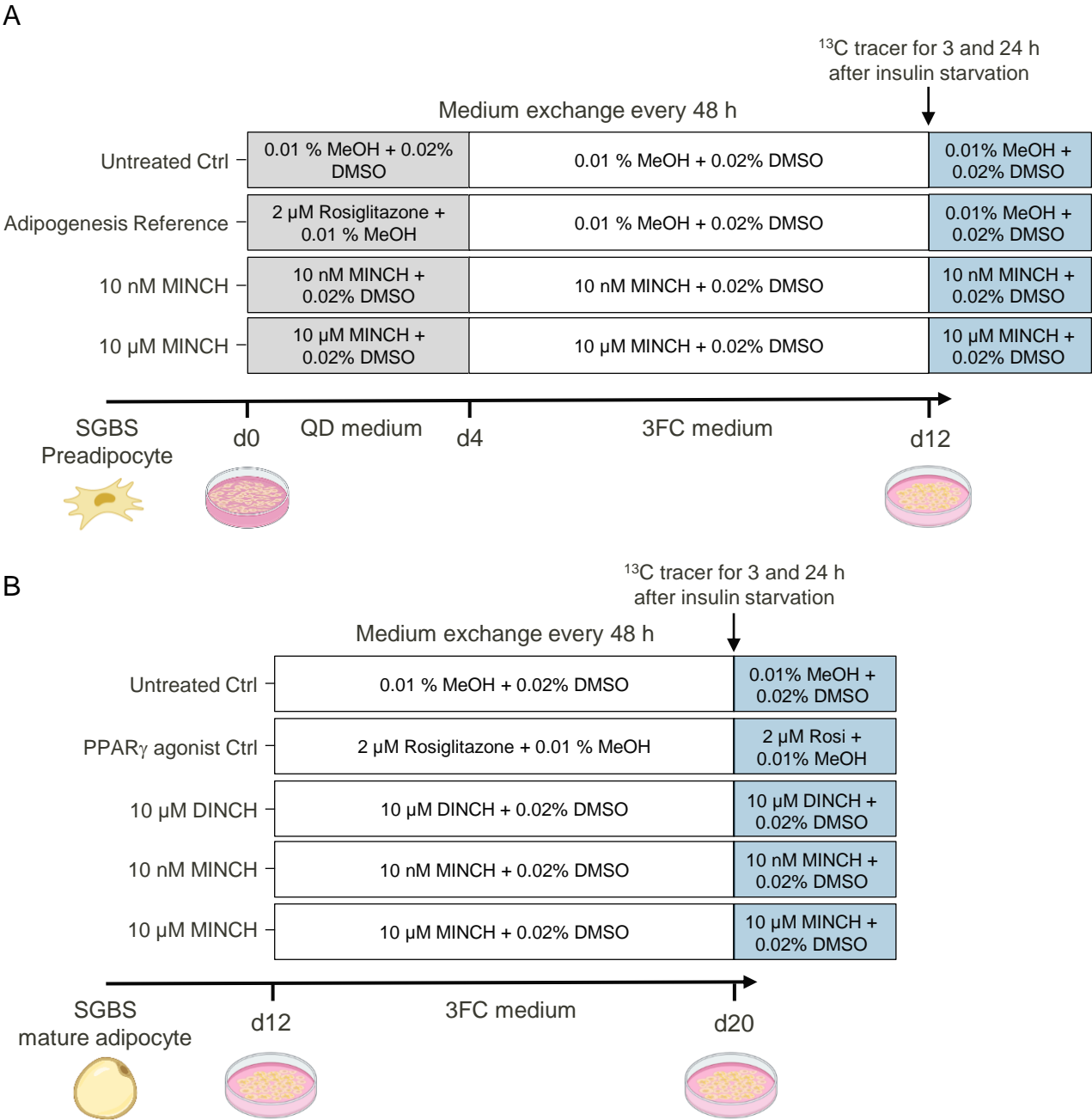
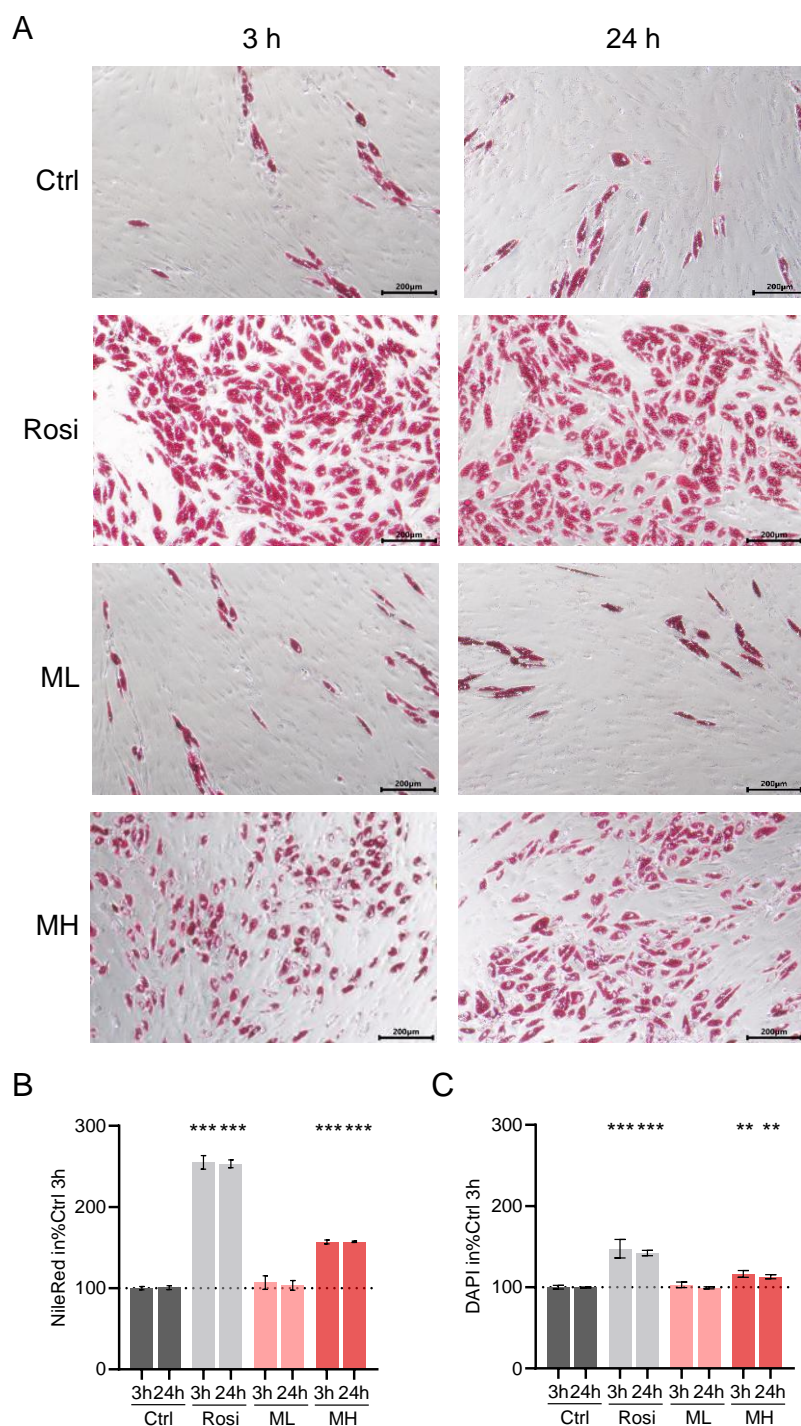


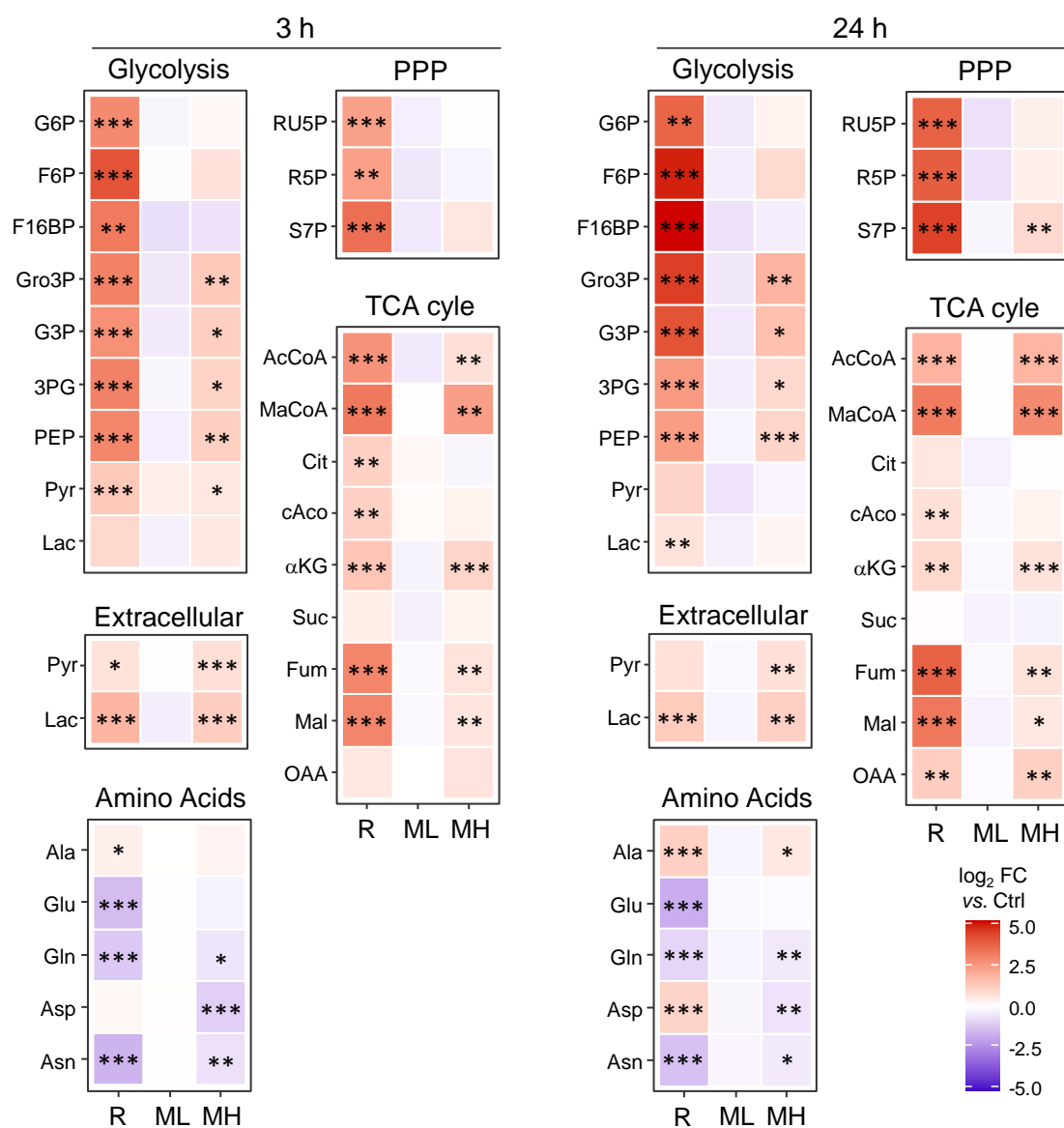
# Supplementary Figures



**Figure S1: Experimental setup for the assessment of the effects of MINCH on the activity of the central carbon metabolism of treated preadipocytes and mature adipocytes.** (A) MINCH exposure (10 nM and 10  $\mu\text{M}$ ) was performed for 12 days in preadipocytes followed by  $^{13}\text{C}$  tracer addition for 3 and 24 h to analyze effects on adipogenesis. Effects were compared to rosiglitazone-differentiated (Rosi) and untreated control (Ctrl) cells. (B) DINCH (10  $\mu\text{M}$ ) and MINCH (10 nM and 10  $\mu\text{M}$ ) exposure was performed in mature adipocytes for 8 days (day 12 to day 20) followed by  $^{13}\text{C}$  tracer addition for 3 and 24 h to assess effects on terminally differentiated adipocytes and effects were compared to rosiglitazone-treated (Rosi) and untreated control (Ctrl) cells.  $^{13}\text{C}$  labeling of treated preadipocytes and mature adipocytes was performed after insulin starvation for 16 h before the end of the treatment with conditioned labeling media supplemented with insulin (20 nM) to promote  $^{13}\text{C}$  tracer incorporation by the insulin-mediated stimulation of glucose uptake and lipid synthesis.



