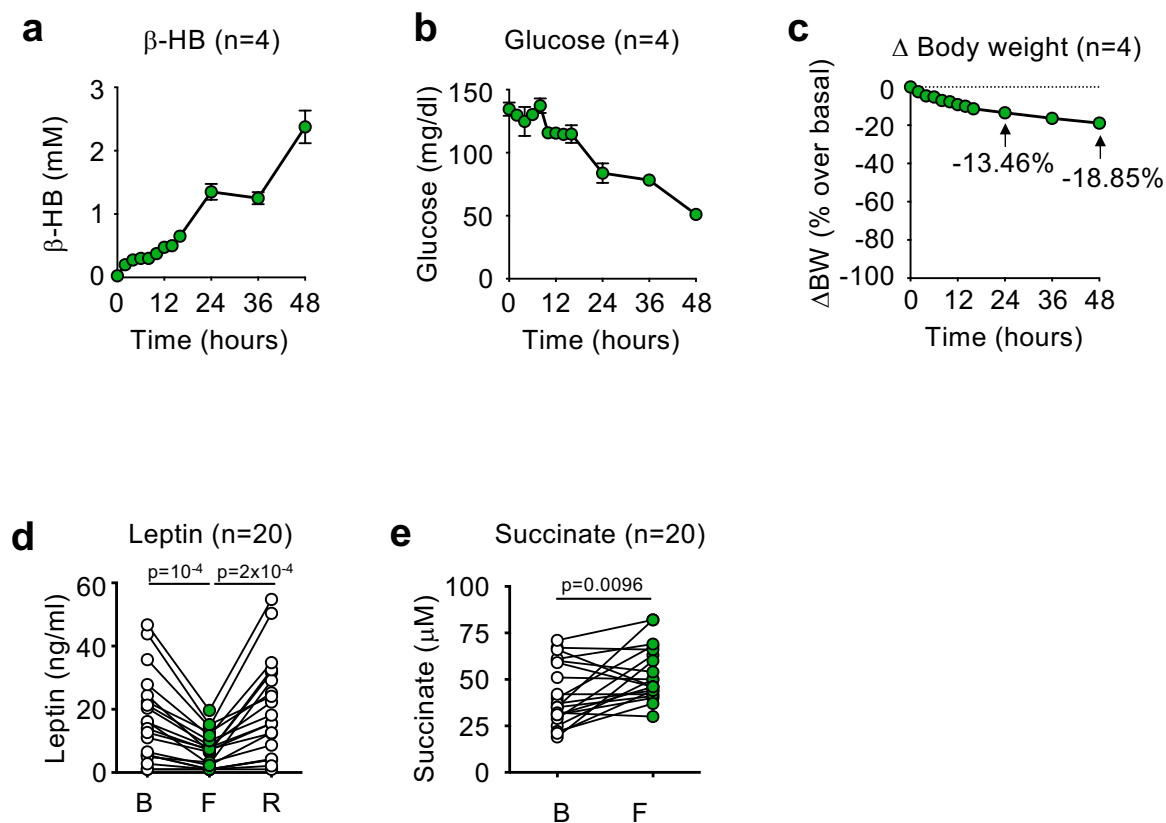


# **Fatty acids homeostasis during fasting predicts protection from chemotherapy toxicity.**

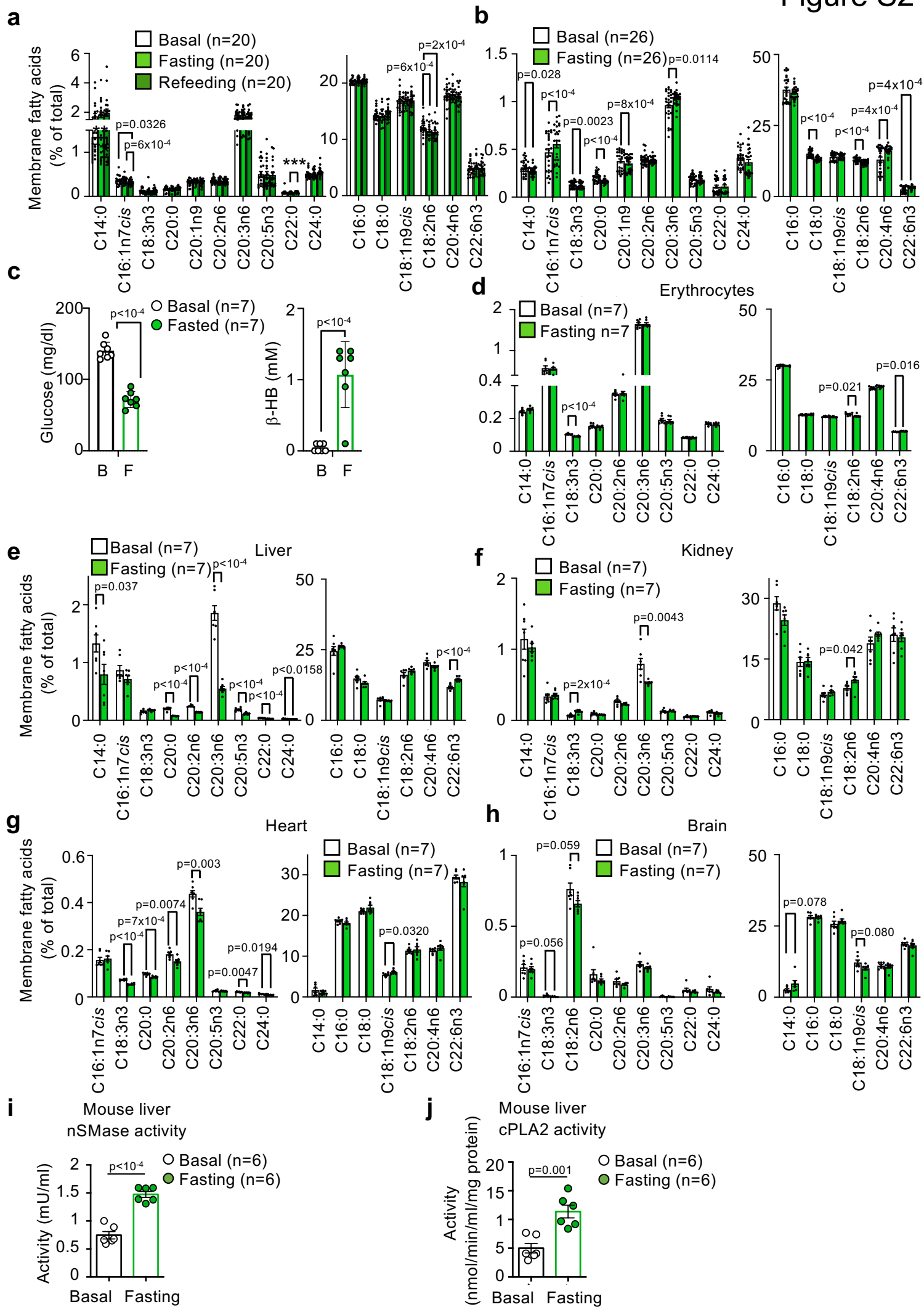
## **Inventory of Supporting Information**

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- Supplementary Figure S1.
- Supplementary Figure S2.
- Supplementary Figure S3.
- Supplementary Figure S4.
- Supplementary Figure S5.
- Supplementary Figure S6.
- Supplementary Figure S7.
- Supplementary Table 1.

