Supplemental Material

Multi-omics Reveal that c-Src Modulates the Mitochondrial Phosphotyrosine Proteome and Metabolism According to Nutrient Availability

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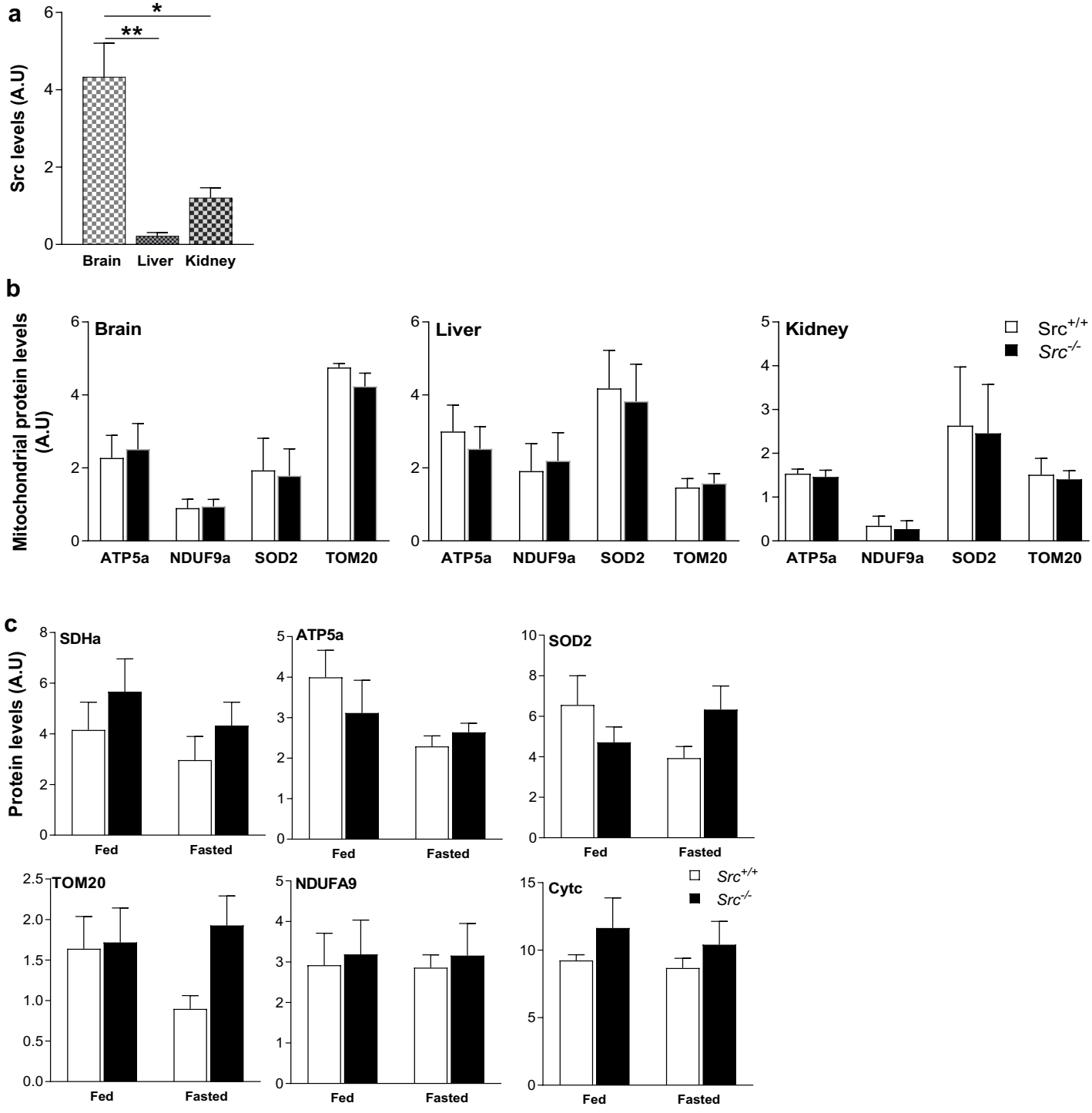