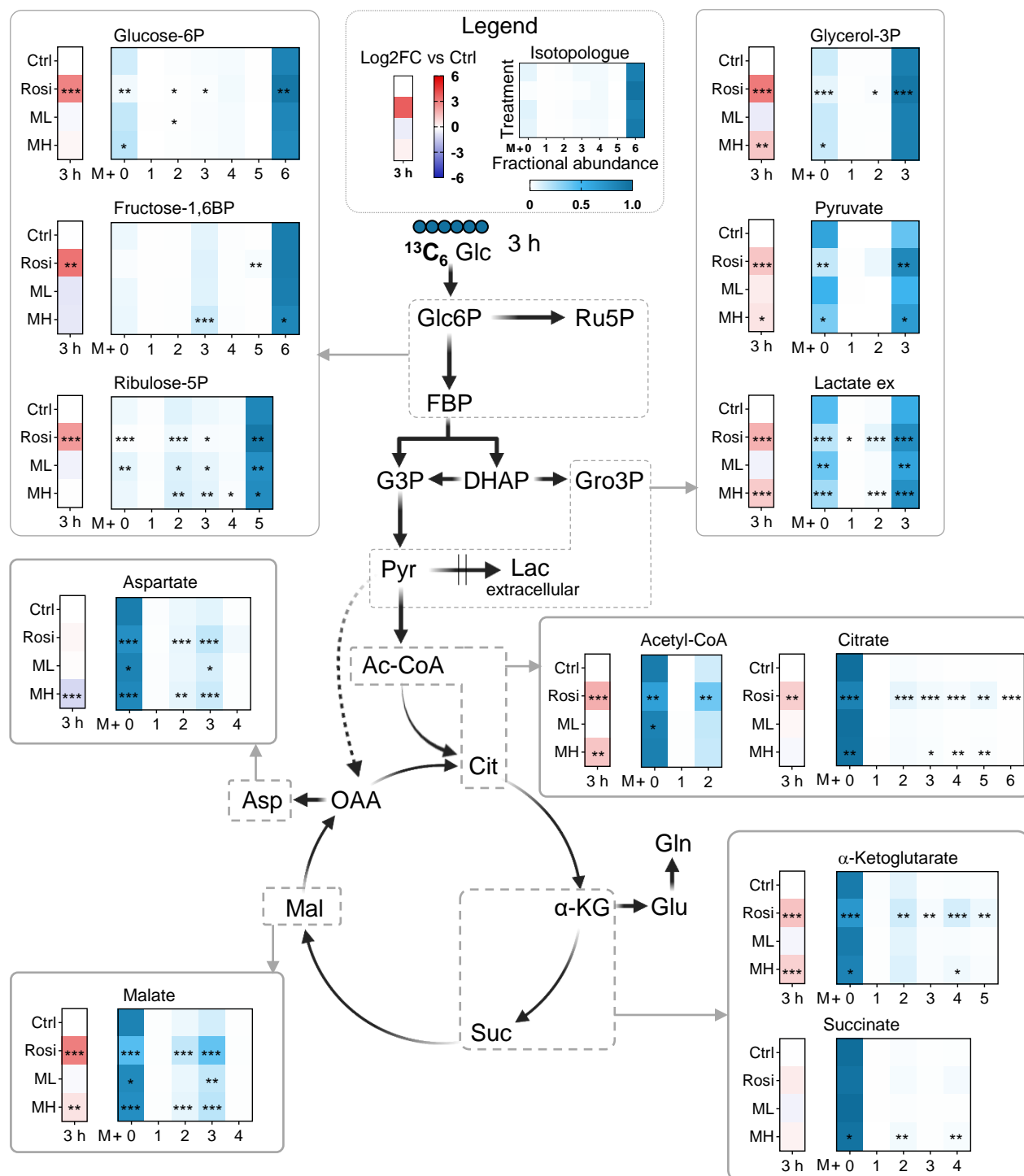
**Figure S2: Lipid accumulation and DNA content of treated SGBS preadipocytes.** (A) Visualization of lipid accumulation via Oil Red O staining and imaging at 10-fold magnification (size bar: 200  $\mu$ m) of MINCH-treated (ML: 10 nM and MH: 10  $\mu$ M), rosiglitazone-differentiated (Rosi), and untreated control (Ctrl) cells after 12 days of preadipocyte treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. (B) Lipid accumulation assessed by measuring Nile red fluorescence of MINCH-treated, rosiglitazone-differentiated, and untreated control cells after 12 days of preadipocyte treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. (C) DNA content assessed by measuring DAPI fluorescence of MINCH-treated, rosiglitazone-differentiated and untreated