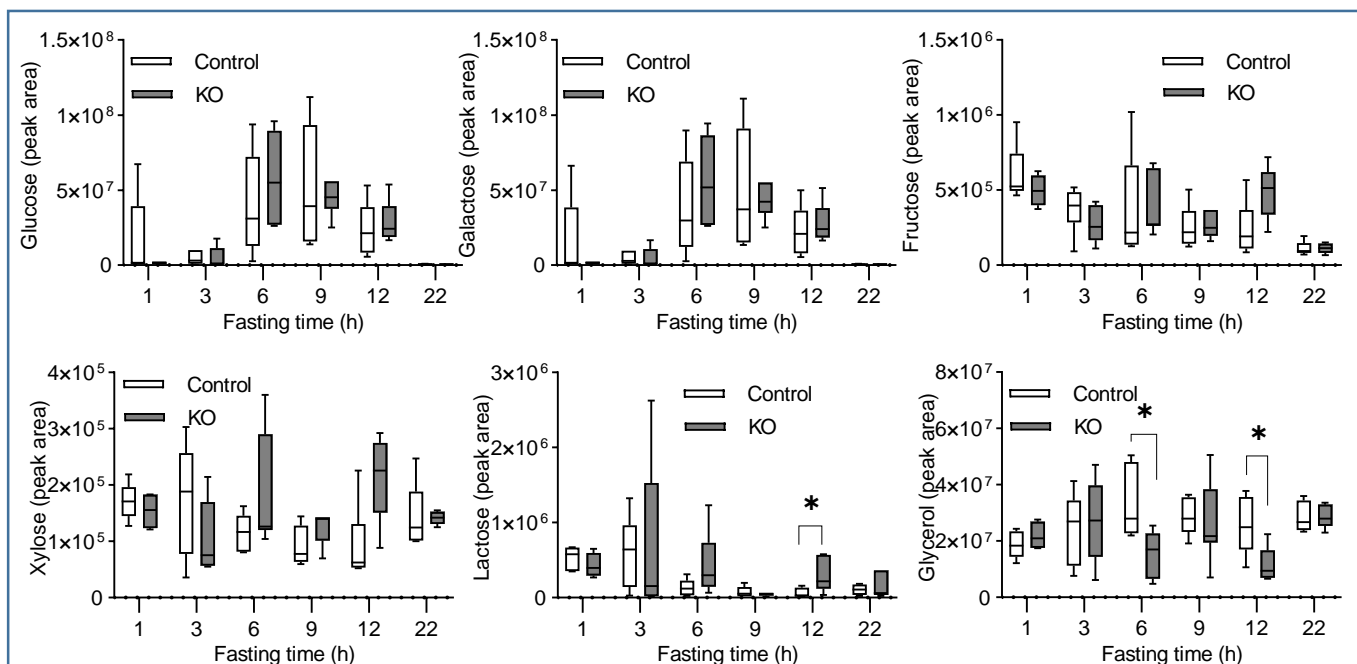
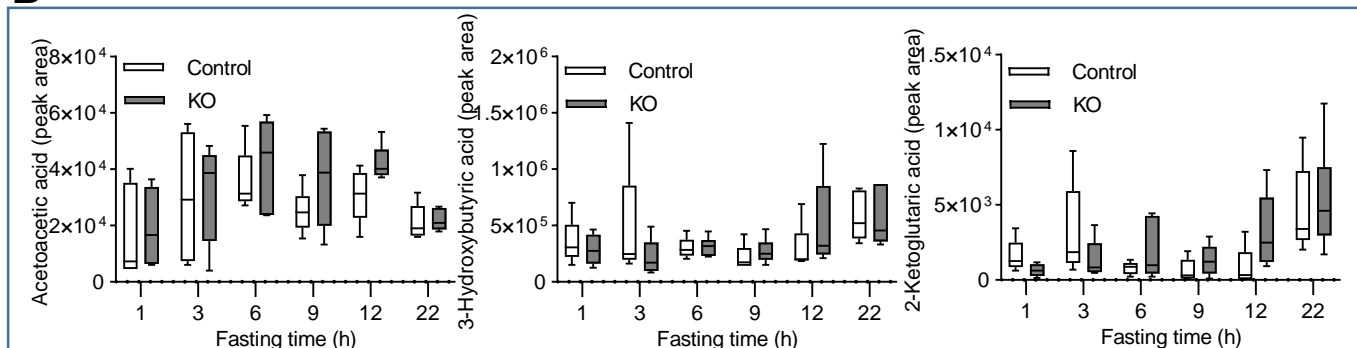


