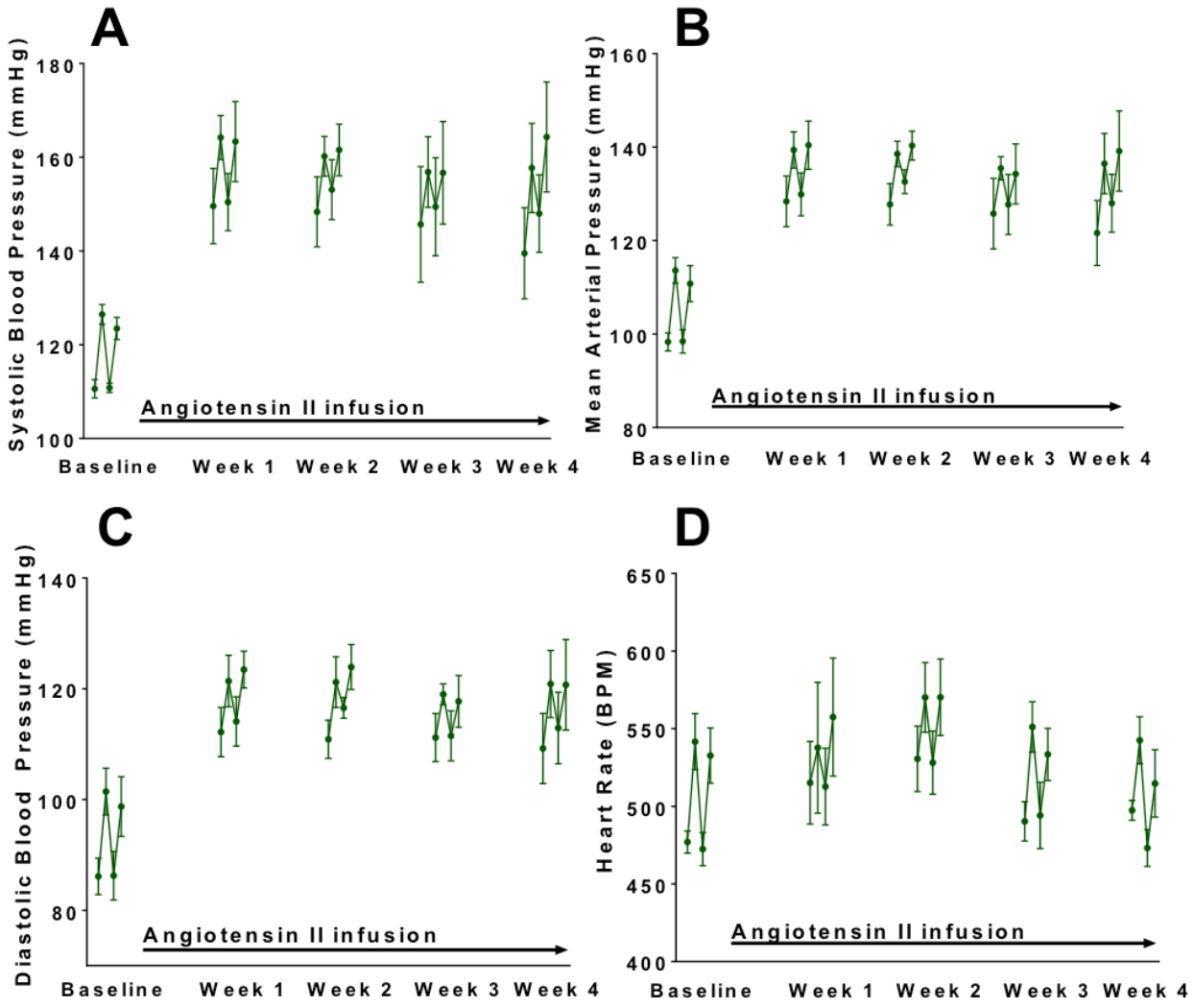
A Salt Sensing Kinase in T Lymphocytes, SGK1, Drives Hypertension and Hypertensive End-Organ Damage

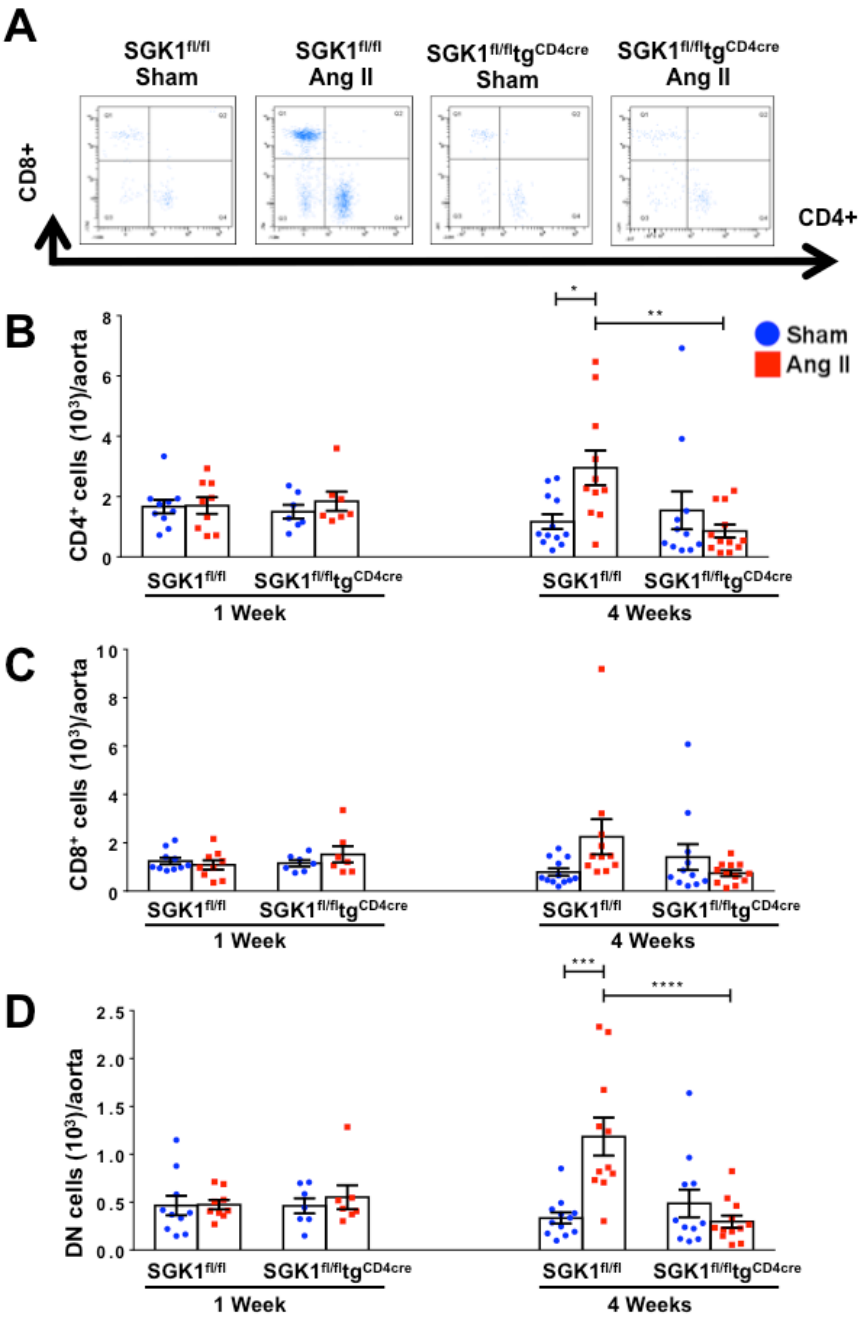
Allison E. Norlander[1], Mohamed A. Saleh[2,3], Arvind K. Pandey[4], Hana A. Itani[2], Jing Wu[1†], Liang Xiao[2], Jay Kang[1], Bethany L. Dale[1], Slavina B. Goleva[1], Fanny Laroumanie[2], Liping Du[5], David G. Harrison[1,2], Meena S. Madhur*[1,2]

[1]Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN 37232, USA.
[2]Division of Clinical Pharmacology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA.