control cells after 12 days of preadipocyte treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. All values are expressed as mean  $\pm$  SD (n = 4). One-way ANOVA followed by Dunnett's post-hoc test compared to the untreated control of the respective time point was performed to calculate statistical significance; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



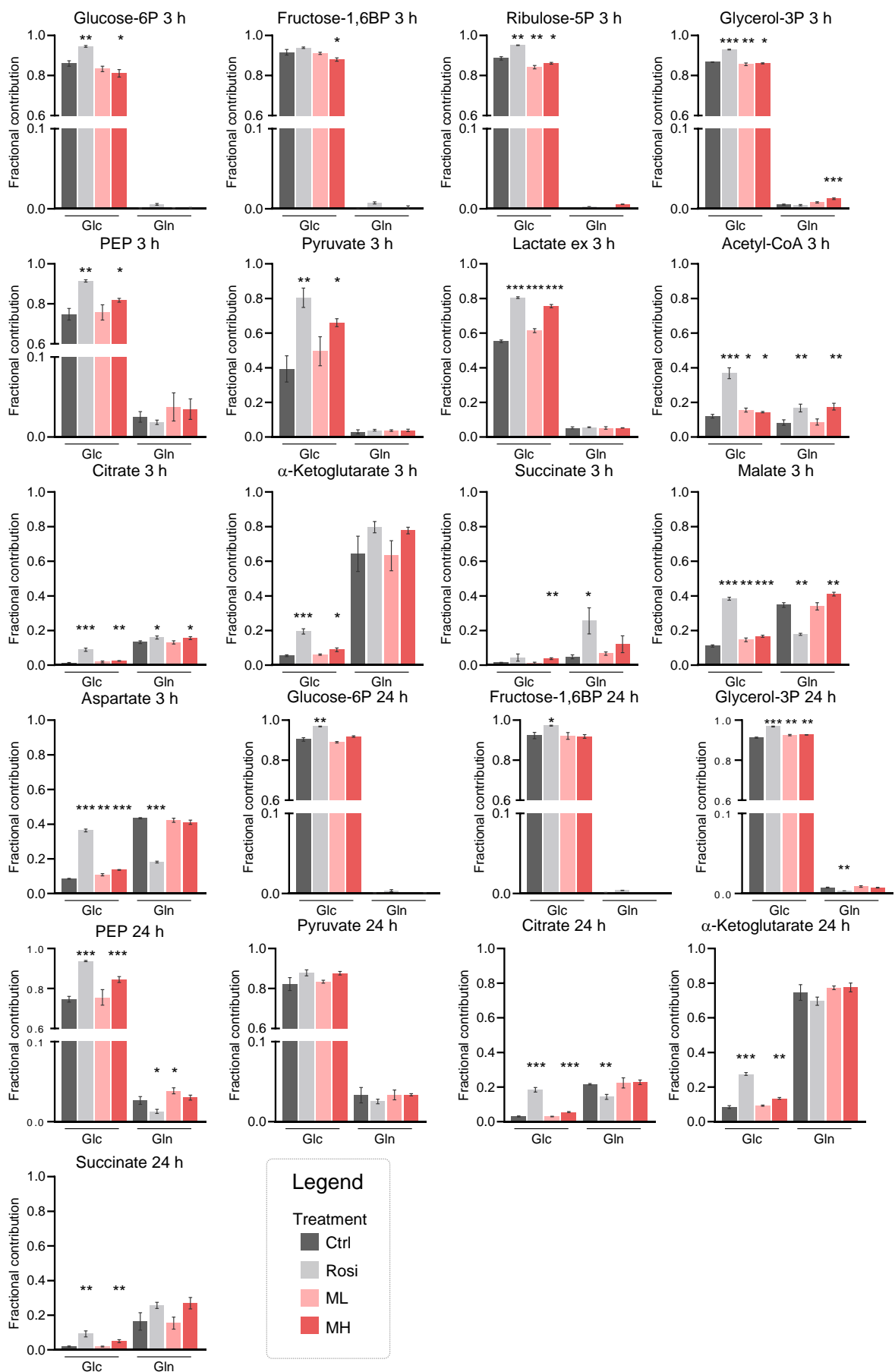
**Figure S3: Metabolite abundances of central carbon metabolism of treated SGBS preadipocytes.**

Metabolite abundances of measured metabolites of the glycolysis, PPP, TCA-cycle, and associated amino acids of MINCH-treated SGBS preadipocytes (ML: 10 nM and MH: 10 μM), SGBS cells treated with rosiglitazone (R) compared to untreated control cells after 12 days of treatment and additional