Supplementary Figure S1. The automated Western blotting analysis of NCoR1 and PPAR α in the liver. (A) The protein expression of NCoR1 and PPAR α in liver extract of Control and KO mice after 1 and 12 h of starvation (n = 3 at each starvation time point). The expression of NCoR1 (B) and PPAR α (C) refer to Control mice at 1 and 12 h of starvation were shown in graphs. β -actin was used as an internal control. Each bar is expressed as the mean \pm SEM. P, Student's t-test; KO: liver-specific *Atg5*-deficient; NCoR1: nuclear receptor co-repressor 1; PPAR α : peroxisome proliferator-activated receptor α .

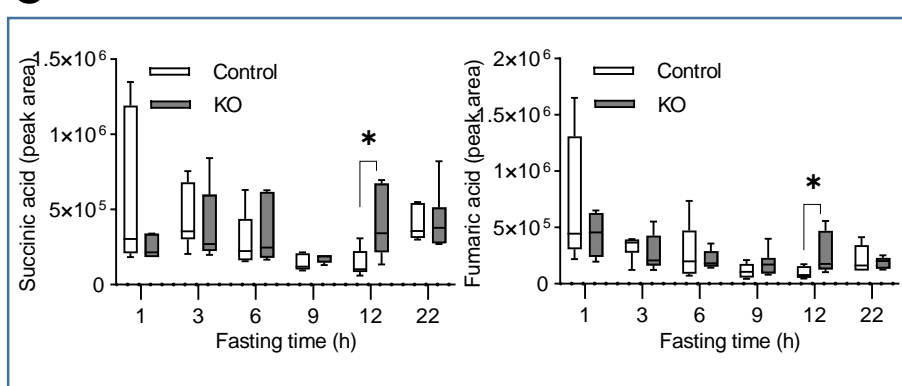
A



B



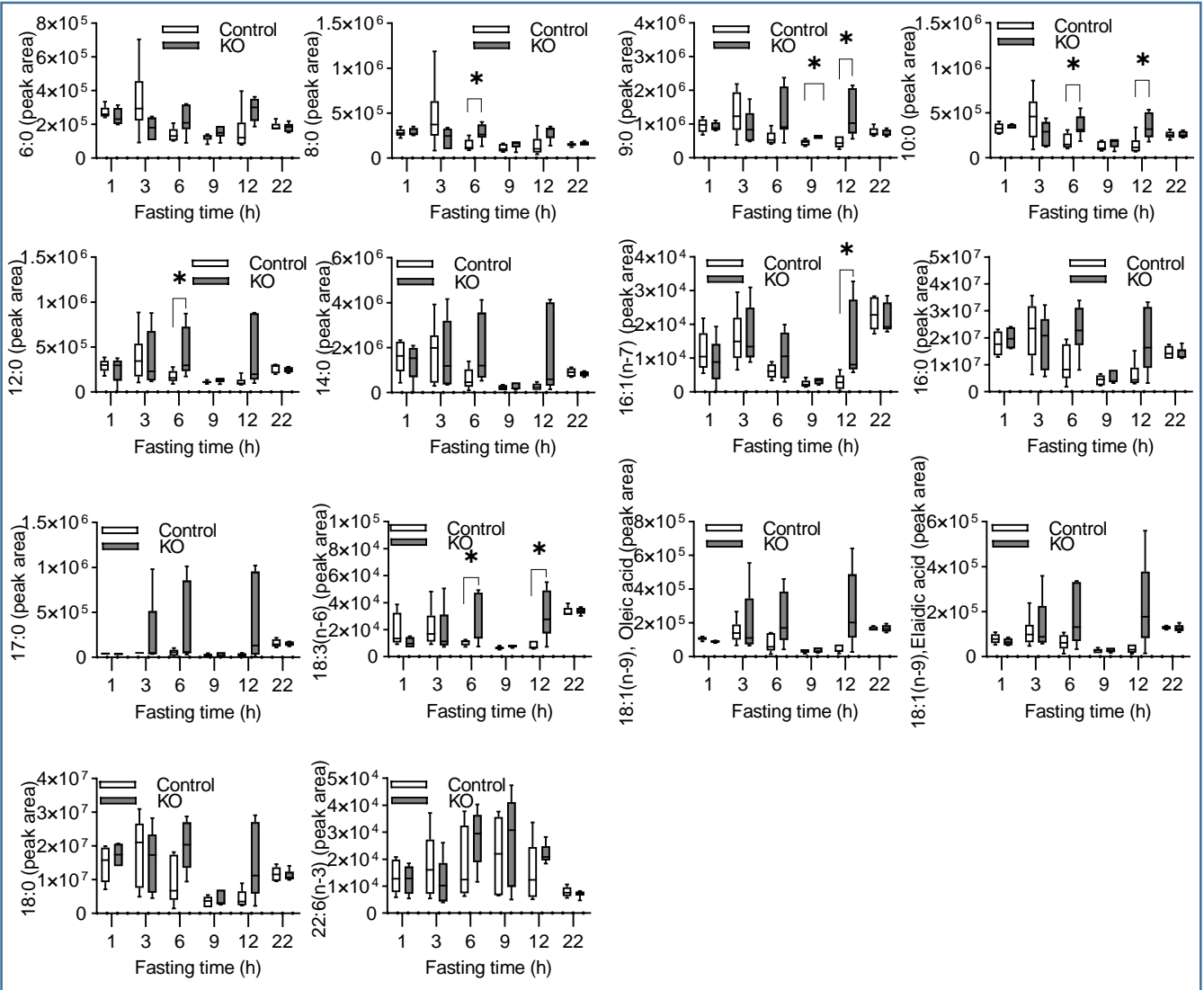
C



Supplementary Figure S2. The trajectory course of serum (A) carbohydrates and glycerol, (B) ketone bodies and (C) Succinic acid and Fumaric acid by the GC-MS/MS analysis with 22-h starvation survivors