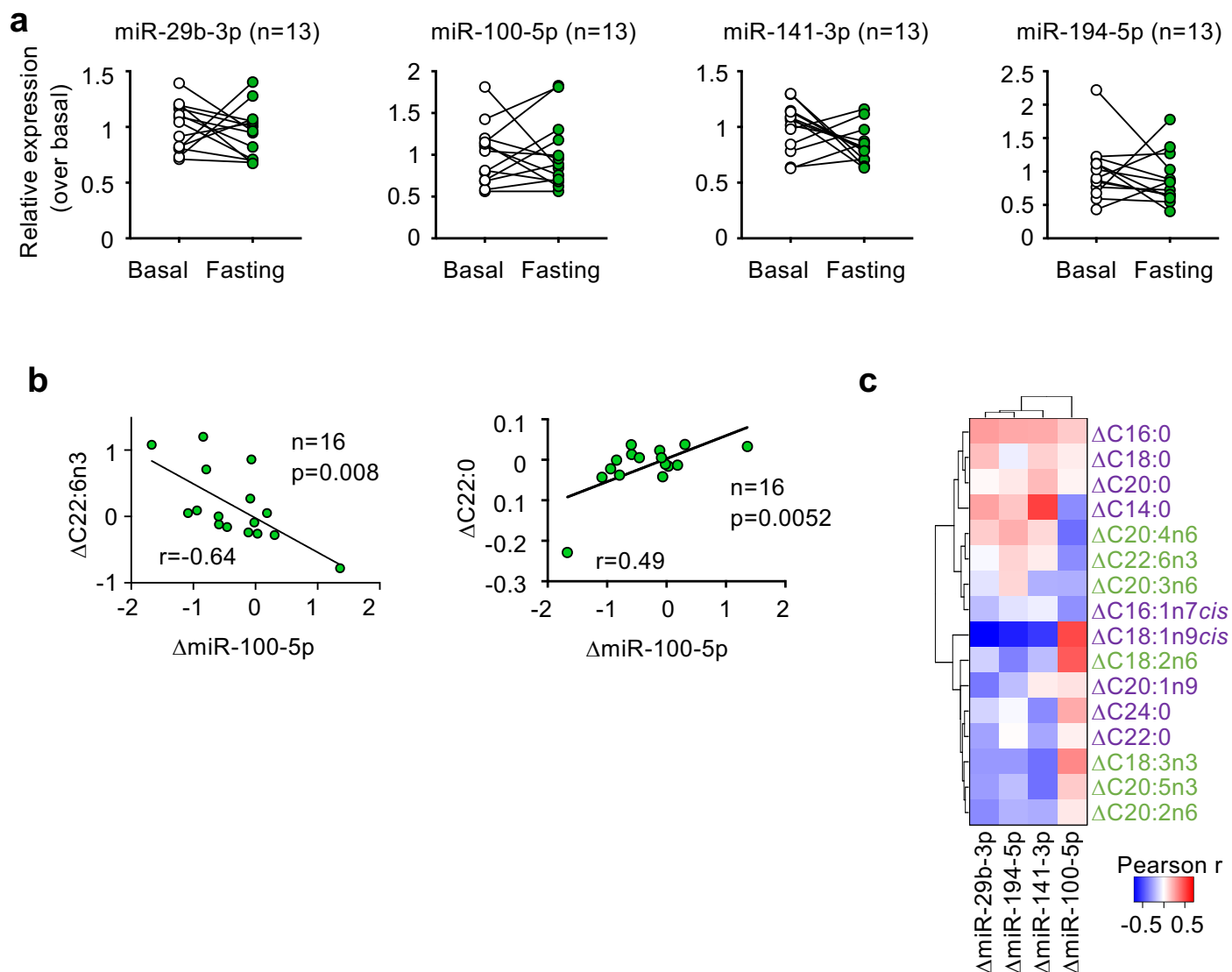


**Supplementary Figure S1. Biochemical and morphometric markers in fasting mice and humans.** (a-c) 12-week-old male mice were put to fast and the blood ketone  $\beta$ -hydroxybutyrate ( $\beta$ -HB, a), blood glucose (b) and the change in body weight ( $\Delta$ BW, expressed as a % of basal weight) compared to the initial weight (c) were recorded at the indicated times. (d-e) Blood levels at the indicated times of leptin (d) or succinate (e) in the human volunteers shown in Figure 1 at the basal point after 12 hours of overnight fasting (B); after 36 hours of fasting (F); and after 12 hours of refeeding and 12 hours of overnight fasting (R). Statistical significance was assessed using the one-way ANOVA with Tukey correction for multiple comparisons (d) or two-tailed paired Student t test (e). The exact p value is provided for each significant comparison. Source data are provided as a Source Data file.

