

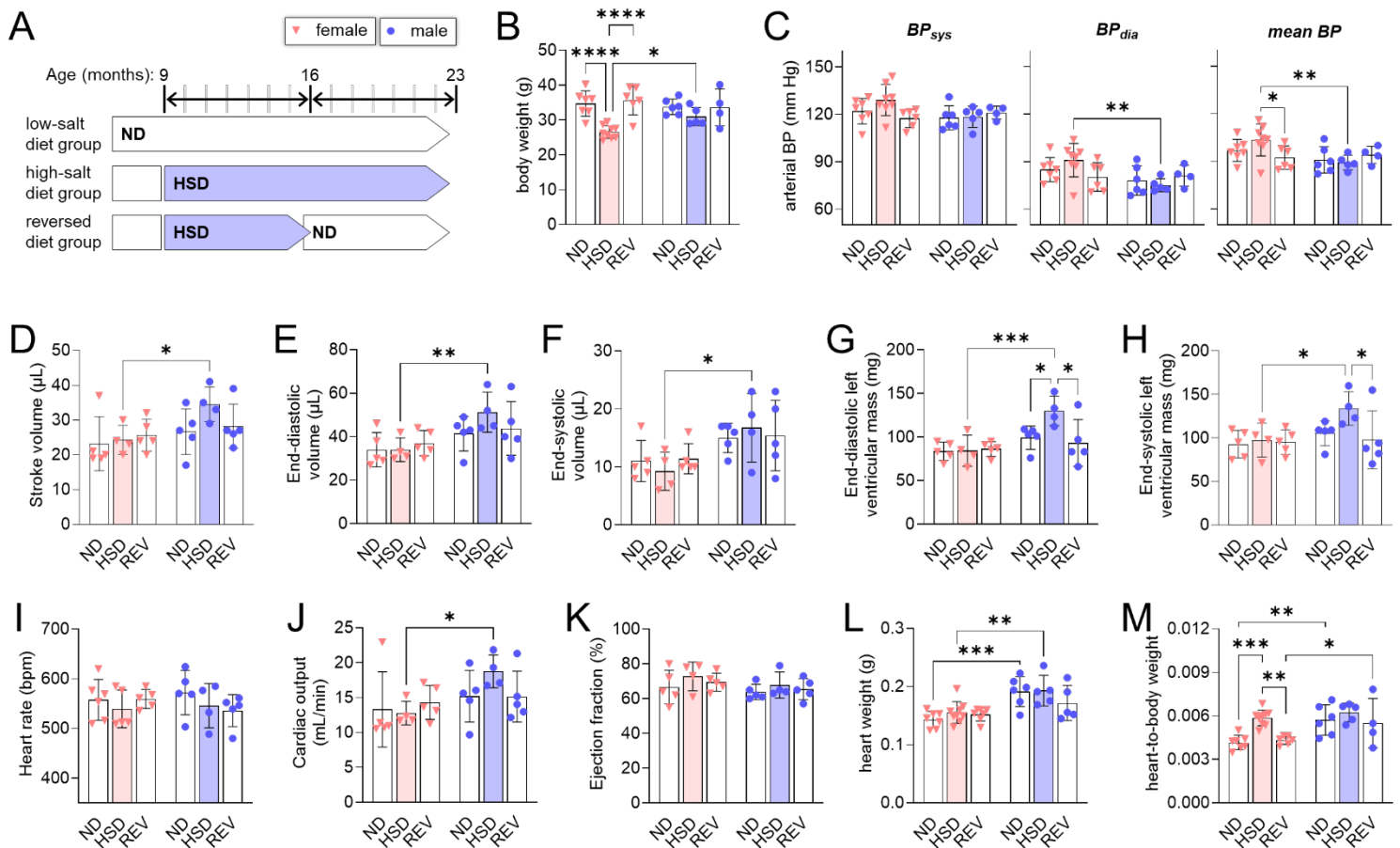
Supplementary material for Göktaş *et al.*

“Sex-specific metabolomic signature of long-term high-salt diet in mice”