incubation with conditioned DMEM/F12 without <sup>13</sup>C label for 3 and 24 h. Abundances are expressed as log<sub>2</sub> fold change (FC) compared to the untreated control of the respective time point (n = 4) and Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance;

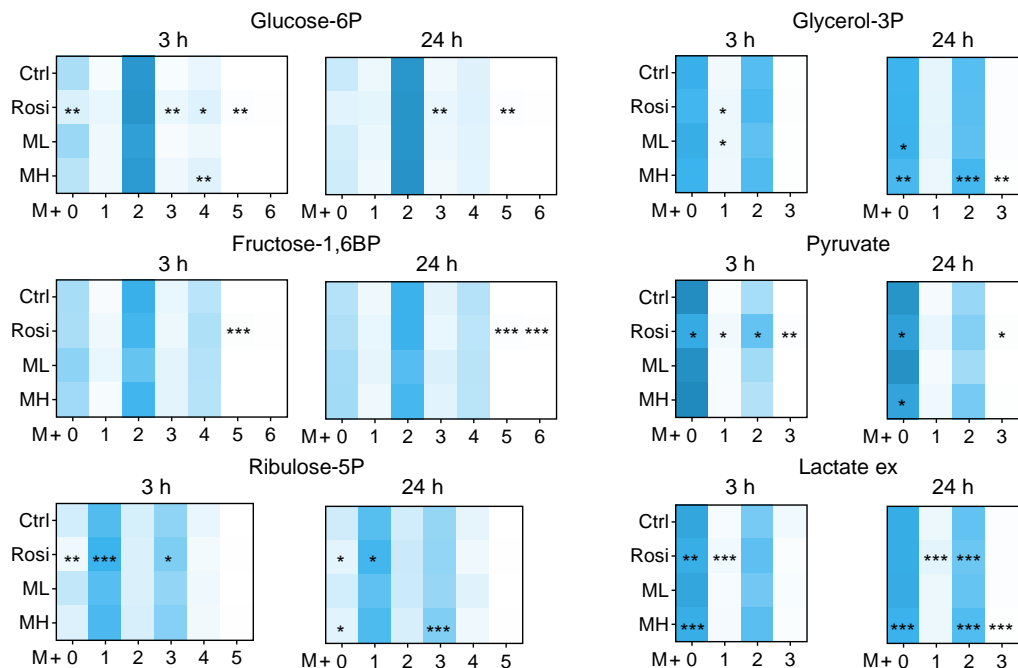
\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Figure S4: Metabolite abundances and isotopologue enrichments 3 h after [U- $^{13}\text{C}$ ]glucose labeling of treated SGBS preadipocytes.** Metabolite abundances and isotopologue enrichment from [U- $^{13}\text{C}$ ]glucose labeling of selected metabolites of the glycolysis, PPP, TCA cycle, associated amino acids and acetyl-CoA of MINCH-treated SGBS preadipocytes (ML – MINCH Low (10 nM) and MH – MINCH High (10  $\mu\text{M}$ )) and