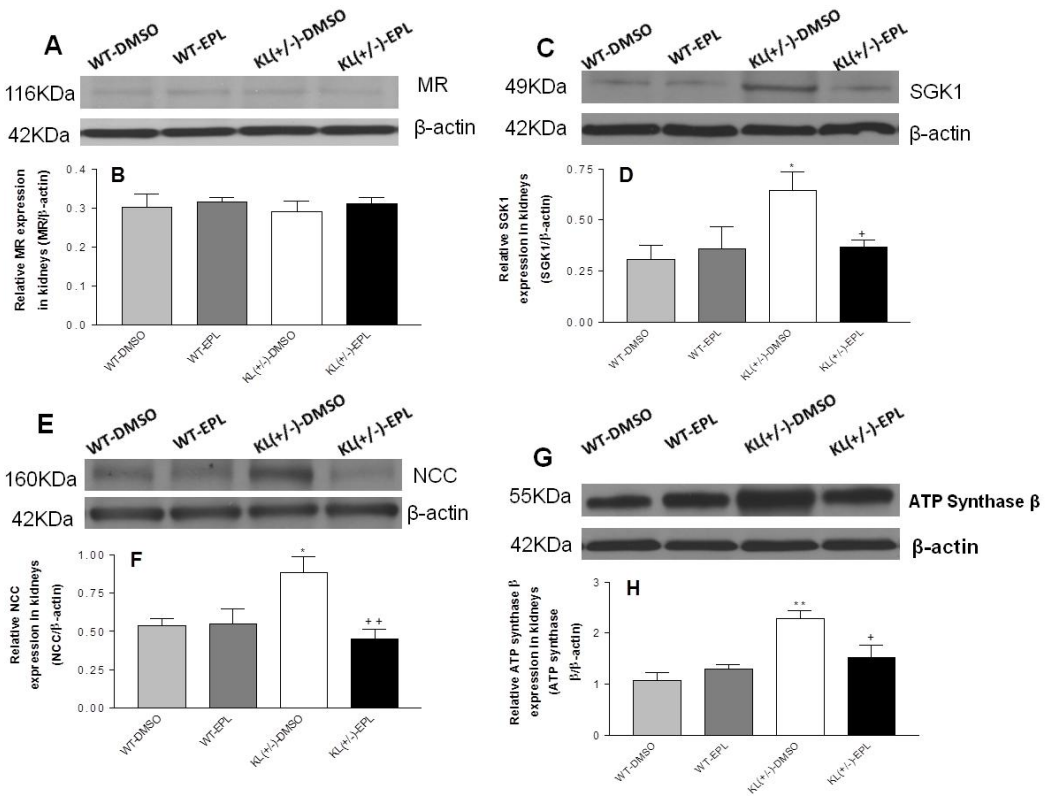
Online Supplemental Data

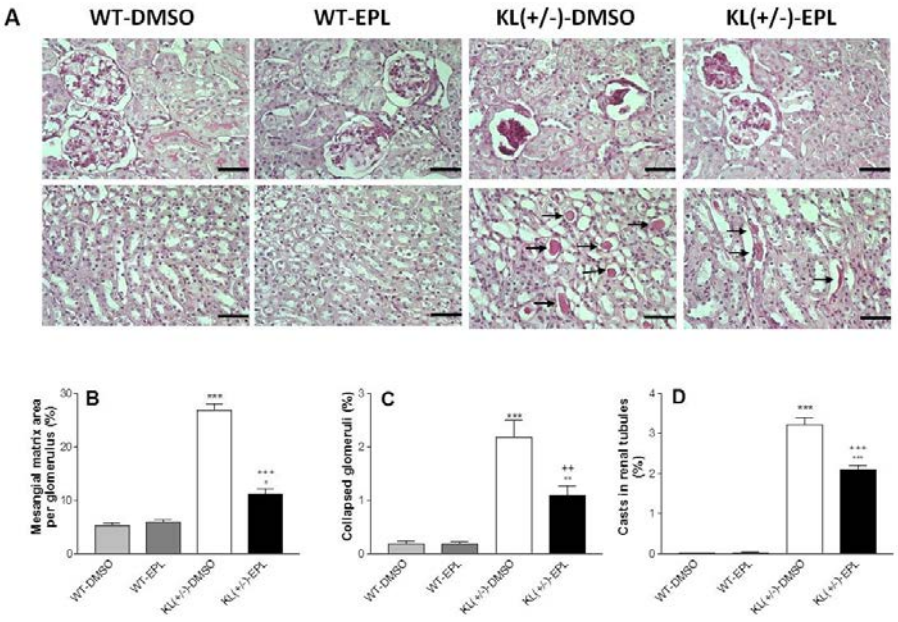


Supplemental Figure 1. Time course schema.

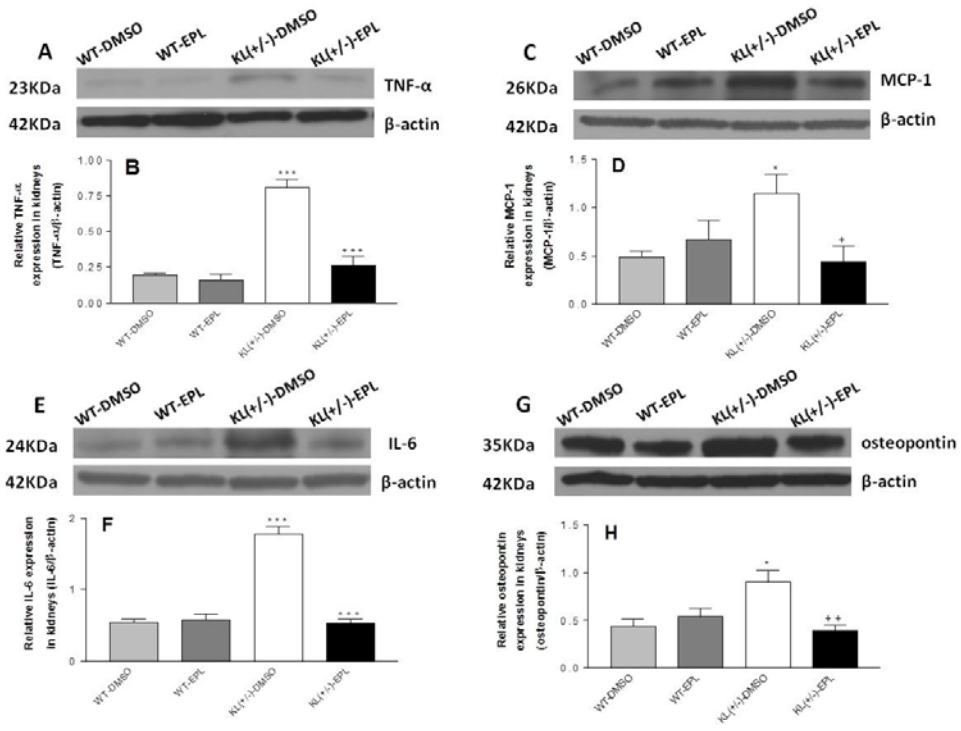


Supplemental Figure 2. Blockade of aldosterone actions abolished upregulation of the SGK1-NCC signaling in kidneys in KL(+/-) mice. **(A)** Representative western blots of mineralocorticoid receptor (MR) protein expression in kidneys. **(B)** Quantitative analysis of MR protein expression. **(C)** Representative western blots of serum and glucocorticoid regulated kinase (SGK1) in kidneys. **(D)** Quantitative analysis of SGK1 protein expression. **(E)** Representative western blots of NaCl cotransporter (NCC) protein in kidneys. **(F)** Quantitative analysis of NCC protein expression. **(G)** Representative western blots of ATP synthase β (ATPase β) protein expression in kidneys. **(H)** Quantitative analysis of ATPase β protein expression. Western blot analysis was carried out when animals were sacrificed at three weeks after treatment with eplerenone. Data=means±SEMs. *p<0.05,**p<0.01 *vs* WT-