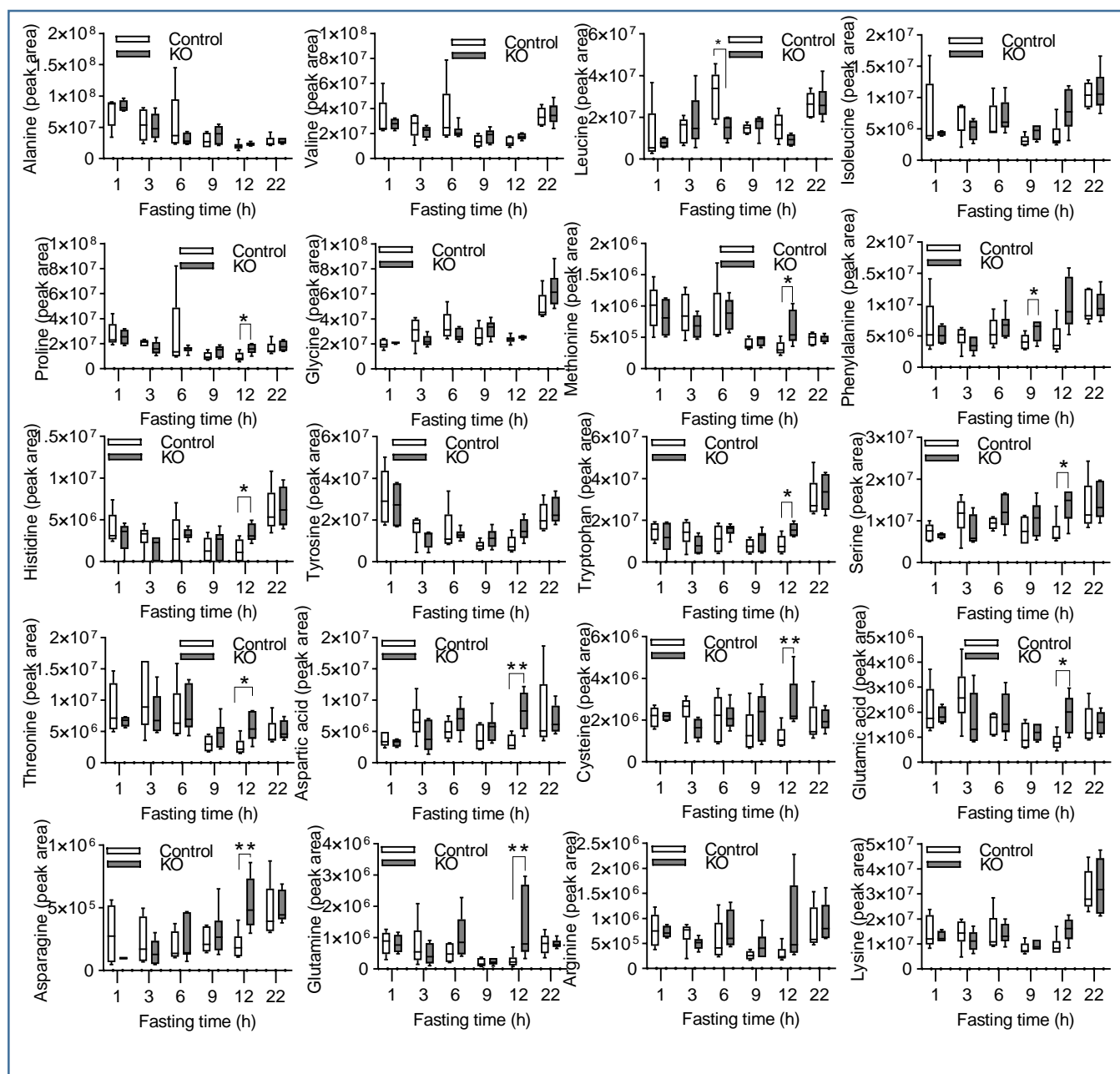
The numbers of mice (starvation time) were as follows: 5 (1 h), 6 (3 h), 5 (6 h), 6 (9 h), 6 (12 h), and 5 (22 h) Control mice and 4 (1 h), 5 (3 h), 7 (6 h), 7 (9 h), 5 (12 h), and 6 (22 h) KO mice. The concentration of metabolites was measured as the peak area of the calibration curve, and the concentration at each starvation time was compared between the groups. Boxes represent the interquartile range (25th to 75th percentiles), and lines within the boxes are the median; error bars represent the 25th percentile minus 1.5 times interquartile range (IQR) and the 75th percentile plus 1.5 times IQR. Mann-Whitney U test; * $P < 0.05$, ** $P < 0.01$. KO: liver-specific *Atg5*-deficient.