[3]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.
[4]Division of Cardiology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA.
[5]Vanderbilt Center for Quantitative Sciences, Vanderbilt University School of Medicine, Nashville, TN 37232, USA.
[†]Current address: Department of Pharmacology, The University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA.

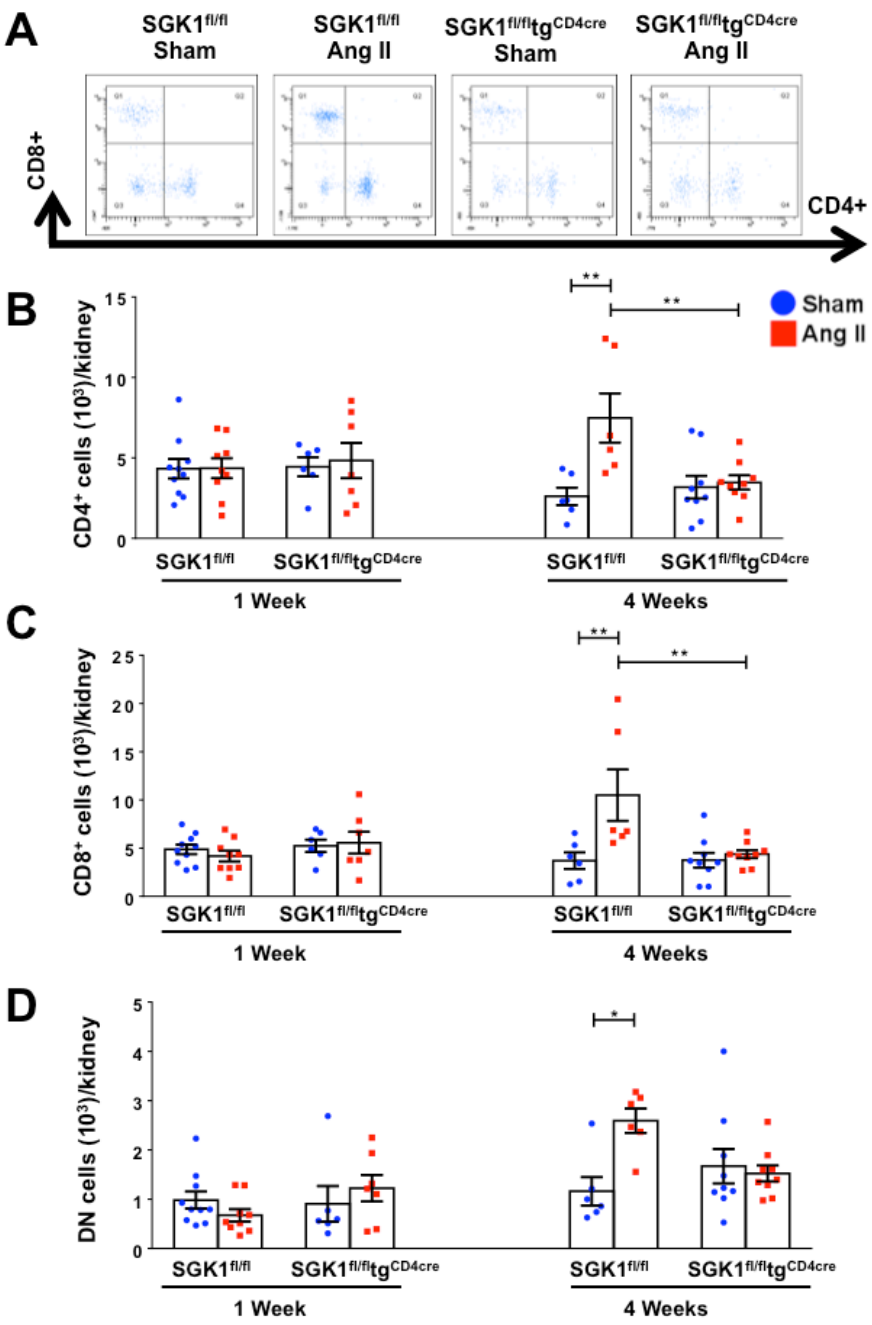
SUPPLEMENTAL MATERIAL



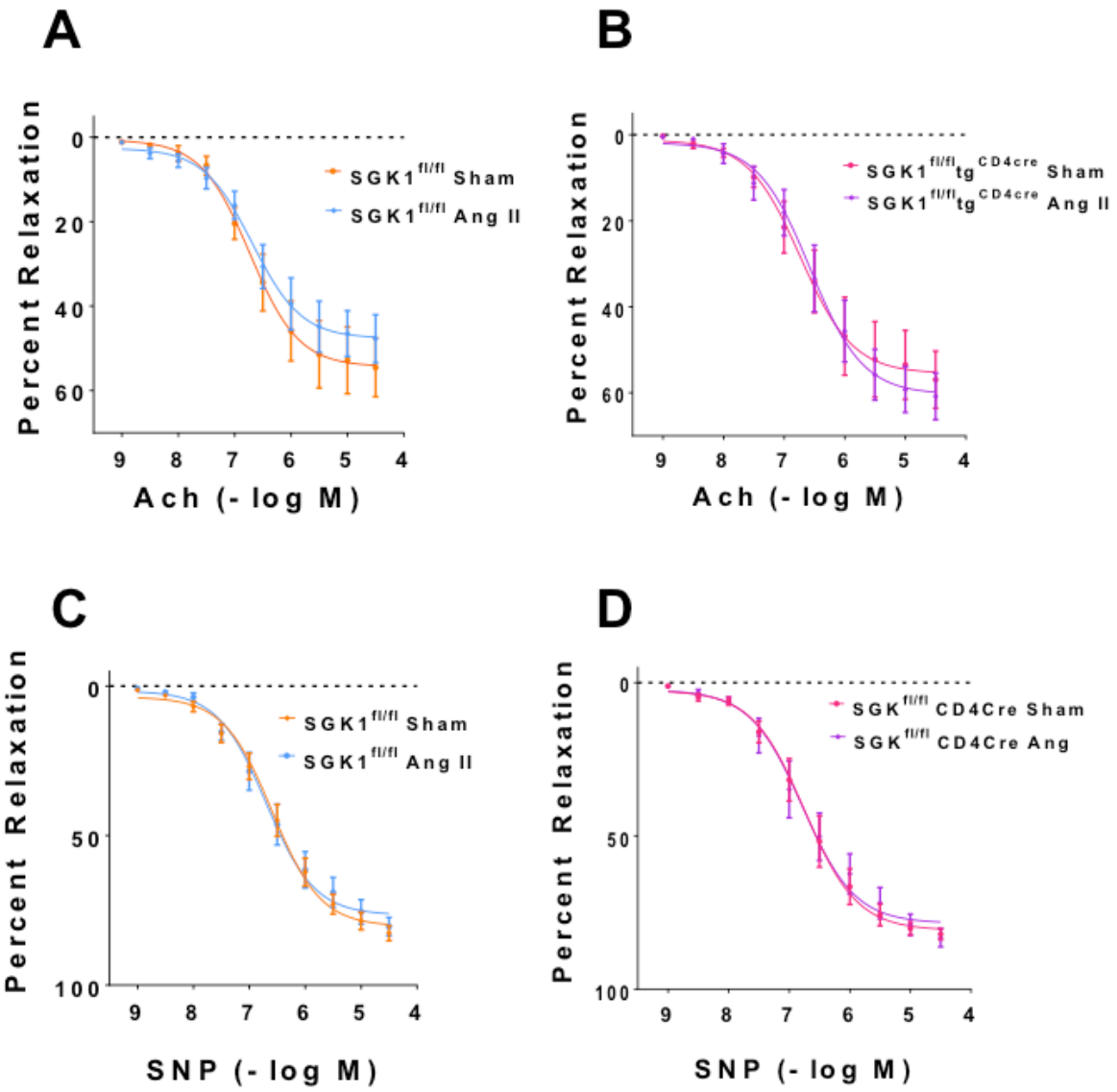
Supplemental Figure 1. Cre recombinase expression in T cells does not affect Ang II-induced blood pressure response. Telemetry recordings of **(A)** systolic blood pressures, **(B)** mean arterial pressures, **(C)** diastolic blood pressures, and **(D)** heart rates in tg^{CD4cre} mice infused with Ang II (490ng/kg/min) for 28 days. Data were recorded for 2 days at baseline and weekly thereafter during Ang II infusion. All data are expressed as mean ± SEM (n = 5).