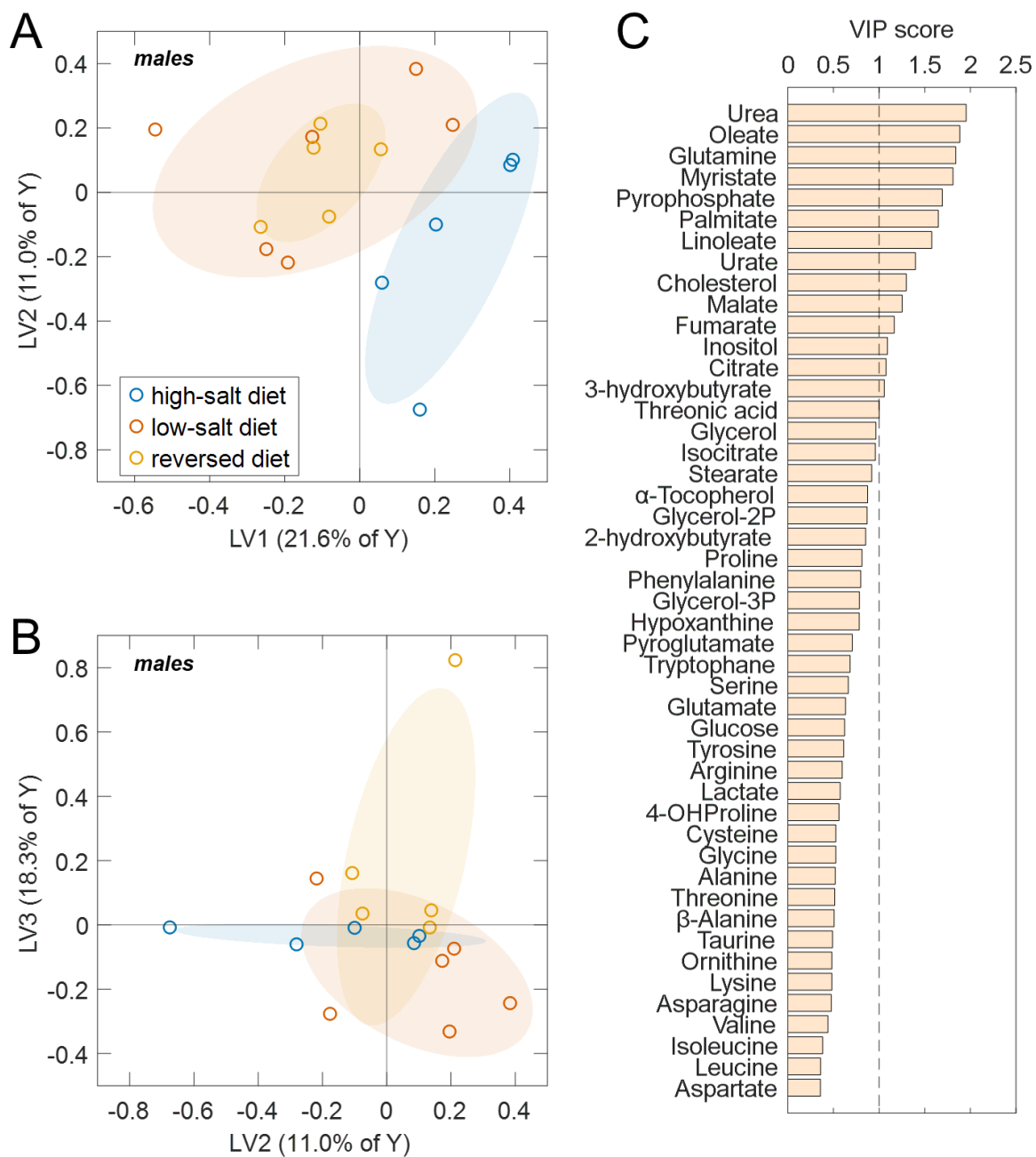
Supplementary Table S1. Results from 2-way ANOVA in Figure 1. Abbreviations: SS, sum of squares; DF, degrees of freedom; MS, mean square; DF_n, DF for numerator (effect); DF_d for denominator (error/residuals). Green shade indicates $P < 0.05$, with darker color representing higher significance.

Supplementary Table S2. Results from 2-way ANOVA in Figure 4. Abbreviations: SS, sum of squares; DF, degrees of freedom; MS, mean square; DF_n, DF for numerator (effect); DF_d for denominator (error/residuals). Green shade indicates $P < 0.05$, with darker color representing higher significance.