A



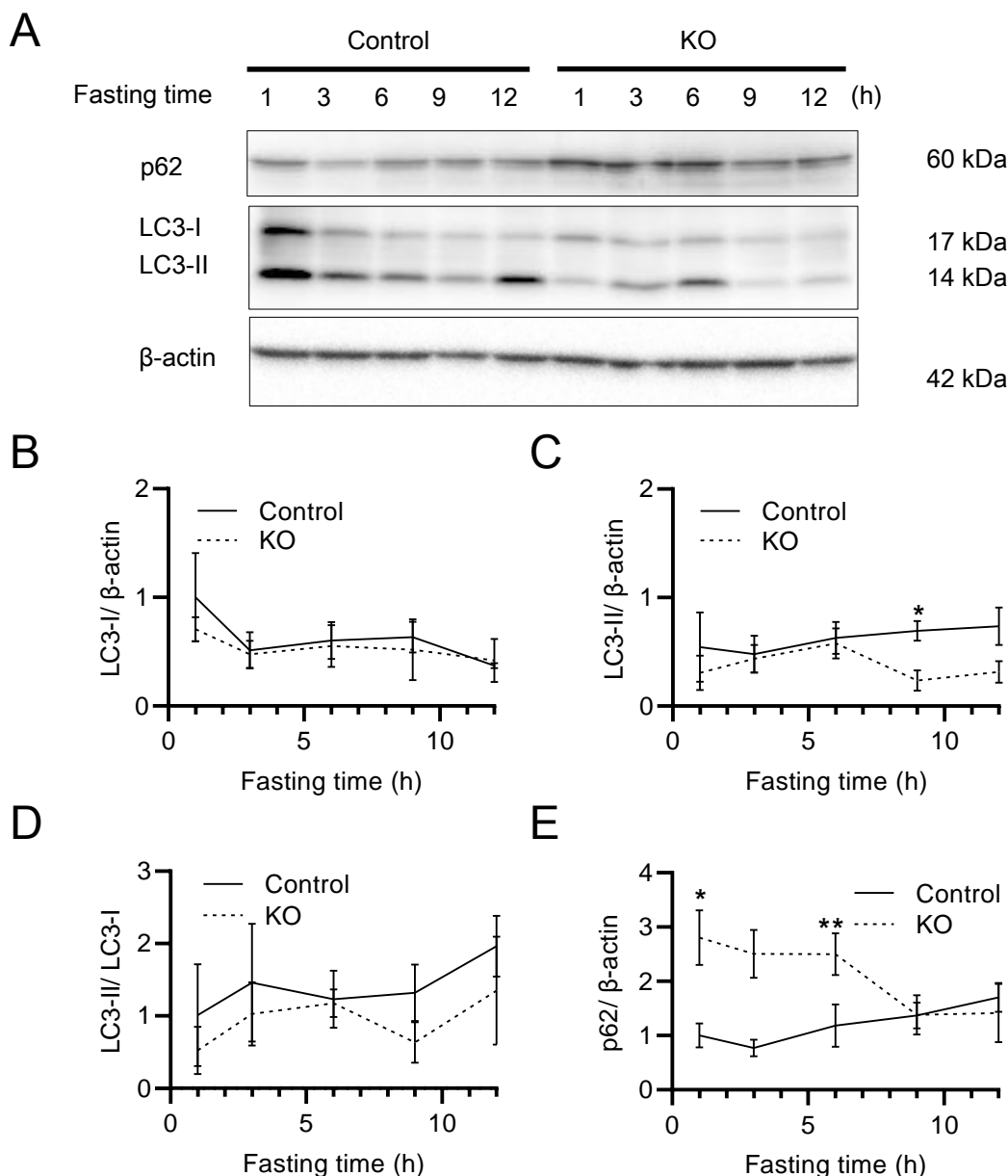
Supplementary Figure S3. The trajectory course of serum FFAs with the GC-MS/MS analysis with 22-h starvation survivors

The numbers of mice (starvation time) were as follows: 5 (1 h), 6 (3 h), 5 (6 h), 6 (9 h), 6 (12 h), and 5 (22 h) Control mice and 4 (1 h), 5 (3 h), 7 (6 h), 7 (9 h), 5 (12 h), and 6 (22 h) KO mice. The concentration of metabolites was measured as the peak area of the calibration curve, and the concentration at each starvation time was compared between the groups. Boxes represent the interquartile range (25th to 75th percentiles), and lines within the boxes are the median; error bars represent the 25th percentile minus 1.5 times interquartile range (IQR) and the 75th percentile plus 1.5 times IQR. Mann-Whitney U test; * $P < 0.05$, ** $P < 0.01$. KO: liver-specific *Atg5*-deficient.



