Vascular oxidation promotes immune activation, fibrosis and hypertension

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Condensed Title: Oxidative stress, immunity and aortic stiffening

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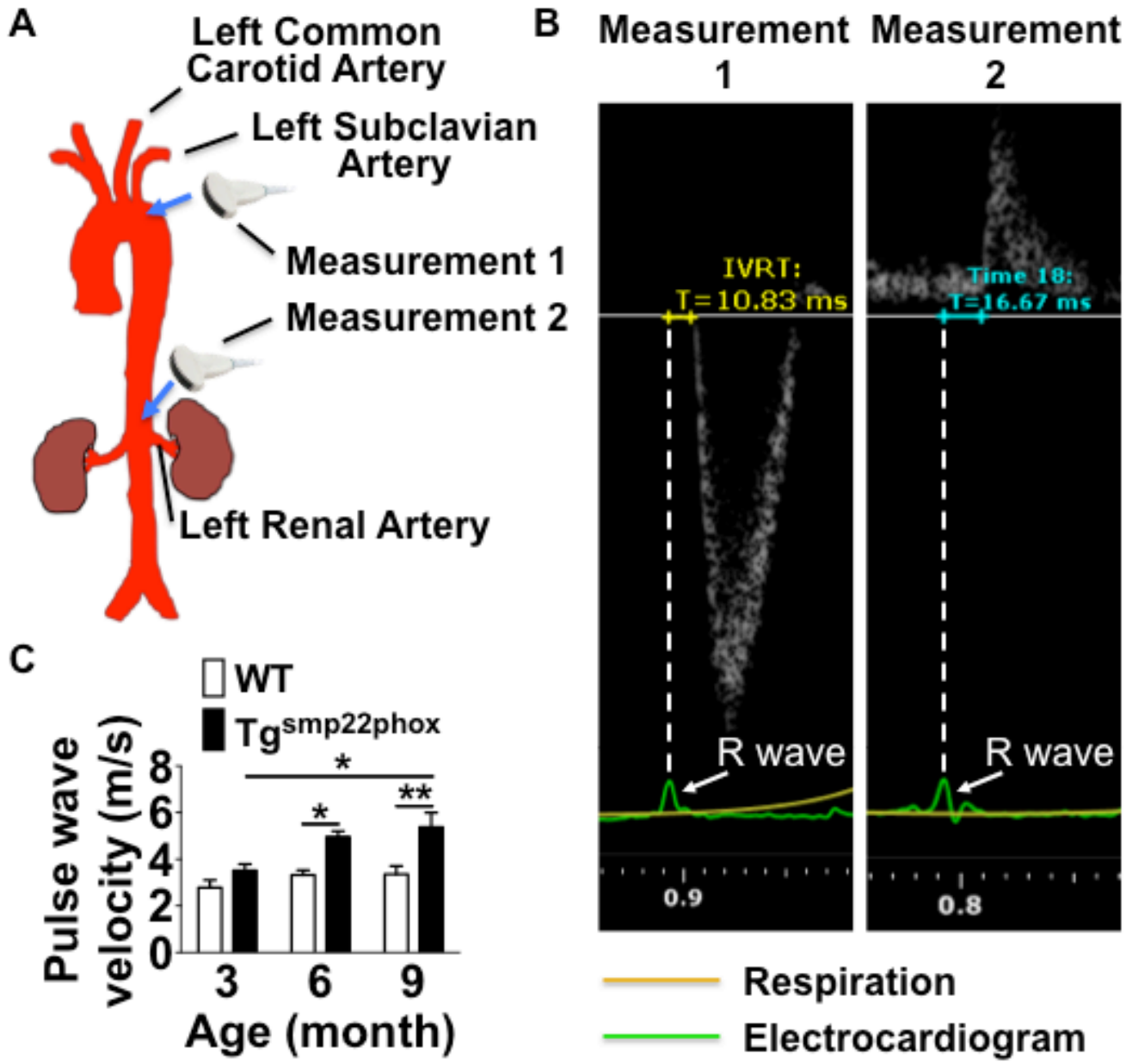
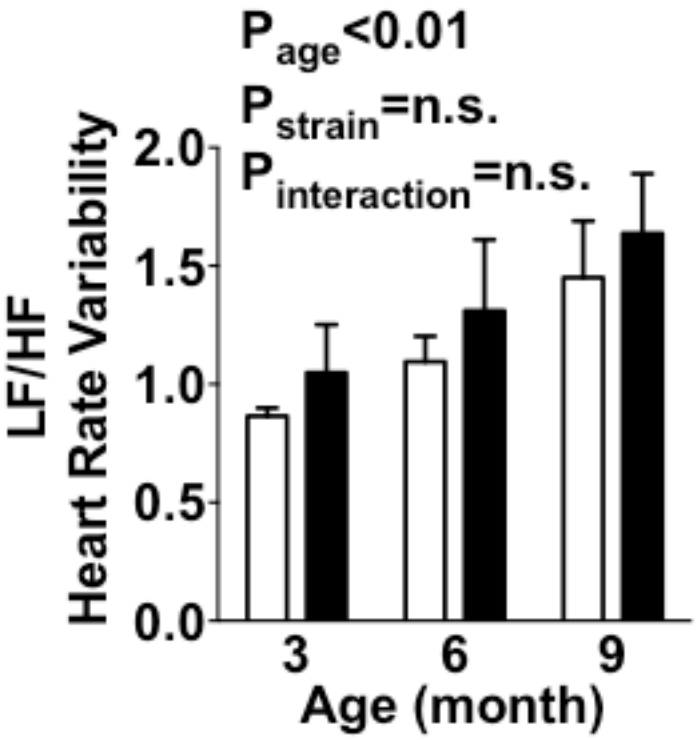
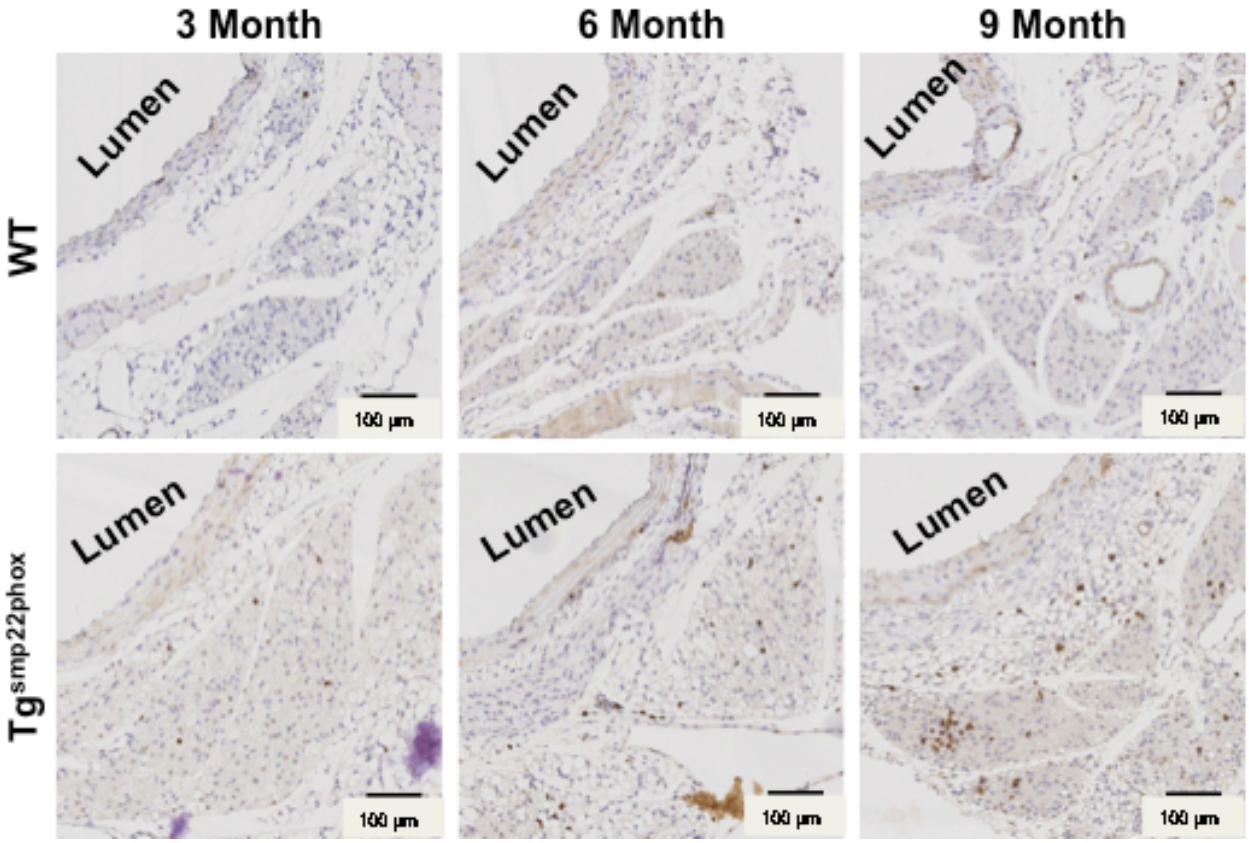


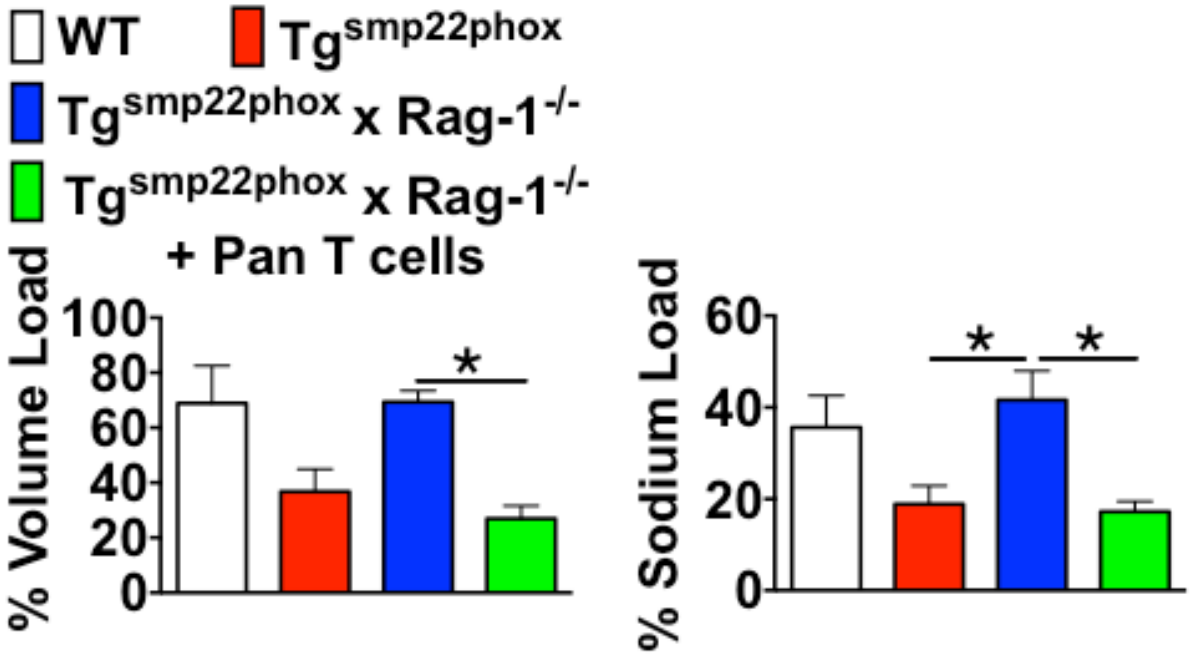
Supplemental Figure 1: The measurement of pulse wave velocity using Doppler ultrasound. Mice were anaethezied with isoflurane and pressure waveforms were recorded simultaneously with electrocardiogram. Panel A shows the sites where Doppler signals were obtained. Arrival times were calculated based on the distances between the foot of the pressure waveform and the peak of R wave for both the proximal and distal locations as shown in panel B. The transit time was the difference in these arrival times and was averaged over 5 cardiac cycles. The distances between these two positions were measured post mortem with a ruler after thoracic and abdominal organs were removed to reveal the whole aorta. PWV (m/s) was calculated by dividing the distance by the transit time. Pulse wave velocities for WT and $tg^{sm/p22phox}$ mice at 3, 6 and 9 months of age are shown in panel C. Data were analyzed using two-way ANOVA (n=6).