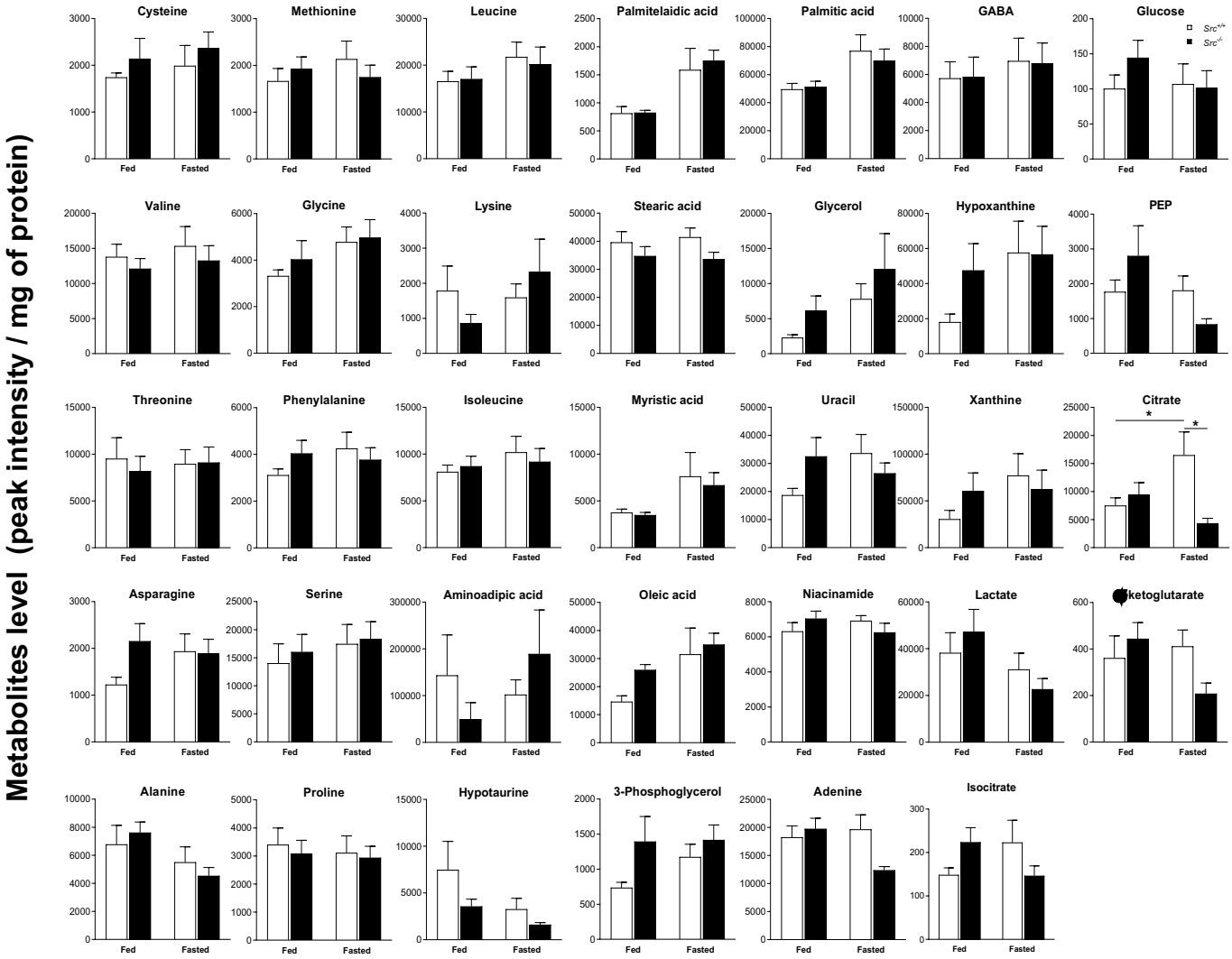

a
Src levels (A.U)
Brain
Liver
Kidney
b
Mitochondrial protein levels (A.U)
Brain
Liver
Kidney
ATP5a
NDUF9a
SOD2
TOM20
Src+/+
Src-/-
c
Protein levels (A.U)
SDHa
ATP5a
SOD2
TOM20
NDUFA9
Cytc
Fed
Fasted