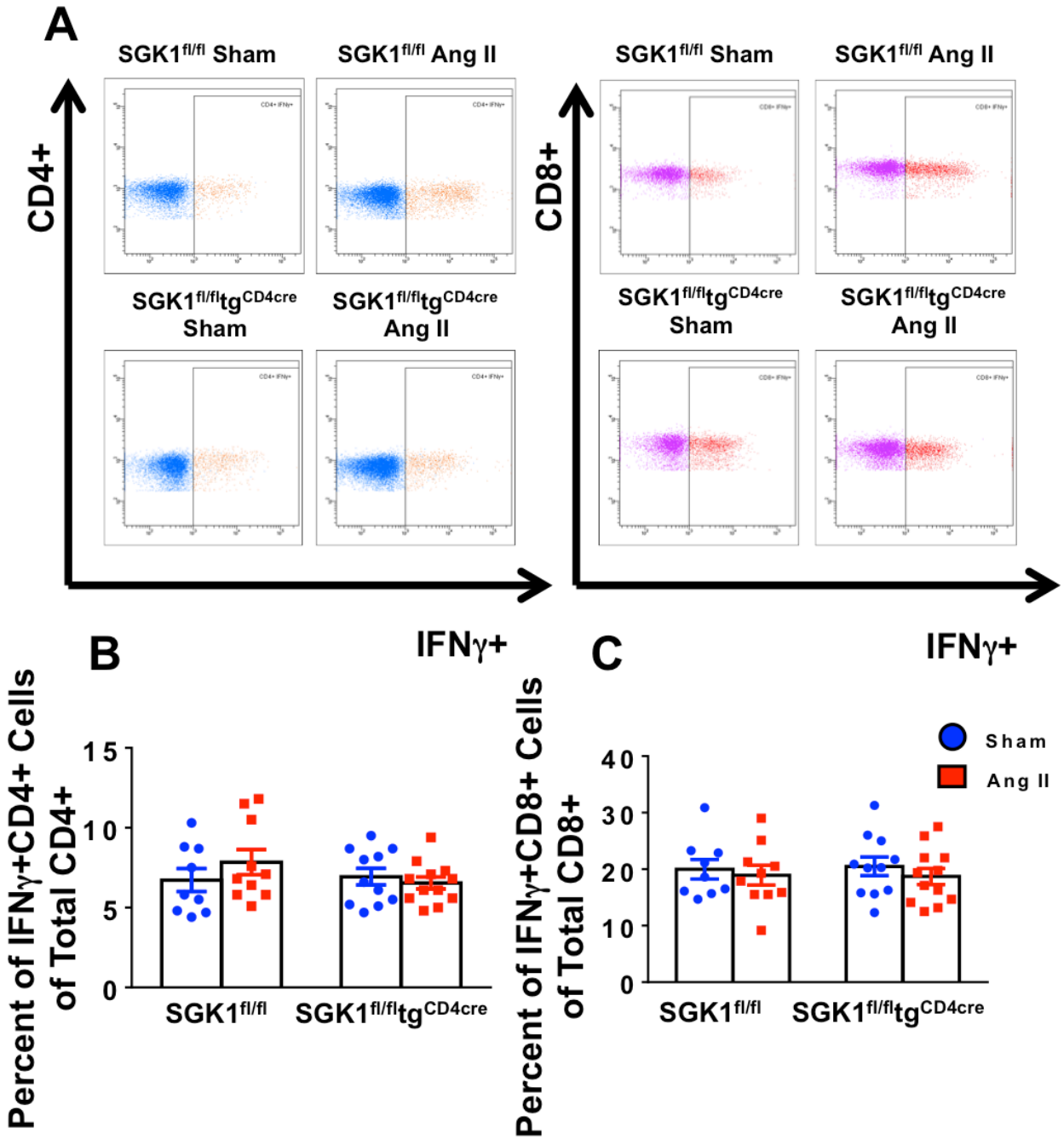
Supplemental Figure 2. T cell SGK1 deficiency prevents the chronic phase of Ang II-induced vascular inflammation. (A) Representative flow cytometry dot plots showing gating strategy for T cell subsets ($CD4^+$, $CD8^+$, and $CD3^+CD4^-CD8^-$ double negative (DN) cells) in single cell suspensions from the thoracic aorta of $SGK1^{fl/fl}$ and $SGK1^{fl/fl}tg^{CD4cre}$ mice infused with Ang II or vehicle (Sham) for 7 or 28 days. **(B-D)** Summary data of absolute numbers of indicated cell types per thoracic aorta (**P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001; two-way ANOVA/Holm-Sidak's post hoc test; n = 7-12 per group). All data are expressed as mean ± SEM.