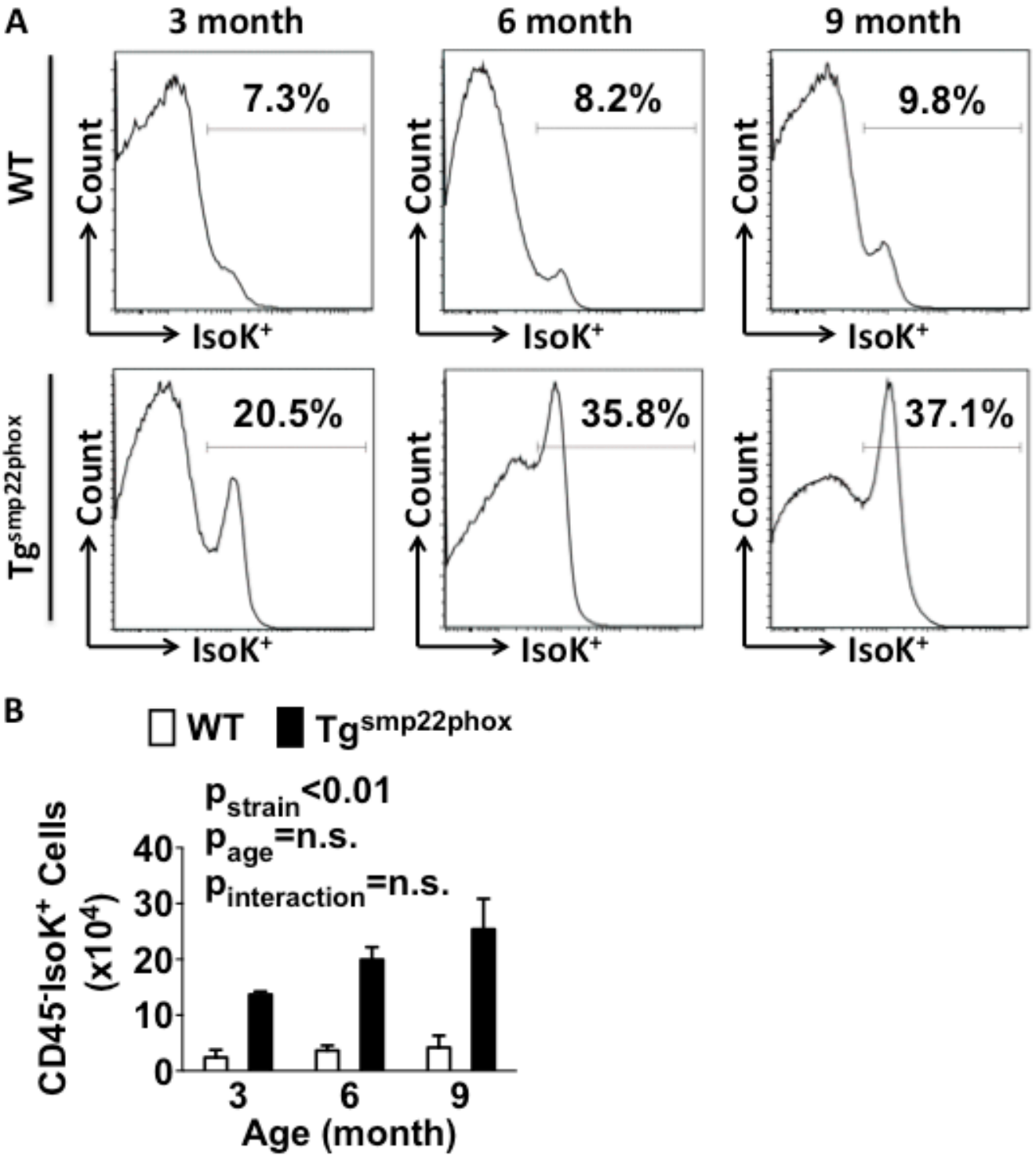
Supplemental Figure 2: The assessment of sympathetic outflow in WT and tg$^{sm/p22phox}$ mice. Continous heart rate recordings were obtained with radio telemetry for power spectral analysis of heart rate variability. The ratio of low frequency (LF) to high frequency (HF) variabilities of the heart rate was used as an indication of sympathetic outflow. WT mice are represented by open bars and tg$^{sm/p22phox}$ mice by filled bars. Data were analyzed using two-way ANOVA (n=6).