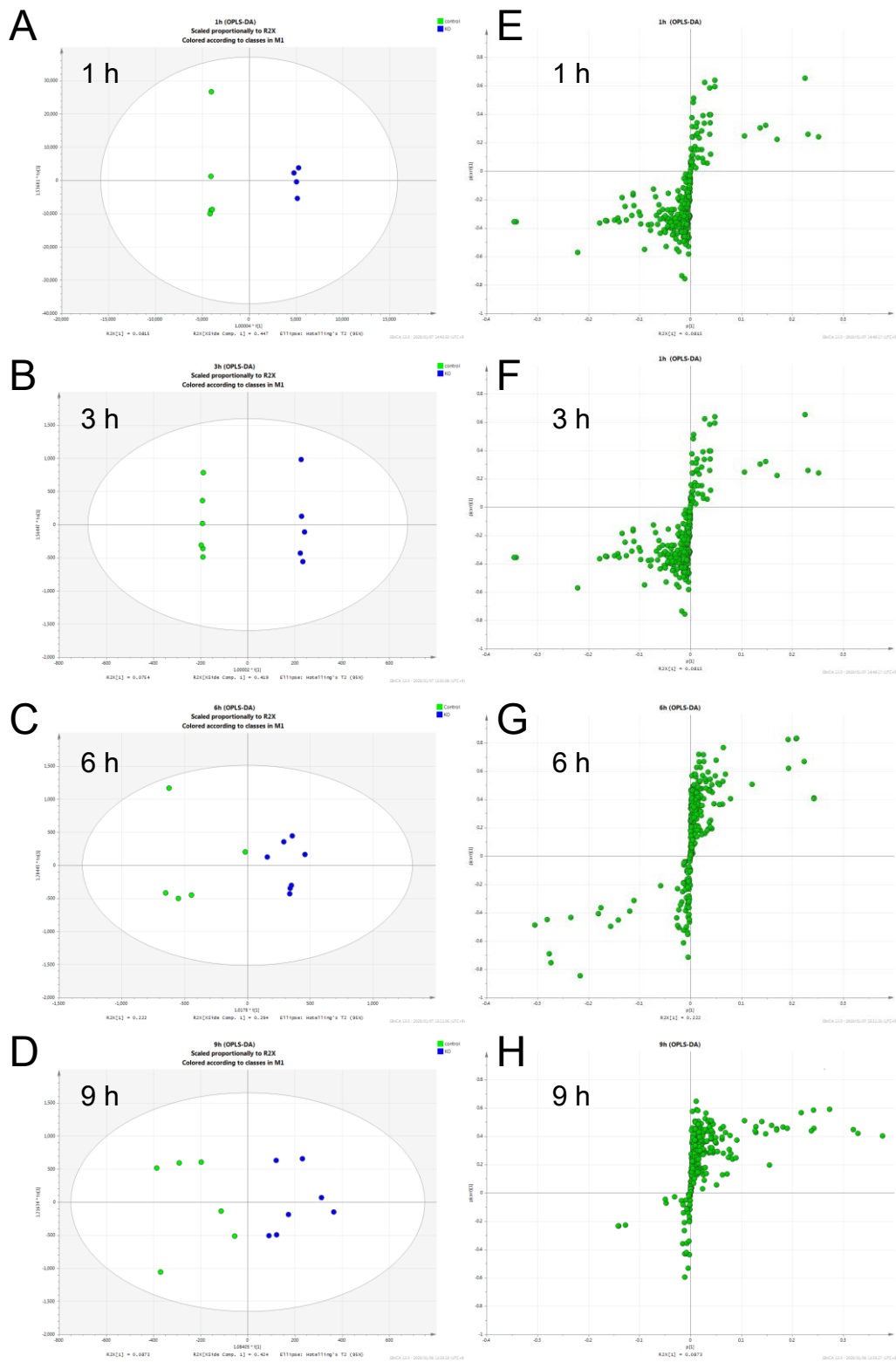
Supplementary Figure S4. The trajectory course of serum 20 protein-coding amino acids with GC-MS/MS analysis with 22-h starvation survivors

The numbers of mice (starvation time) were as follows: 5 (1 h), 6 (3 h), 5 (6 h), 6 (9 h), 6 (12 h), and 5 (22 h) Control mice and 4 (1 h), 5 (3 h), 7 (6 h), 7 (9 h), 5 (12 h), and 6 (22 h) KO mice. The concentration of metabolites was measured as the peak area of the calibration curve, and the concentration at each starvation time was compared between the groups. Boxes represent the interquartile range (25th to 75th percentiles), and lines within the boxes are the median; error bars represent the 25th percentile minus 1.5 times interquartile range (IQR) and the 75th percentile plus 1.5 times IQR. Mann-Whitney U test ; * $P < 0.05$. KO: liver-specific *Atg5*-deficient.