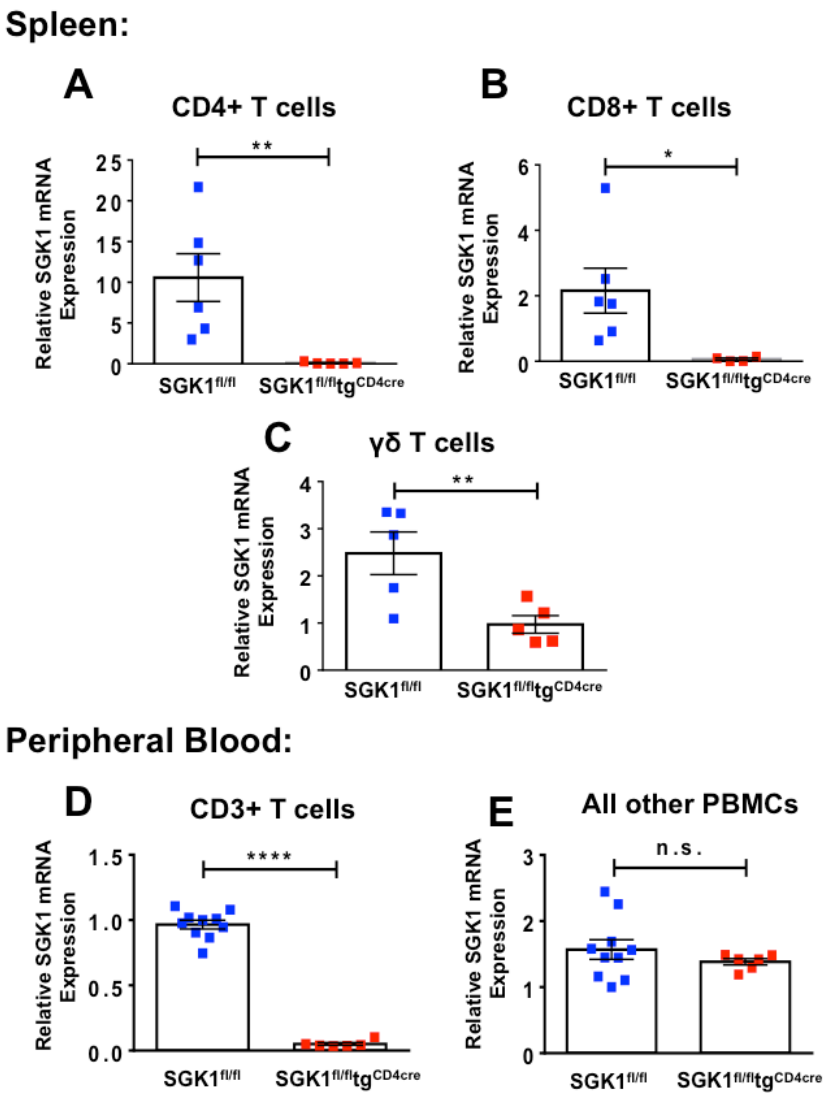
Supplemental Figure 3. T cell SGK1 deficiency prevents the chronic phase of Ang II-induced renal inflammation. (A) Representative flow cytometry dot plots showing gating strategy for T cell subsets ($CD4^+$, $CD8^+$, and $CD3^+CD4^-CD8^-$ double negative (DN) cells) in single cell suspensions from one kidney of $SGK1^{fl/fl}$ and $SGK1^{fl/fl}tg^{CD4cre}$ mice infused with Ang II or vehicle (Sham) for 7 or 28 days. **(B-D)** Summary data of absolute numbers of indicated cell types per kidney (**P*<0.01, ***P*<0.01; two-way ANOVA/Holm-Sidak's post hoc test; n=6-10 per group). All data are expressed as mean ± SEM.



