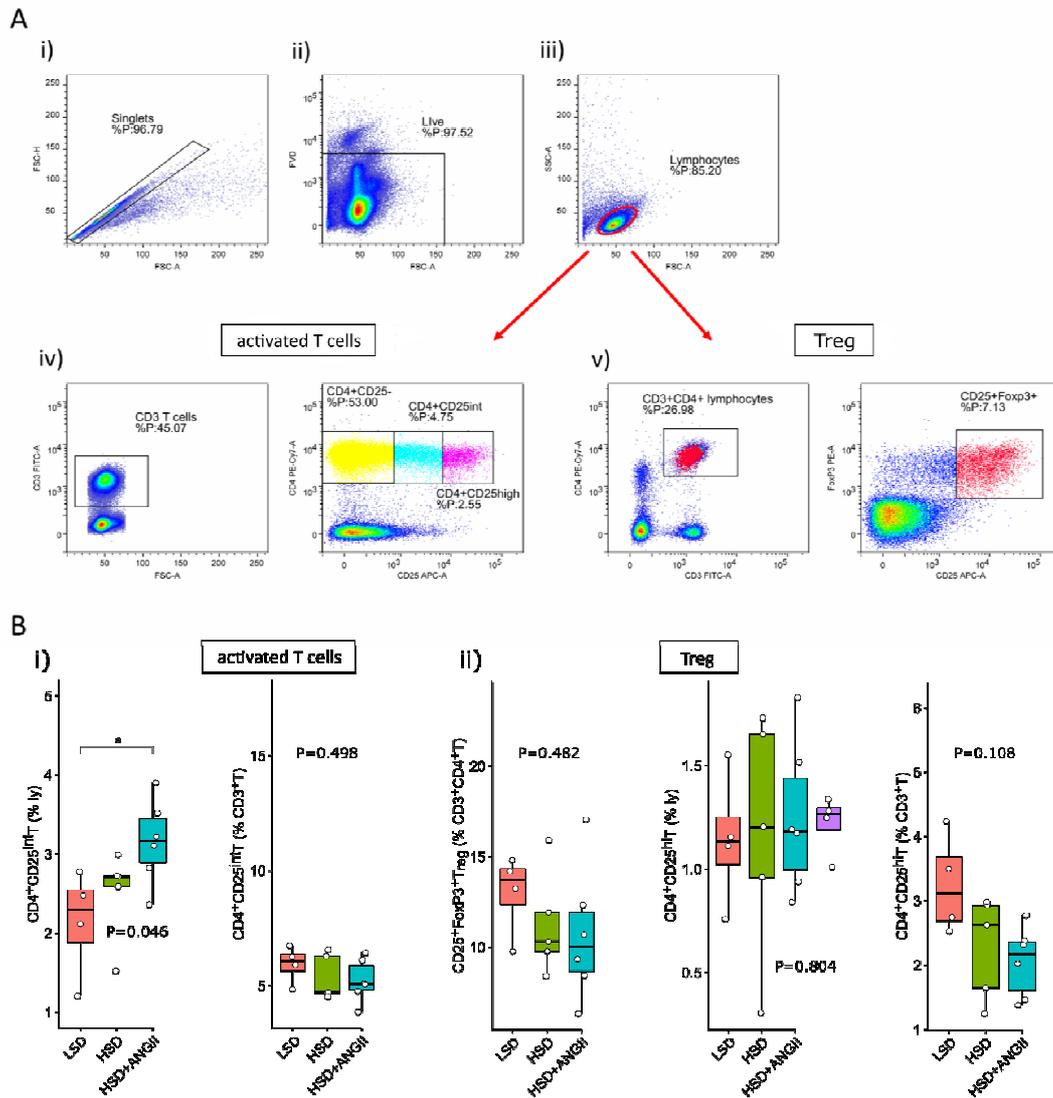


Supplemental Material

Early low-grade inflammation induced by high-salt diet in Sprague Dawley rats involves Th17/Treg axis dysregulation, vascular wall remodeling, and a shift in the fatty acid profile

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Supplementary Figure 1. Distribution of peripheral regulatory T cells (Treg) and recently activated CD4 cells following 7-day salt loading

Panel A shows representative dot plots illustrating gating strategy, including exclusion of doublets using forward scatter area (FSC-A) versus forward scatter width (FSC-W) analysis (A-i), gating on live cells negative for amine-reactive fixable viability dye (A-ii), lymphocytes (A-iii) and CD3⁺ T cells (A-iv). T lymphocytes were further analyzed for the expression levels of CD25 and Foxp3 revealing representation of recently activated T helper cells (B-i) or regulatory T cells (B-ii) in the peripheral blood. The lower and upper edges of a box represent the first (Q1) and third quartiles (Q3), respectively; the horizontal line inside the box indicates the median (M); the whiskers extend to the most extreme values inside inner fences, $M \pm 1.5(Q3 - Q1)$; and the circles represent values outside the inner fences

(outliers). Each dot represents one animal. Group-wise significance was tested using the Kruskal–Wallis test (P), followed by Conover’s post-hoc test (horizontal lines, pairwise differences). *P < 0.05. Non-significant P-values were suppressed.

Supplementary Table 1. Fatty acid species observed within this study

fatty acids	Common name
C14:0	Myristic acid
C16:0	Palmitic acid
C16:1 (ω -7)	Palmitoleic acid
C18:0	Stearic acid
C18:1 (ω -9)	Oleic acid
C18:1 (ω -7)	Vaccenic acid
C 18:2 (ω -6)	Linoleic acid
C18:3 (ω -3)	α -Linolenic acid
C20:4 (ω -6)	Arachidonic acid
C24:0	Lignoceric acid
C24:1 (ω -9)	Nervonic acid
C22:6 (ω -3)	Cervonic acid

Supplementary Table 2. Enrichment analysis

Pathways/Ontology	Padj.	Process
GO BP 2021	6.50E-03	Muscle filament sliding (GO:0030049)
	6.50E-03	Actin-myosin filament sliding (GO:0033275)
	9.50E-03	Glycosaminoglycan catabolic process (GO:0006027)
Hallmark 2020	2.00E-04	Myogenesis
	2.00E-04	Epithelial mesenchymal transition

Pathway enrichment analysis showing the top enriched terms in the chosen library for up-regulated proteins (Enrichr software), along with their corresponding false discovery rate adjusted P-values (min. size ≥ 2). No significant pathway (Padj. < 0.05) emerged for down-regulated proteins.

Supplementary Table 3. Blood pressure and body mass

	LS	HS	HS + ANGII	P =
Systolic BP (mmHg)	129.0 [127.6, 131.5]	128.9 [128.4, 131.3]	125.4 [123.6, 126.9]	0.186
Diastolic BP (mmHg)	89.7 [86.1, 91.5]	92.4 [88.7, 95.3]	87.1 [84.6, 93.3]	0.901
Body mass (g)	400.5 [372.5, 407.5]	395.5 [357.8, 440.8]	401.5 [395.5, 418.0]	0.881
MAP (mmHg)	101.6 [99.5, 104.0]	105.6 [102.4, 106.7]	100.3 [97.7, 103.9]	0.750

Data are presented as median (interquartile range). The differences were tested using one-way ANOVA test, $P \leq 0.05$ was considered significant, $n = 6$ animals per group. LS – low-salt diet group; HS – high-salt diet group; HS + ANGII – HS animals receiving angiotensin II infusion.