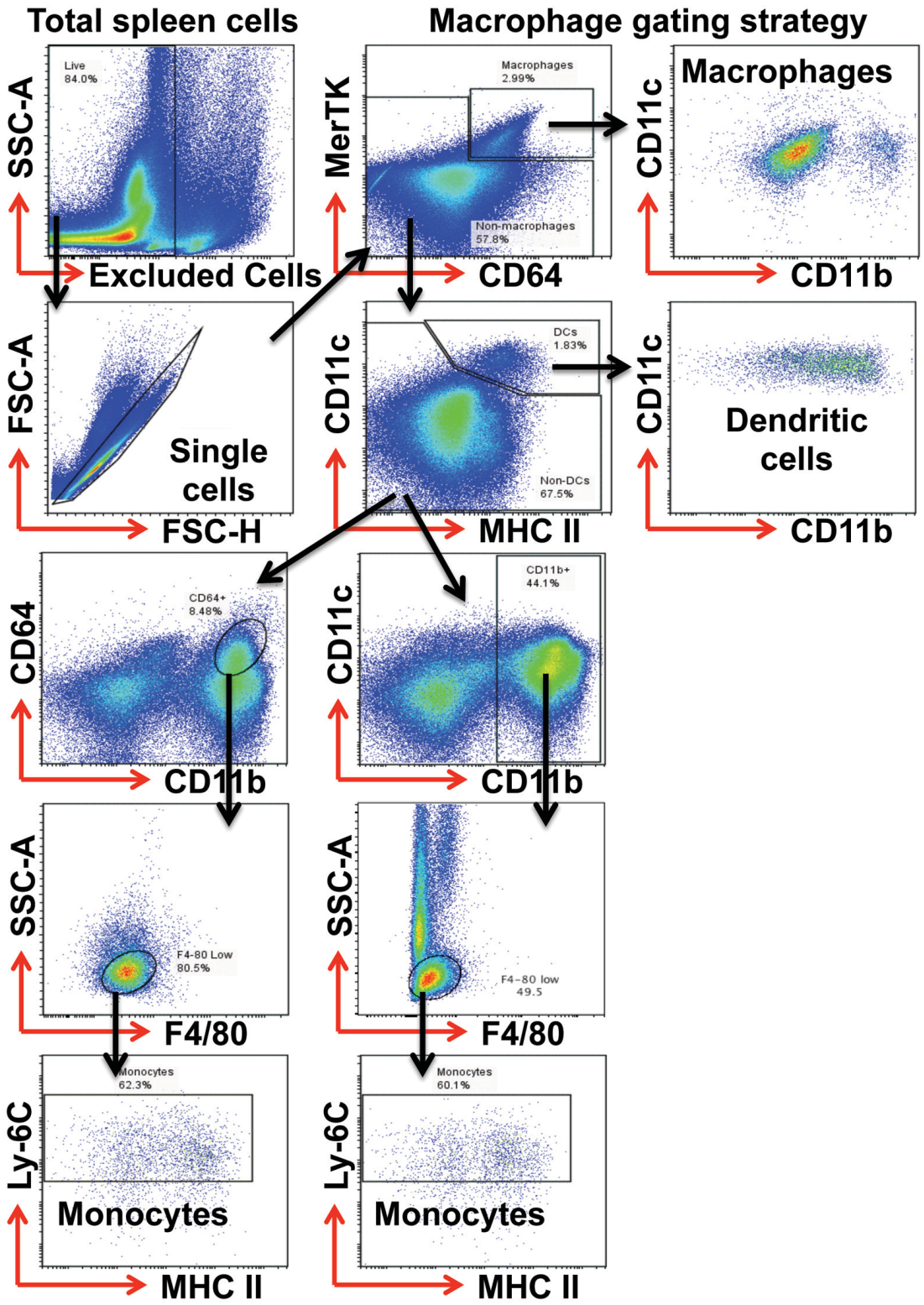
Supplemental Figure 3: Aortic T cell presence in WT and tg$^{sm/p22phox}$ mice. Thoracic aortas were fixed with 4% formalin, sliced into 6 µm sections and stained for CD3 to identify T lymphocytes (dark brown). Images are shown at 12x magnification. All images are shown at Scale bar indicates 100 µm.