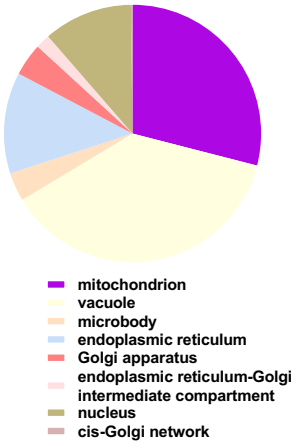
Figure S1. Deletion of Src does not alter levels of mitochondrial proteins. (a) Quantification of data presented in Fig. 1a showing levels of Src in brain, liver and kidney mitochondria. (**b**) Quantification of data presented in Fig. 1j showing the level of different mitochondrial proteins in brain, liver and kidney mitochondria. **(c)** Quantification of data presented in Fig. 2j, showing the level of different mitochondrial proteins in liver of $Src^{+/+}$ and $Src^{-/-}$ mice fed or fasted. Data are presented as mean ± s.e.m. Data are not significantly different ($p \geq 0.05$) determined by Student's T test (a and b) or by two-way ANOVA followed by post-hoc Tukey's test (c).



Guedouari et al, Fig. S2

Figure S2. Hepatic metabolites not significantly different among *Src*$^{+/+}$ and *Src*$^{-/-}$ mice fed *ad libitum* or fasted determined by two-way ANOVA. Data are presented as mean ± s.e.m. (n = 7).

a


Cellular localization
mitochondrion
vacuole
microbody
endoplasmic reticulum
Golgi apparatus
endoplasmic reticulum-Golgi intermediate compartment
nucleus
cis-Golgi network