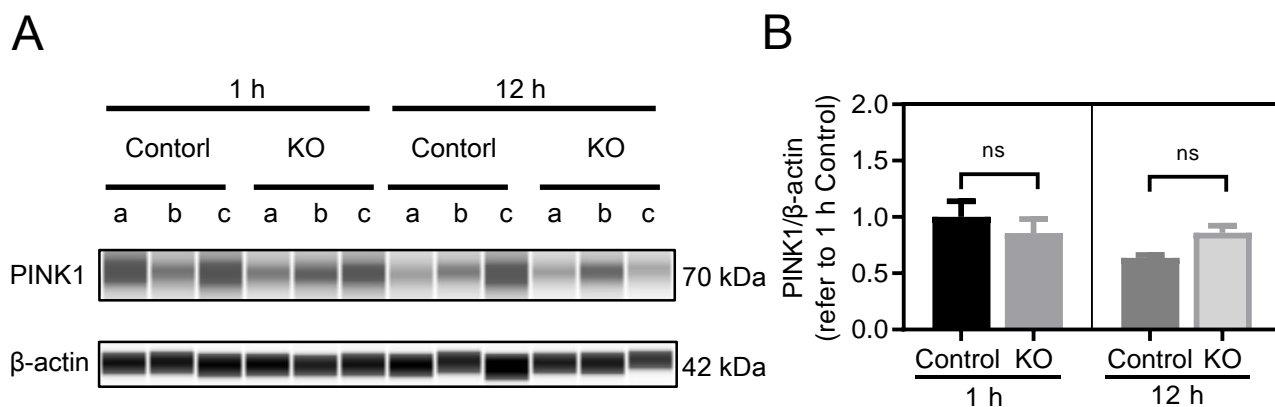
Supplementary Figure S5. The Western blotting analysis of LC3, and p62 in extract protein of muscles.

(A) The protein expression of LC3-I, LC3-II, and p62 in muscle after 1–12 h starvation ($n = 4$ at each starvation time). β -actin was used as an internal control. The full-length blots are presented in Supplementary Figure S7. The relative expression of LC3-I (B) and LC3-II (C), LC3-II/LC3-I ratio (D), and p62 (E) are shown in graphs. Each bar is expressed as the mean \pm SEM. Student's t-test; * $P < 0.05$, ** $P < 0.01$. KO: liver-specific *Atg5*-deficient; LC3: microtubule-associated protein 1A/1B-light chain 3; LC3-I: cytosolic form of LC3; LC3-II: phosphatidylethanolamine conjugate form of LC3.



Supplementary Figure S6. Multivariate statistical and pathway analyses (OPLS-DA) of serum metabolites at each starvation timepoint

(A-D) Score scatter plots of serum metabolites from the liver-specific *Atg5*-deficient (KO, blue dot) and Control groups (green dot) at 1 h (A), 3 h (B), 6 h (C), and 9 h (D) of starvation. A differential metabolic pattern was noted between KO and Control mice by the two plots clearly separated on the score scatter plot. (E-H) S-plots of serum metabolites from the KO and Control groups at 1 h (E), 3 h (F), 6 h (G), and 9 h (H) of starvation. The number of significant metabolites with $|p(\text{corr})| > 0.7$ at each timepoint was less than that of 12-hour starvation. OPLS-DA: orthogonal partial least squares discriminant analysis; KO: liver-specific *Atg5*-deficient.



Supplementary Figure S7. The automated Western blotting analysis of PINK1 in the liver.

(A) The protein expression of PINK1 in liver extract of Control and KO mice after 1 and 12 h of starvation ($n = 3$ at each starvation time point). (B) The expression of PINK1 and at 1 and 12 h of starvation refer to 1 h Control mice were shown in graphs. β -actin was used as an internal control. Each bar is expressed as the mean \pm SEM. P, Student's t-test; KO: liver-specific *Atg5*-deficient; PINK1: PTEN-induced putative kinase 1.

Supplementary Table S1. Metabolites significantly differed according to S-plot analysis Control vs. KO mice at each starvation time.

starvation duration	A	N	R ² X (cum)	R ² Y (cum)	Q ² (cum)
1 h	1+5+0	9	0.986	0.999	0.116
3 h	1+7+0	11	0.989	0.999	0.161
6 h	1+2+0	12	0.694	0.850	0.433
9 h	1+2+0	13	0.769	0.796	0.0238
12 h	1+4+0	11	0.960	0.990	0.163

OPLS-DA: orthogonal partial least squares discriminant analysis; KO: liver-specific *Atg5*-deficient.

Supplementary Table S2. Metabolites significantly differed according to S-plot analysis Control vs. KO mice at each starvation time.