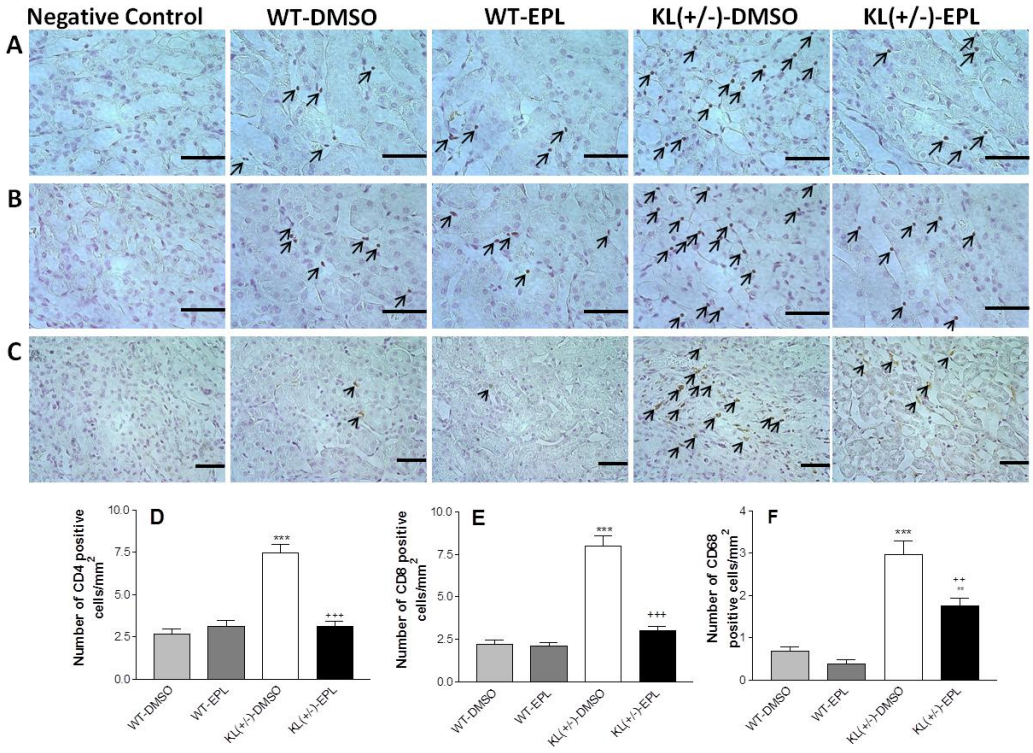
DMSO; +p<0.05,++p<0.01 *vs* KL(+/-)-DMSO. n=7 mice/group.



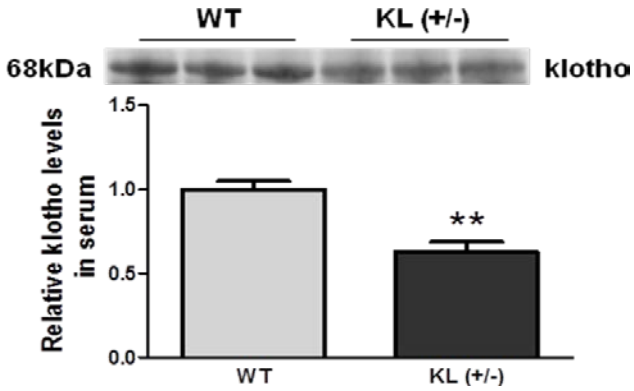
Supplemental Figure 3. Blockade of aldosterone receptors attenuated renal glomerular damage in KL(+/-) mice. (A) Representative photomicrographs of Periodic Acid Schiff-stained kidney sections (top row) and HE staining of renal tubules (bottom row). Glomerulosclerosis (red staining), glomerular collapse, tubular atrophy and dilation, and cast formation (indicated by arrows) were markedly increased in KL(+/-) mice *vs* the WT mice. Eplerenone alleviated renal damage. **(B)** Semiquantitative analysis of mesangial matrix staining in glomeruli. **(C)** Quantitative analysis of collapsed glomeruli. **(D)** Semiquantitative analysis of cast formation in renal tubules. Staining was carried out when animals were sacrificed at three weeks after treatment with eplerenone. Scale bars=50 μm. Data=means±SEMs. *p<0.05, **p<0.001, ***p<0.001 *vs* WT-DMSO; ++p<0.01, +++ p<0.001 *vs* KL(+/-)-DMSO. n=5 mice/ group.