Supplemental Figure 4. Vascular function is not impaired after 1 week of Ang II infusion. SGK1$^{fl/fl}$ controls and SGK1$^{fl/fl}$tg^{CD4cre} mice were infused with Ang II or vehicle (Sham) for 7 days. Endothelium-dependent relaxation to increasing doses of acetylcholine (Ach) was measured in **(A)** SGK1$^{fl/fl}$ mice and **(B)** SGK1$^{fl/fl}$tg^{CD4cre} mice, and endothelium-independent relaxation to increasing doses of sodium nitroprusside (SNP) was measured in **(C)** SGK1$^{fl/fl}$ mice and **(D)** SGK1$^{fl/fl}$tg^{CD4cre} mice. Data were analyzed by linear regression; n=5-7 per group. All data are expressed as mean ± SEM.



Supplemental Figure 5. Frequency of splenic Th1/Tc1 cells is not altered by Ang II infusion or loss of T cell SGK1. (A) Representative flow cytometry dot plots for $CD4^+IFN\gamma^+$ Th1 cells and $CD8^+IFN\gamma^+$ Tc1 cells in splenic single cell suspensions from $SGK1^{fl/fl}$ and $SGK1^{fl/fl}tg^{CD4cre}$ mice infused with Ang II or vehicle (Sham) for 28 days. **(B-C)** Summary data of percentages of $CD4^+IFN\gamma^+$ Th1 cells out of total $CD4^+$ cells and $CD8^+IFN\gamma^+$ Tc1 cells out of total $CD8^+$ cells in the indicated groups. All data are expressed as mean ± SEM (n=9-12 per group).



Supplemental Figure 6. SGK1 expression in splenic T cell subsets and peripheral blood T and non-T cell fractions in SGK1$^{fl/fl}$ controls and SGK1$^{fl/fl}$tg^{CD4cre} mice. Naïve CD4^{+} T cells **(A)**, naive CD8^{+} T cells **(B)**, or γδ T cells **(C)** were isolated from spleens of SGK1$^{fl/fl}$ control and SGK1$^{fl/fl}$tg^{CD4cre} mice and cultured for 3 days on anti-CD3/anti-CD28 coated plates in the presence of Th17 polarizing cytokines plus an excess 40mM NaCl to maximize SGK1 expression which was then quantified by qRT-PCR. SGK1 expression was also quantified by qRT-PCR from peripheral blood CD3^{+} T cells and all other PBMCs isolated from SGK1$^{fl/fl}$ control and SGK1$^{fl/fl}$tg^{CD4cre} mice. *P<0.05, **P<0.01, ****P<0.0001; Student's t-test; n = 4-10 per group. All data are expressed as mean ± SEM.

Sodium Channels and Transporters Expressed by T cells
Epithelial Sodium Channel α,β,γ subunits (ENaC)
Sodium/Chloride Cotransporter (NCC)
Sodium/Calcium Exchanger 1 (NCX1)
Sodium/Calcium Exchanger 2 (NCX2)
Sodium/Hydrogen Exchanger 1 (NHE1)
Sodium/Hydrogen Exchanger 6 (NHE6)
Sodium/Potassium/2 Chloride Cotransporter 1 (NKCC1)
Voltage-gated Sodium Channel 5A (SCN5A)

Supplemental Table 1. Sodium channels and transporters expressed by T cells. Pan $CD3^+$ T cells were isolated from spleens of C57Bl/6J wild type mice. The indicated sodium channels/transporters were detected by RT-PCR.