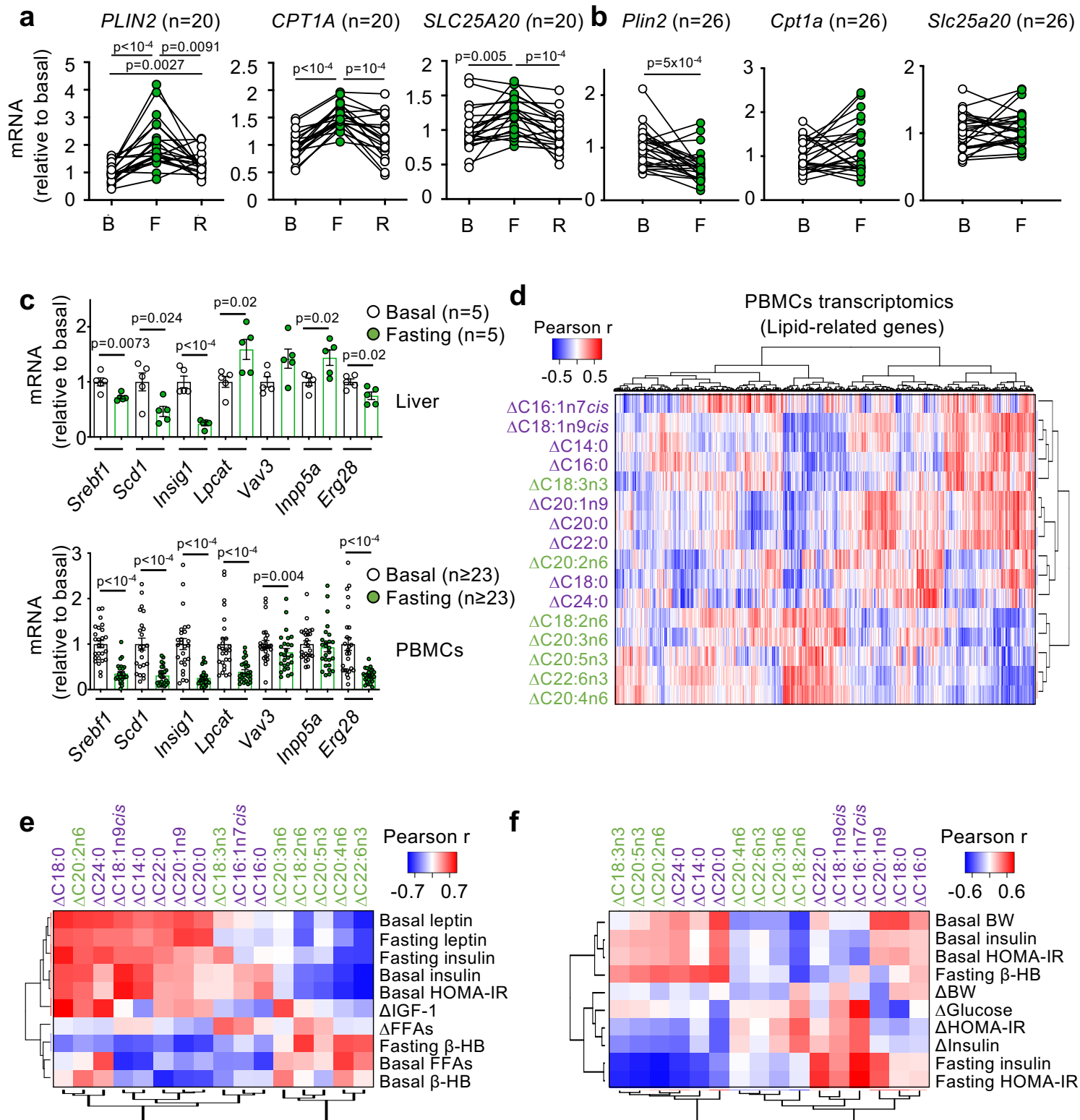


**Supplementary Figure S2. Membrane fatty acids during fasting in human erythrocytes and mouse tissues.**

(a-b) Quantification of the % of erythrocyte membrane fatty acids in the human (a) and mouse (b) cohorts shown in Figures 1 and 2 at the indicated time points: Basal, after 12 hours of fasting (humans, a) or in fed condition (mice, b); Fasting, after 36 hours (humans, a) or 24 hours (mice, b) of fasting; Refeeding: after 12 hours of refeeding and 12 hours of fasting (humans, a). (c-h) Two groups of 12-week-old male mice were either kept under normal feeding (Fed) or fasted for 24 hours (Fasted), and then sacrificed to obtain blood and tissue samples. Correct fasting was verified by measuring blood glucose and  $\beta$ -HB (c). The indicated membrane fatty acids from cell membranes of erythrocytes (d), liver (e), kidney (f), heart (g) and brain (h) were measured. (i, j) Activities of the neutral sphingomyelinase (nSMase, i) or the cytosolic phospholipase A2 (cPLA2, j) in livers from mice fed normally (Fed) or fasted for 24 hours (Fasting). Bars represent the average of the indicated number of individuals. Error bars represent the standard error of the mean. Statistical significance was assessed using one-way ANOVA with Tukey correction for multiple comparisons (a), and the paired (b) or unpaired (c-j) two-tailed Student t test. The exact p value is provided for significant comparisons. Source data are provided as a Source Data file.



**Supplementary Figure S3. Circulating miRNA changes during short-term fasting.** (a) Expression of the indicated miRNAs measured in plasma from mice at the basal point and after a 24 hour-fasting. (b) Linear correlation of the changes with fasting (fasting *minus* basal) of the indicated parameters measured in human plasma samples. (c) Heatmap showing the Pearson correlation  $r$  values between changes (fasting minus basal) of all the analyzed erythrocyte plasma membrane fatty acids and changes with fasting of the indicated circulating miRNAs significantly altered with fasting in mice. Each dot represents data from one precise individual. Lines in (a) link the paired observations from the same individual. Statistical significance was calculated using the two-tailed paired Student  $t$  test (a) or the Pearson correlation (b). The exact  $p$  value is provided for significant comparisons. Source data are provided as a Source Data file.