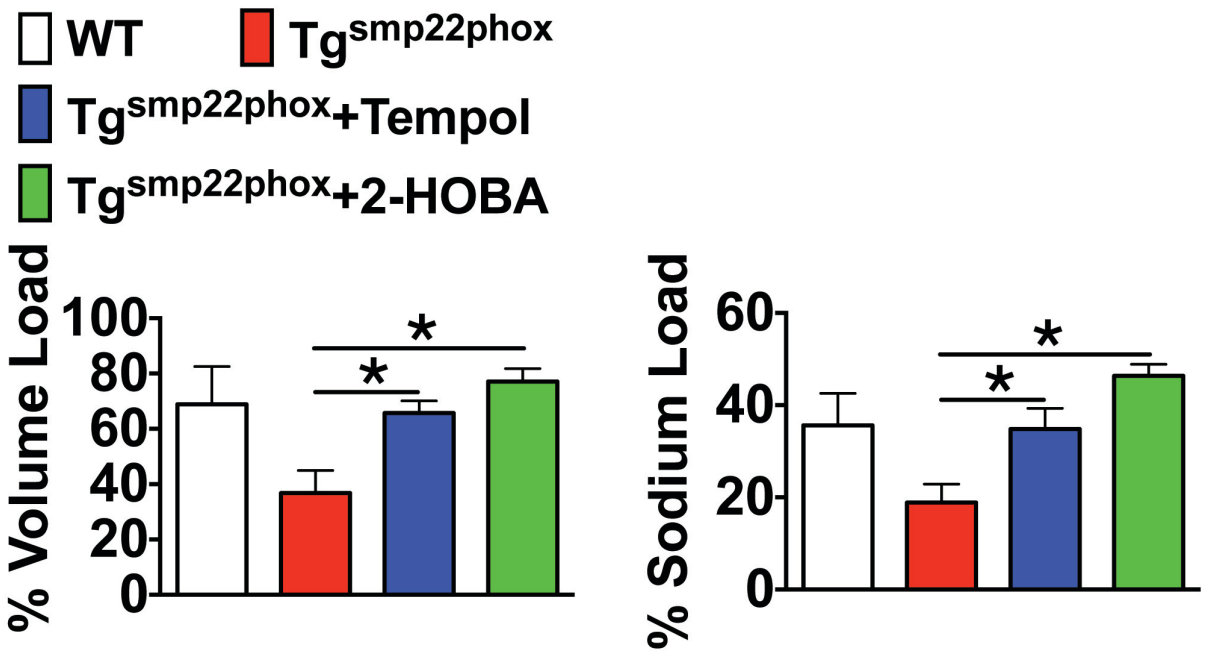
Supplemental Figure 4: The role of T cells in the development of renal dysfunction in tg$^{sm/p22phox}$ mice. Pan T cells were isolated from the spleen of 3 month-old WT mice and adoptively transferred to age-matched tg$^{sm/p22phox}$ x Rag-1$^{-/-}$ mice to re-constitute the T cell population. At 9 months of age, mice received a single i.p. injection of normal saline equal to 10% of body weight and urine was collected over the subsequent 4 hours for analysis of volume and sodium content. Values are presented as a percent of the amount administered. Data were analyzed with one-way ANOVA (n=6-8).