b

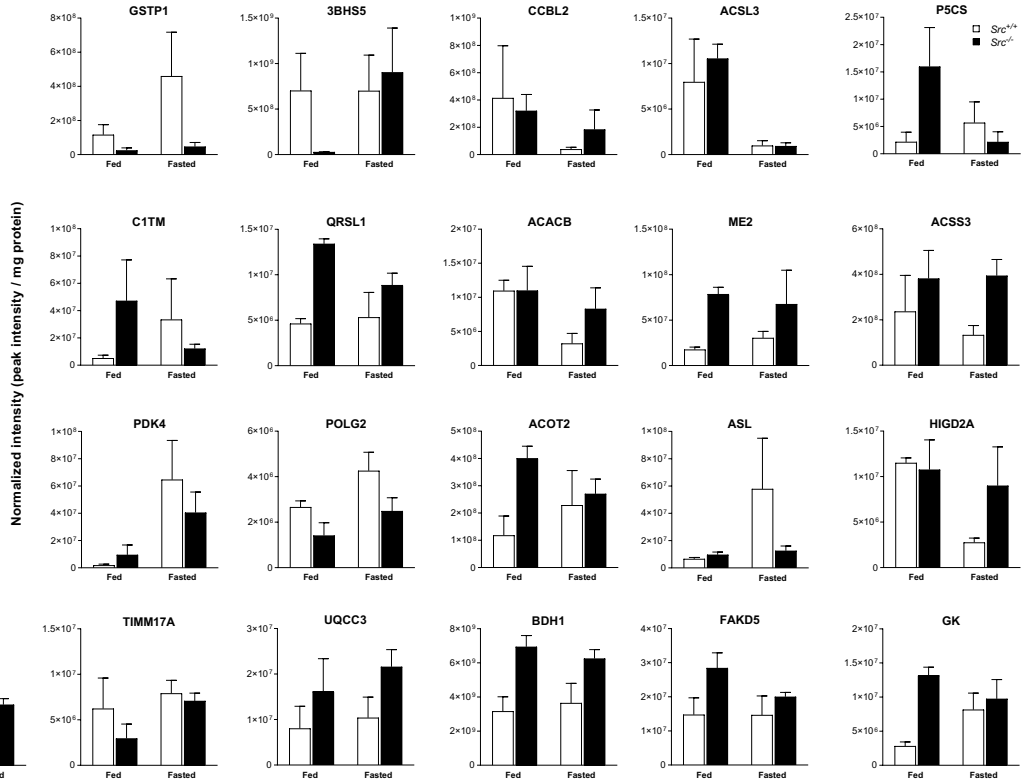
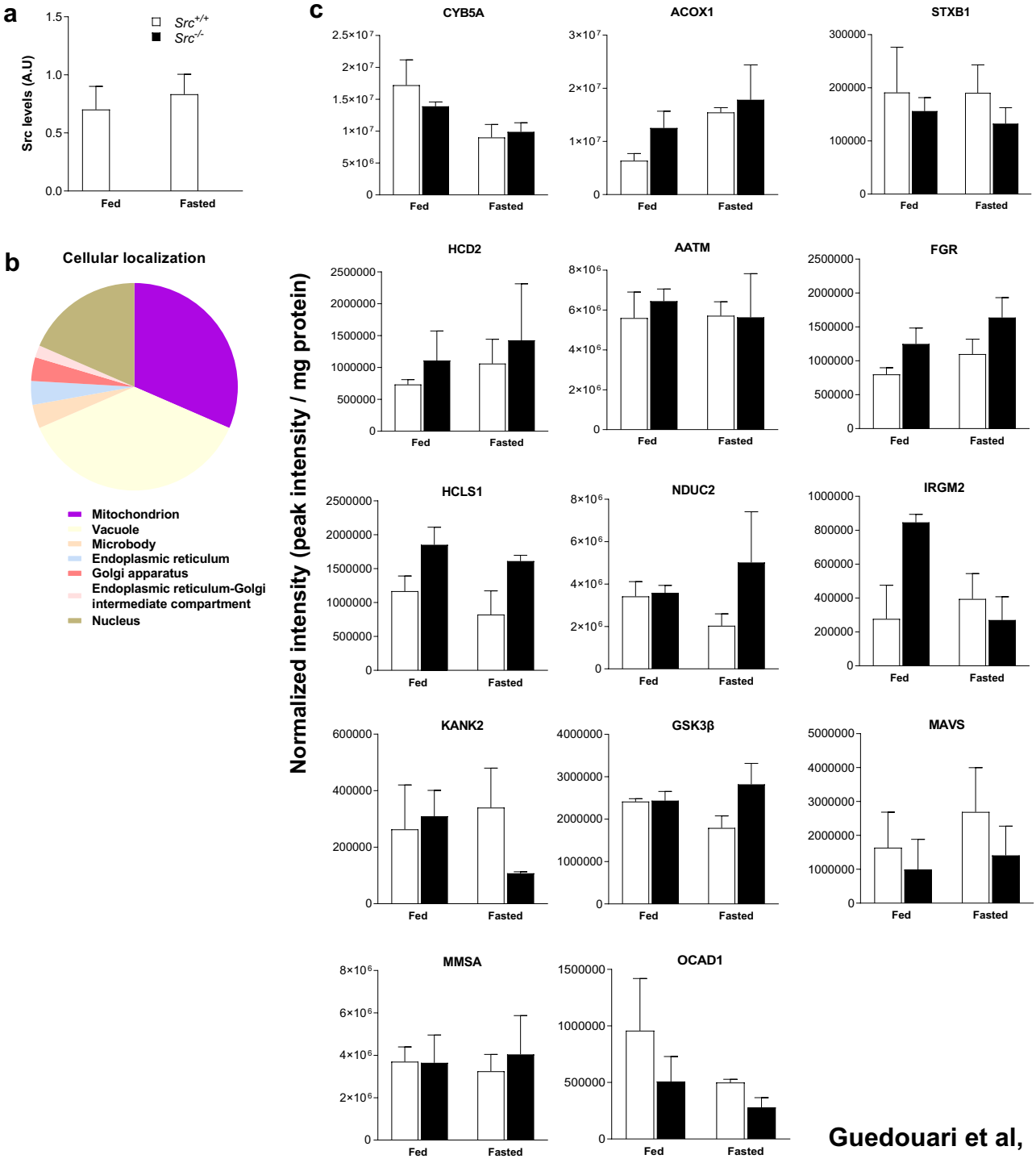