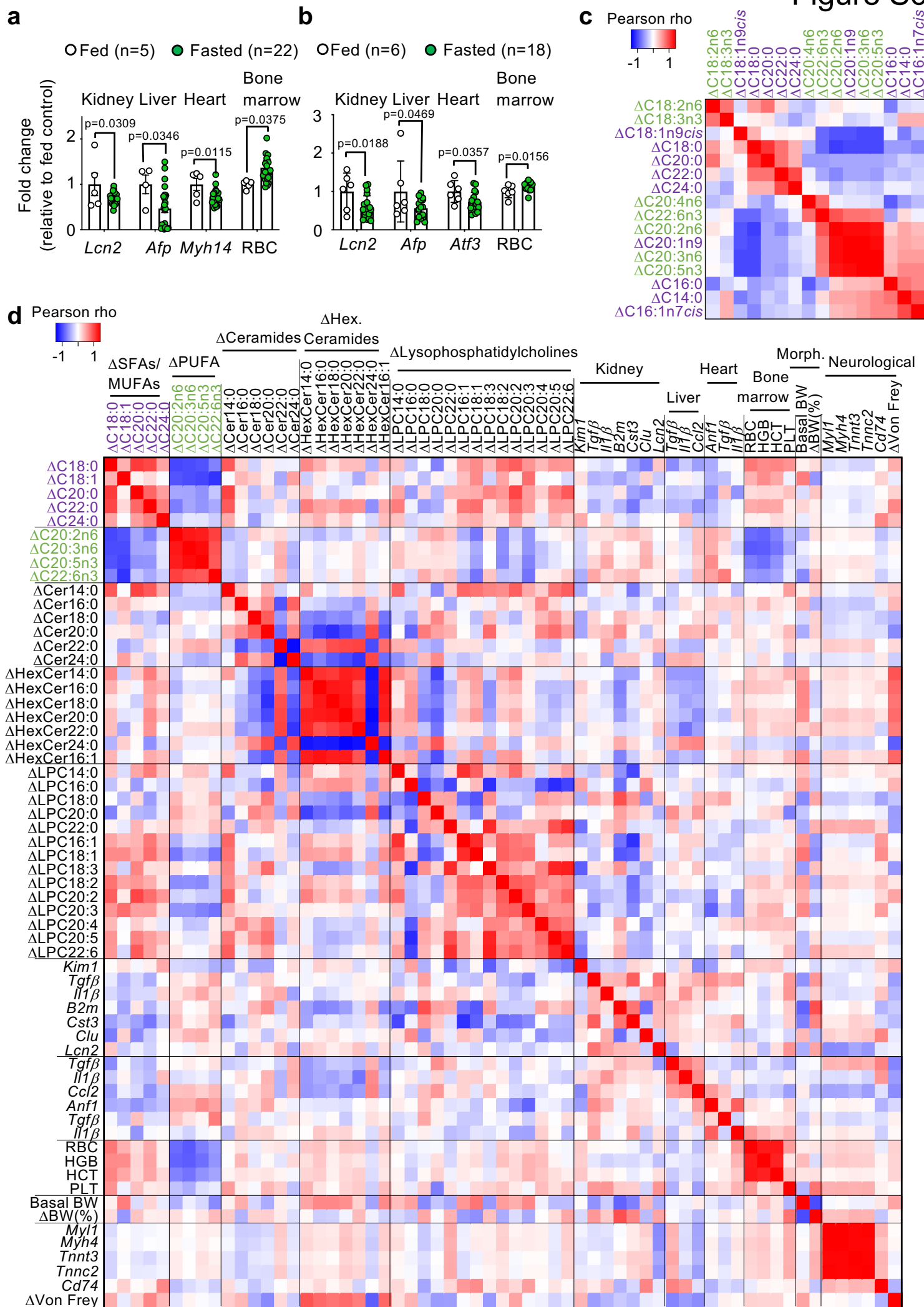


**Supplementary Figure S4. Changes in gene expression during fasting in humans and mice.** (a, b) Gene expression at PBMCs from humans (a) or mice (b) at the indicated times of the indicated PPAR $\alpha$  target genes. (c) mRNA expression of the indicated genes in livers (top panel) or PBMCs (bottom panel) of mice on normal feeding (Basal) or fasted for 24 hours (Fasting). (d) Heatmap depicting the correlation of changes with fasting of all lipid-related genes detected in the RNAseq from human peripheral blood mononuclear cells (a total of 923 genes) and the changes with fasting of the indicated erythrocyte membrane fatty acids (SFAs and MUFAs are written in purple and PUFAs are written in green). (e, f) Pearson correlations of changes in erythrocyte membrane fatty acids with the most significant biochemical and morphometric parameters shown in Table 3 for humans (e) and mice (f). HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. FFAs: free fatty acids.  $\beta$ -HB:  $\beta$ -hydroxybutyrate. BW: body weight. Line-connected dots represent the paired data for each individual (a and b), while bars represent the average of the indicated replicates (c). Error bars represent the standard error of the mean. Statistical significance was assayed using the one-way ANOVA with Tukey correction for multiple comparisons (a); the unpaired (c, top panel) or paired (b, c-bottom panel) two-tailed Student t test; or Pearson correlations (d-f). The exact p value is provided for significant comparisons. Source data are provided as a Source Data file.