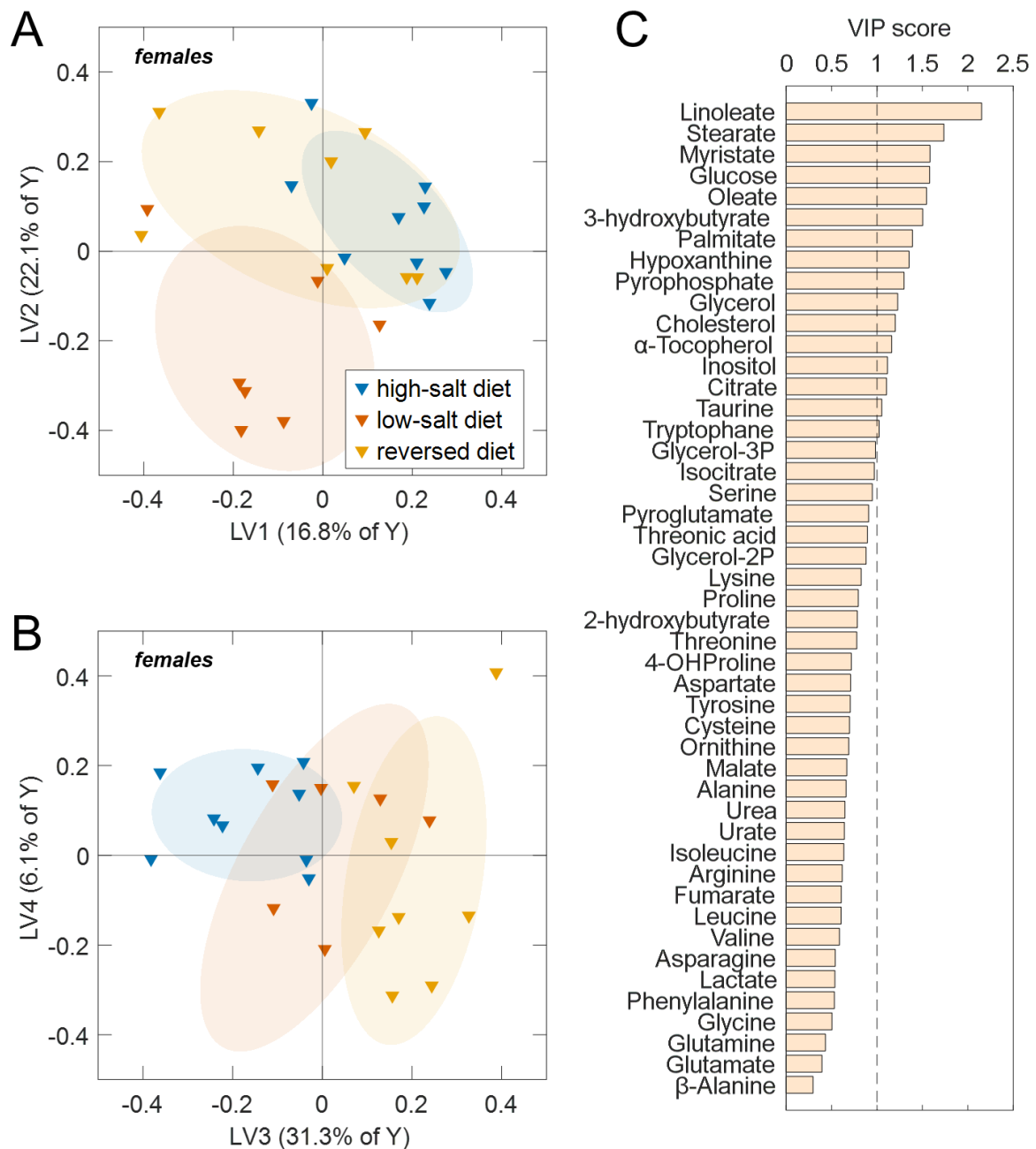
Supplementary Figure S1. Sex-specific cardiovascular effects of chronic high-salt intake and impact of diet reversal. (A) Schematic summary of the study design with interventions with normal diet (ND) and high-salt diet (HSD) during adulthood and aging. (B) Body weight at the end of the study. (C) Systolic, diastolic and mean arterial blood pressure (BP) depicting salt-sensitive hypertension in females. Stroke volume (D), end-diastolic volume (E), end-systolic volume (F), end-diastolic left ventricular mass (G), and end-systolic left ventricular mass (H) indicating cardiac remodeling and structural changes in males. (I) Heart rate in beats per minute (bpm). (J) Cardiac output showing HSD-induced functional remodeling in males. (K) Preserved ejection fraction in both sexes. (L) Small variations in heart weight, but (M) large differences in heart weight-to- body weight ratio. Data is shown as datapoints representing each mouse overlaid onto the respective mean $\pm$ SD. Triangles and dots represent female and male mice respectively. Significant differences from Holm-Šídák's multiple comparisons tests (after significant ANOVA effects) are indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.