Normalized intensity (peak intensity / mg protein)
GSTP1
3BHS5
CCBL2
ACSL3
P5CS
Src+/+
Src-/-
C1TM
QRSL1
ACACB
ME2
ACSS3
PDK4
POLG2
ACOT2
ASL
HIGD2A
MIC25
FADS1
TIMM17A
UQCC3
BDH1
FAKD5
GK
Fed
Fasted

Figure S3. Proteomic characterization of *Src*$^{+/+}$ and *Src*$^{-/-}$ mice according to nutrient availability. (**a**) Cellular localization of all proteins detected by nanoLC-MS/MS analyses. (**b**) Hepatic mitochondrial proteins not significantly different among *Src*$^{+/+}$ and *Src*$^{-/-}$ mice fed *ad libitum* or fasted determined by two-way ANOVA. Only proteins with VIP score ≥ 2 are shown. Data are presented as mean ± s.e.m. (n = 3). Means are not significantly different ($p \geq 0.05$).



Guedouari et al, Fig. S4

Figure S4. Phosphotyrosine proteomic characterization of *Src*$^{+/+}$ and *Src*$^{-/-}$ liver mitochondria during fasting. (**a**) Quantification of Src levels shown in Fig 5a. (**b**) Cellular localization of tyrosine-phosphorylated proteins identified by nanoLC-MS/MS in *Src*$^{+/+}$ and *Src*$^{-/-}$ mice fed *ad libitum* and fasted during 24h. (**c**) Hepatic mitochondrial phosphoproteins not significantly different among *Src*$^{+/+}$ and *Src*$^{-/-}$ mice fed *ad libitum* or fasted determined by two-way ANOVA. Only proteins with VIP score ≥ 1 are shown. Data are presented as mean ± s.e.m. (n = 3). Means are not significantly different ($p \geq 0.05$).

Due to the size of the Supplementary Tables, please use the following links to download and open the Excel-file.

Supplementary Table 1. Dataset listing the detected proteins in liver enriched-mitochondrial fractions derived from Src+/+ and Src-/- mice fed ad libitum or fasted during 24h using nanoLC-MS/MS.
https://www.cellphysiolbiochem.com/Articles/000237/SM/Supplementary_Table_1.xlsx

Supplementary Table 2. Dataset listing the mitochondrial proteins (according to Uniprot annotations) detected by nanoLC-MS/MS in liver enriched-mitochondrial fractions derived from Src+/+ and Src-/- mice fed ad libitum or fasted during 24h.
https://www.cellphysiolbiochem.com/Articles/000237/SM/Supplementary_Table_2.xlsx

Supplementary Table 3. Mitochondrial proteins with VIP score ≥ 1 driving the proteomic signature of Src+/+ and Src-/- mice fed ad libitum or fasted during 24h.
https://www.cellphysiolbiochem.com/Articles/000237/SM/Supplementary_Table_3.xlsx

Supplementary Table 4. Dataset listing the detected phosphotyrosine proteins in liver enriched-mitochondrial fractions derived from Src+/+ and Src-/- mice fed ad libitum or fasted during 24h using nanoLC-MS/MS. Phosphorylation site: numbers in parenthesis indicate the phosphorylation probability.
https://www.cellphysiolbiochem.com/Articles/000237/SM/Supplementary_Table_4.xlsx

Supplementary Table 5. Dataset listing the detected mitochondrial phosphotyrosine proteins (according to Uniprot annotations) in liver enriched-mitochondrial fractions derived from Src+/+ and Src-/- mice fed ad libitum or fasted during 24h using nanoLC-MS/MS.
https://www.cellphysiolbiochem.com/Articles/000237/SM/Supplementary_Table_5.xlsx