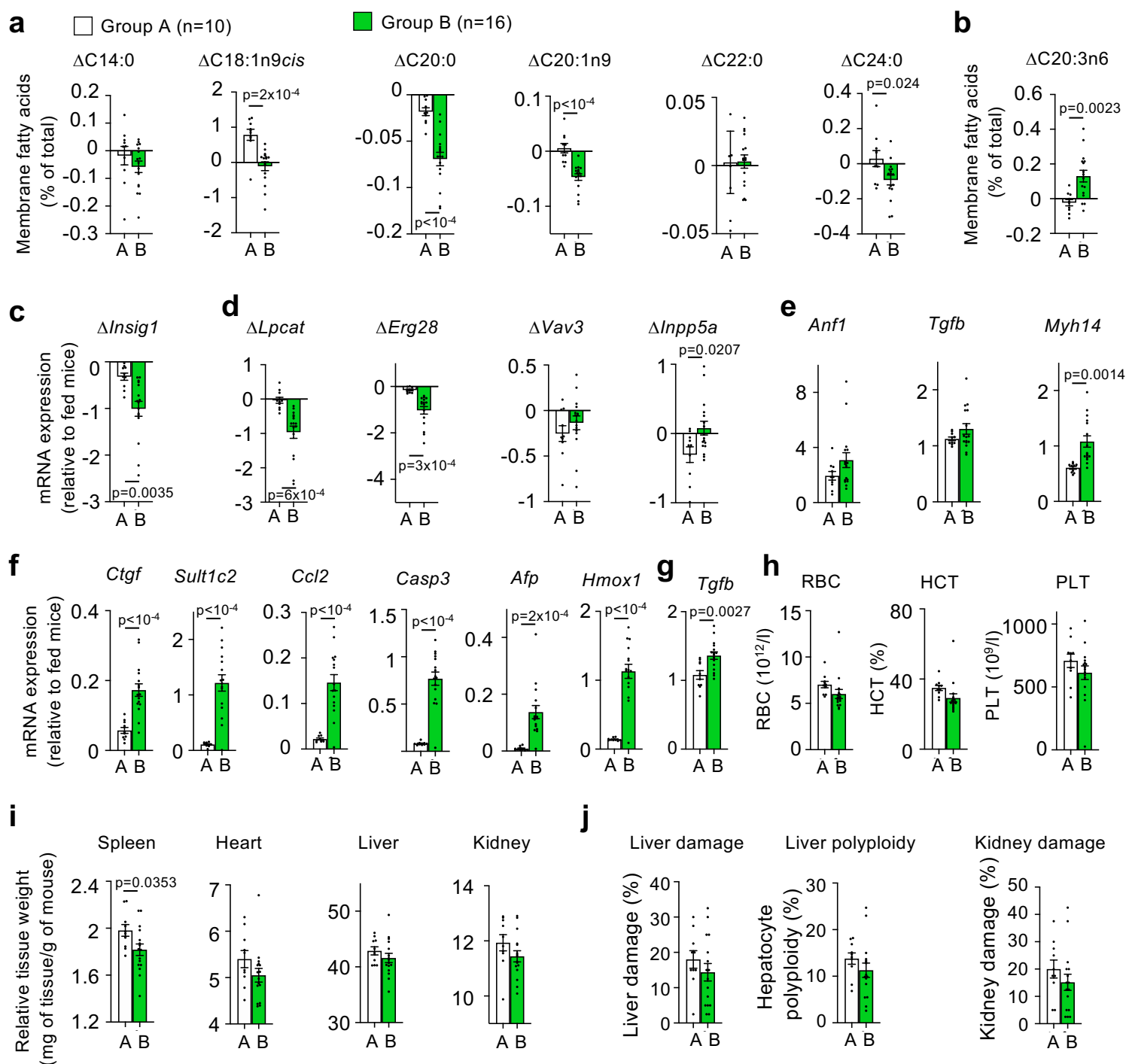


**Supplementary Figure S5. Different fasting protocols protect from different chemotherapy toxicities. (a, b)** Expression of the indicated toxicity-reporter genes in liver (a) or blood neutrophils counts (b) from mice shown in Figure 5 either fed ad libitum or fasted for 24 hours before and 24 hours after i.p. inoculation mice of saline (S) or 15 mg/kg oxaliplatin (CT), and sacrificed 5 days after chemotherapy administration. **(c-g)** Expression of the indicated toxicity-reporter genes in heart (c), kidney (d) or liver (e); counts of the indicated lymphocyte populations (f; Mon: monocytes; WBC: white blood cells; Lym: lymphocytes); and body weight (g) at the indicated time points after chemotherapy (CT) administration from mice either fed ad libitum or fasted for 24 hours before and 24 hours after i.p. inoculation of saline (S) or 15 mg/kg doxorubicin (CT). **(h-m)** Expression of the indicated toxicity-reporter genes in liver (h), heart (i) or kidney (j); blood platelet counts (PLT, k); body weight (l); and neurotoxicity biomarkers (m) including the scores for the neurotoxicity-measuring experiment von Frey test (m, panel to the left) and expression of the indicated toxicity genes in dorsal root ganglia (m, panels to the right) from mice either fed ad libitum or fasted for 12 hours before and 12 hours after i.p. inoculation of saline (S) or 15 mg/kg oxaliplatin (CT), and sacrificed 5 days after chemotherapy administration. Bars and line-connected dots represent the average of  $n \geq 5$  mice per treatment group. Error bars represent the standard error of the mean. Statistical significance was assayed using the one-way ANOVA test (a-f, h-k, m) or the repeated measures two-way ANOVA test (g, l) with Tukey correction for multiple comparisons. When possible, the exact p value is provided for significant comparisons; otherwise, \*,  $p < 0.05$ , \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$ . Source data are provided as a Source Data file.