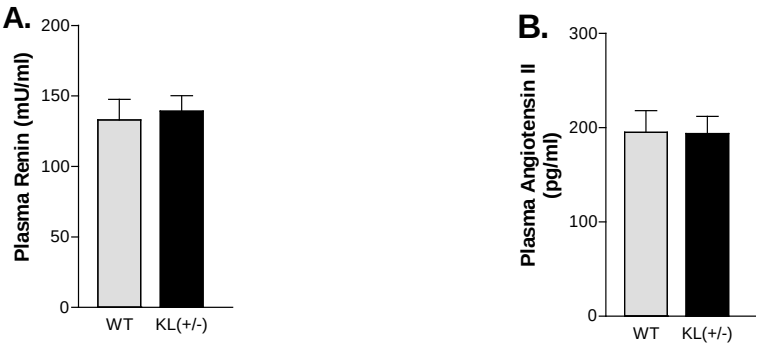
Supplemental Figure 4. Blockade of aldosterone receptors abolished upregulation of proinflammatory cytokine and chemokine expression in kidneys in KL(+/-) mice. **(A)** Representative western blots of tumor necrosis factor-α (TNF-α) in kidneys. **(B)** Quantitative analysis of TNF-α protein expression. **(C)** Representative western blots of monocyte chemotactic protein-1 (MCP-1) in kidneys. **(D)** Quantitative analysis of MCP-1 expression. **(E)** Representative western blots of Interleukin-6 (IL-6) in kidneys. **(F)** Quantitative analysis of IL-6 expression. **(G)** Representative western blots of osteopontin in kidneys. **(H)** Quantitative analysis of osteopontin expression. Western blot analysis were carried out when animals were sacrificed at three weeks after treatment with eplerenone. Data=means±SEMs. **p*<0.05, ****p*<0.001 *vs* WT-DMSO, +*P*<0.05, ++*P*<0.01,+++*P*<0.001 *vs* KL(+/-)-DMSO. n=7 mice/group.



Supplemental Figure 5. Blockade of aldosterone receptors inhibited T cell and macrophage infiltration in kidneys of KL(+/-) mice. **(A)** Representative photomicrographs of CD4 immunostaining in kidney sections (arrows indicating CD4-positive cells). **(B)** Representative photomicrographs of CD8 immunostaining in kidney sections (arrows indicating CD8-positive cells). **(C)** Representative photomicrographs of CD68 immunostaining in kidney sections (arrows indicating CD68 positive cells). **(D)** Semi-quantitative analysis of the CD4-positive cells. (**E)** Semi-quantitative analysis of the CD8-positive cells. **(F)** Semi-quantitative analysis of the CD68-positive cells. Immunostaining were carried out when animals were sacrificed at three weeks after treatment with eplerenone. Scale bars=50 μm. Data=means±SEMs. ***p*<0.01, ****p*<0.001 *vs* WT-DMSO; ++*p*<0.01,+++*p*<0.001 *vs* KL(+/-)-DMSO. n=5 mice/group.



Supplemental Figure 6. Western blot analysis of serum levels of klotho protein in WT and KL(+/-) mice. Serum was immunoprecipitated before western blot analysis. Data=means±SEMs. ***p*<0.01 *vs* WT. n=5 mice/group.



Supplemental Figure 7. Plasma levels of renin and angiotensin II in WT and KL(+/-) mice measured using ELISA. No significant difference was found between WT and KL(+/-) mice. n=5 mice/group.