Var ID (Primary)	metabolite	p[1]	p(corr)	Var ID (Primary)	metabolite	p[1]	p(corr)
1 h, control > KO				12 h, control < KO			
107	Dihydrouracil-TMS	-0.01141	-0.75722	263	Ornithine-4TMS	0.17683	0.74577
388	Oleic acid-TMS	-0.01732	-0.73572	403	Cystine-4TMS	0.02478	0.74305
3 h, control > KO				122	Threitol-4TMS	0.01018	0.74165
155	O-Phosphoethanolamine-3TMS	-0.01174	-0.77125	396	Tryptophan-3TMS	0.17128	0.73822
253	2-Deoxy-glucose-4TMS	-0.02405	-0.70130	196	Lyxose-meto-4TMS	0.02697	0.73355
6 h, control < KO				175	Glutamic acid-3TMS	0.06575	0.73231
312	Allose-meto-5TMS	0.20847	0.83128	431	Serotonin	0.03702	0.73056
322	Glucose-meto-5TMS	0.20847	0.83126	247	Dihydroorotic acid-3TMS	0.01064	0.72521
319	Galactose-meto-5TMS	0.20769	0.82792	190	N-Acetylaspartic acid-2TMS	0.01014	0.72162
313	Mannose-meto-5TMS	0.19118	0.82189	86	Serine-3TMS	0.16310	0.72124
309	N-Acetylglutamine-3TMS	0.06403	0.76646	440	Batyl alcohol-2TMS	0.00919	0.71959
355	Gluconic acid-6TMS	0.01658	0.71804	333	Glucuronic acid-meto-5TMS	0.05643	0.71790
60	Octanoic acid-TMS	0.02562	0.71698	437	Inosine-4TMS	0.06958	0.71788
6 h, cotrol > KO				328	Mannitol-6TMS	0.02347	0.71638
62	2-Aminoethanol-3TMS	-0.21632	-0.84693	339	Galacturonic acid-meto-5TMS	0.05714	0.71597
63	Leucine-2TMS	-0.27356	-0.75216	358	Glucaric acid-6TMS	0.01198	0.71474
40	Glyceraldehyde-meto-2TMS	-0.00423	-0.71479	359	Allantoin-3TMS	0.01721	0.71434
12 h, control < KO				223	Aconitic acid-3TMS	0.00594	0.71427
243	Ribonic acid-5TMS	0.02938	0.79801	144	Threonine acid-4TMS	0.07346	0.71411
199	Homocysteine-3TMS	0.01356	0.78809	189	2-Ketoglutaric acid-oxime-3TMS	0.00241	0.71336
265	Dopamine-3TMS	0.04672	0.78808	348	Galacturonic acid-meto-5TMS	0.05448	0.71330
137	4-Aminobutyric acid-3TMS	0.03628	0.78633	208	Taurine-13C2-3TMS	0.13807	0.71319
150	Creatinine-3TMS	0.09444	0.78323	113	Citramalic acid-3TMS	0.00809	0.71142
128	3-Aminoglutaric acid-2TMS	0.15668	0.78298	149	2-Hydroxyglutaric acid-3TMS	0.02619	0.70978
130	Aspartic acid-3TMS	0.14681	0.78063	242	1-Methylhistamine dihydrochloride	0.02127	0.70818
451	Guanosine-5TMS	0.01388	0.77790	106	Decanoic acid-TMS	0.02908	0.70778
169	3-Aminoglutaric acid-3TMS	0.01918	0.77303	300	Allantoin-4TMS	0.02817	0.70701
226	Orotic acid-3TMS	0.01075	0.77204	254	2-Aminopimelic acid-3TMS	0.05809	0.70661
200	Asparagine-3TMS	0.03729	0.75772	74	Proline-2TMS	0.16142	0.70607
241	Homovanillic acid-2TMS	0.00715	0.75634	344	Glucuronic acid-meto-5TMS	0.02416	0.70395
277	Tagatose-meto-5TMS	0.00947	0.75407	454	Maltose-meto-8TMS	0.05947	0.70199
80	Catechol-2TMS	0.00375	0.75161	447	Lactose-meto-8TMS	0.00384	0.70140
206	Taurine-3TMS	0.17443	0.75102	452	Trehalose-8TMS	0.11327	0.70117
276	1,5-Anhydro-glucitol-4TMS	0.01269	0.75064	91	Threonine-3TMS	0.11286	0.70069
				457	Maltose-meto-8TMS	0.05003	0.70019
				12 h, control > KO			
				400	Tryptamine-3TMS	-0.04133	-0.82715

KO: liver-specific *Atg5*-deficient; TMS: trimethylsilyl.