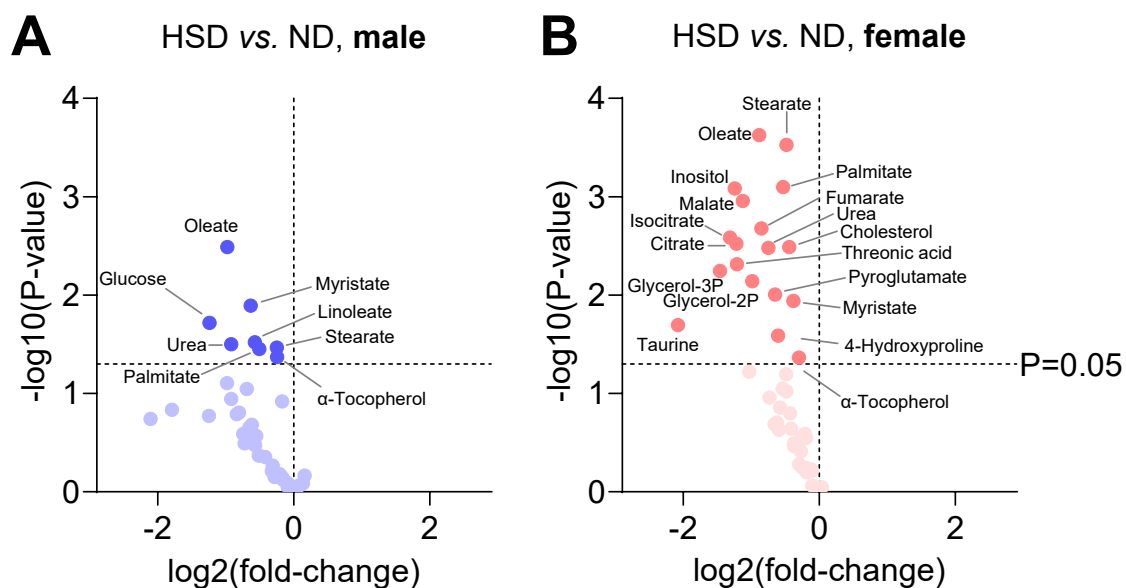
Supplementary Figure S2. Partial least squares discriminant analysis (PLS-DA) for male mice using 3 latent variables (LVs) that account for 50.9% of the variance explained. (A-B) Visualization of the distribution of samples in the latent space in score plots of LV1 *versus* LV2 (A) and LV2 *versus* LV3 (B). Each point represents an individual mouse in high-salt (blue), low-salt (orange) and reversed (yellow) diet groups. Ellipsoids represent SD of the respective group. (C) VIP score for each metabolite. The dashed line represents VIP=1.



Supplementary Figure S3. Partial least squares discriminant analysis (PLS-DA) for female mice using 4 latent variables (LVs) that account for 74.4% of the variance explained. (A-B) Visualization of the distribution of samples in the latent space in score plots of LV1 *versus* LV2 (A) and LV3 *versus* LV4 (B). Each point represents an individual mouse in high-salt (blue), low-salt (orange) and reversed (yellow) diet groups. Ellipsoids represent SD of the respective group. (C) VIP score for each metabolite. The dashed line represents VIP=1.



Supplementary Figure S4. Volcano plots of high-salt diet-induced changes on metabolite levels (HSD *versus* ND) in male (A) and female (B) mice. X-axis displays the fold-change, Y-axis displays P-values resulting from 2-tailed Student t-tests. The horizontal dashed line represents $P=0.05$.



Supplementary Figure S5. Correlation analysis of PC2 score (A) and top 10 metabolites in PC2 (B) with cardiovascular parameters previously measured in male and female mice from the 3 diet intervention groups (all treatments). Letters indicate significance: ^a $P < 0.05$, ^b $P < 0.01$, ^c $P < 0.001$. Abbreviations: ESV, end-systolic volume; EDV, end-diastolic volume; ES-LVM, end-systolic left ventricular mass; ED-LVM, end-diastolic left ventricular mass.