Figure S6



**Supplementary Figure S6. Lipidomics of mouse erythrocyte membranes and its association with chemotherapy toxicity.** (a) Expression of the indicated toxicity biomarker genes in the indicated tissues, in the mice shown in Figure 6, subjected to 15 mg/kg oxaliplatin inoculation and either fed ad libitum (Basal) or fasted for 24 hours before and 24 hours after (Fasting) oxaliplatin administration. (b) Expression of the indicated toxicity biomarker genes in the indicated tissues, in a new cohort of mice subjected to the same treatment as in (a). (c) Heatmap representing the Pearson correlation coefficients ( $r$ ) between the changes with fasting of erythrocyte membrane fatty acids of the mice shown in (b) after the first 24 hours of fasting. (d) Heatmap representing the Pearson correlation coefficients ( $r$ ) between the changes with 24 hours of fasting ( $\Delta$ ) of the indicated lipids carrying one single fatty acid (total fatty acids, ceramides, hexosyl-ceramides and lysophosphatidylcholines), and the indicated toxicity biomarkers obtained at sacrifice. RBC: red blood cells. HGB: hemoglobin. HCT: hematocrit. PLT: platelets. BW: body weight. Bars represent the average of the indicated number of mice per treatment group. Error bars represent the standard error of the mean. Statistical significance was assayed using the unpaired two-tailed Student t test. The exact p value is provided for significant comparisons. Source data are provided as a Source Data file.



**Supplementary Figure S7. Distribution of biomarkers of fasting and chemotherapy toxicity in mice according to the fasting-response groups.** (a-j) Groups A and B, identified in Figures 4f and 7a, were compared for the indicated parameters: changes with fasting of the levels of the indicated SFAs and MUFAs (a) or PUFAs (b) in the erythrocyte membranes; changes with fasting of the expression of the indicated genes in PBMCs (c, d); expression of the indicated toxicity genes in heart (e), liver (f) and kidney (g) 5 days after chemotherapy administration; hematological parameters (h; RBC: red blood cells; HCT: hematocrit; PLT: platelets); relative weights of the indicated tissues (i); and histological findings as described in Figure 5 (j). Bars represent the mean of n=10 (group A) and n=16 (group B). Error bars represent the standard error of the mean. Statistical significance was assessed using the unpaired two-tailed Student t test. The exact p value is provided for significant comparisons. Source data are provided as a Source Data file.

# Supplementary Table 1

Human
Parameter
Age
Height
Basal body weight
BMI
Total fat %
Lean mass %
Visceral fat classification
Basal Metabolism
Waist
Systolic pressure
Diastolic pressure
Heart rate
Basal glucose
Fasting glucose
$\Delta$ Glucose
Basal insulin
Fasting insulin
$\Delta$ Insulin
Basal HOMA-IR
Fasting HOMA-IR
$\Delta$ HOMA-IR
Basal leptin
Fasting leptin
$\Delta$ Leptin
Basal $\beta$ -HB
Fasting $\beta$ -HB
$\Delta\beta$ -HB
Basal FFAs
Fasting FFAs
$\Delta$ FFAs
Basal IGF1
Fasting IGF1
$\Delta$ IGF1

Mouse
Parameter
Basal body weight
$\Delta$ Body weight
Basal glucose
Fasting glucose
$\Delta$ Glucose
Basal insulin
Fasting insulin
$\Delta$ Insulin
Basal HOMA-IR
Fasting HOMA-IR
DHOMA-IR
Fasting $\beta$ -HB

**Supplementary Table 1:** list of the morphometric, physiological and biochemical parameters used to generate Table 3