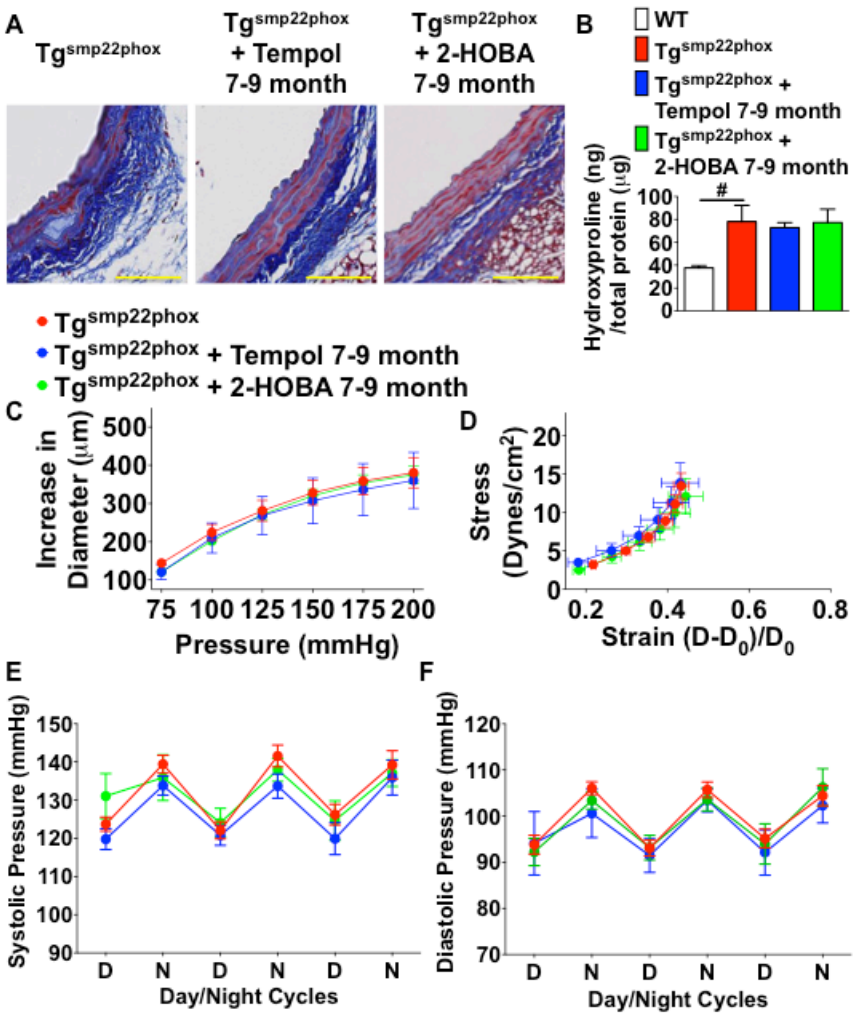
Supplemental Figure 5: Isoketal-adduct content of aortic cells in WT and tg$^{sm/p22phox}$ mice. Flow cytometry and intracellular staining with the D-11 antibody was used to detect isoketal-adducts in CD45^{-} cells. A) Example histograms of D-11 staining at 3 , 6 and 9 months of age in WT and tg$^{sm/p22phox}$ mice. B) Mean data comparing total number of isoketal-adduct containing cells. Data were analyzed using two-way ANOVA (n=3-5).



Supplemental Figure 6: Gating strategy for macrophages, dendritic cells and monocytes in the spleen. Single cells suspension was prepared by mechanical dissociation and enzymatic digestion of mouse spleens. A common channel was employed to exclude dead cells, $CD3^+$ T cells and $CD19^+$ B cells, and cells of interest were identified as illustrated.



Supplemental Figure 7: Effect of superoxide or isoketal scavenging on renal sodium and volume excretion in tg$^{sm/p22phox}$ mice. Tg$^{sm/p22phox}$ mice received either Tempol or 2-hydroxybenzylamine (2-HOBA) in the drinking water from 3-9 months of age. At 9 months, mice were given a single i.p. injection of normal saline equal to 10% of body weight and urine (left panel) and sodium (right panel) excretion in the subsequent 4 hours were monitored. These data were analyzed with one-way ANOVA (n=6-8).



Supplemental Figure 8: Two-month treatment Tempol or 2-HOBA does not reverse established aortic stiffening or hypertension. $Tg^{sm/p22phox}$ mice received either Tempol or 2-hydroxybenzylamine (2-HOBA) in the drinking water from 7-9 months of age and were studied at 9 months of age. A) Masson's trichome stains to highlight collagen of perfusion-fixed thoracic aortas sections. Collagen is represented by blue staining. Images are 20x magnification and the scale bars indicate 100 µm. B) Aortic collagen quantification by hydroxyproline assay. C and D) Freshly-isolated aortas were mounted on a myograph system in Ca^{2+}-free buffer to determine pressure-diameter relationships. Stress-strain relationships were constructed from intraluminal pressure, wall thickness, inner and outer diameters. These parameters were measured at 25 mmHg step changes in pressure from 0-200 mmHg. E and F) Telemetry blood pressure of WT mice, $tg^{sm/p22phox}$ mice or $tg^{sm/p22phox}$ mice treated from 7-9 month of age. Data were analyzed using one-way ANOVA (n=6).