SGBS cells treated with rosiglitazone (Rosi) 3 h after incubation with conditioned DMEM/F12 with (isotopologue enrichment) or without (abundances)  $^{13}\text{C}$  label on day 12. Metabolite abundances were normalized to the DNA content determined by DAPI fluorescence and are presented as log<sub>2</sub> fold changes compared to the control (n = 4). Isotopologue enrichments are represented as relative fractional abundances after correction of natural isotope abundance (n = 4). Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance compared to the untreated control; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

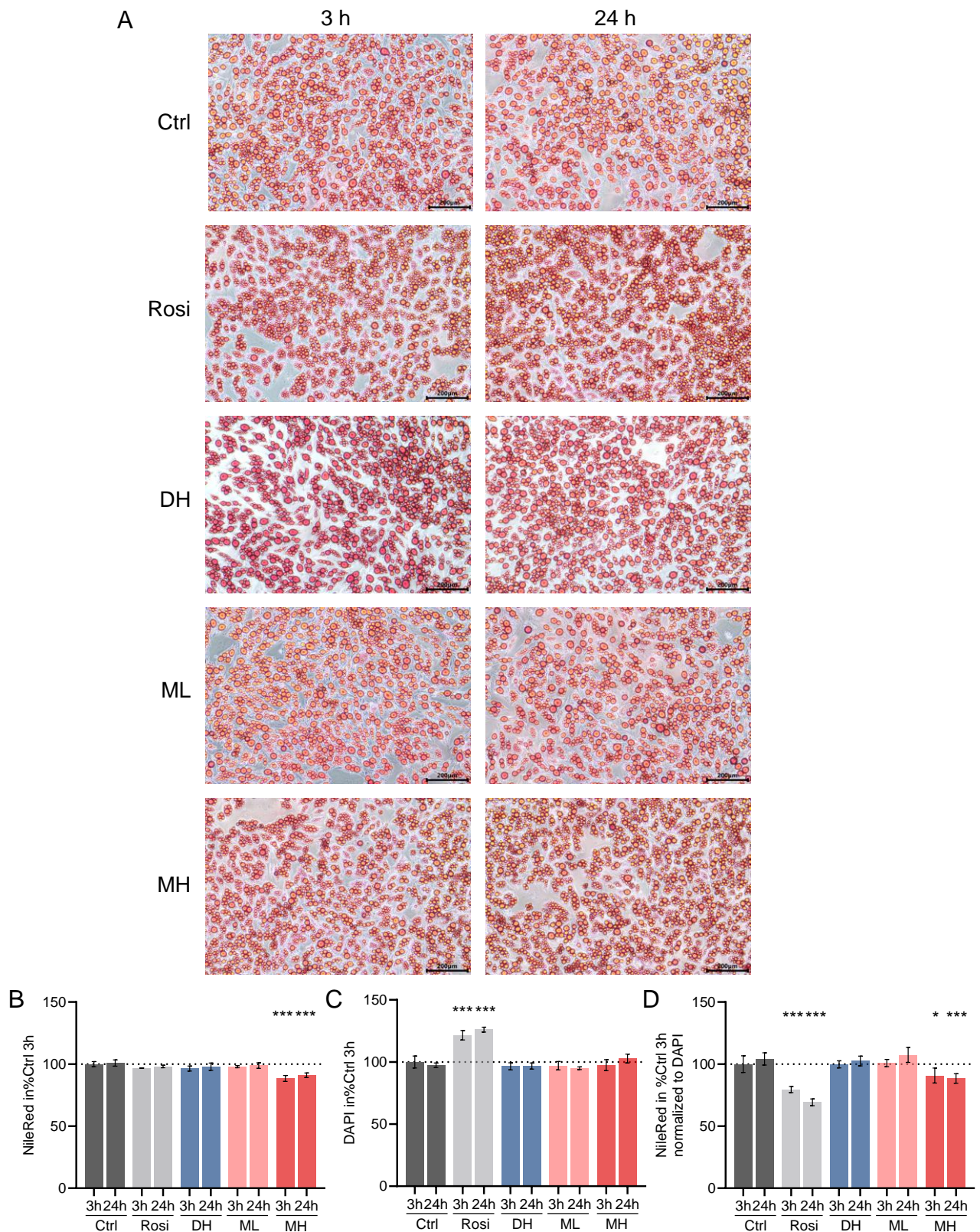


**Figure S5: Fractional contribution from glucose and glutamine to metabolites of treated SGBS preadipocytes.**  $^{13}\text{C}$  fractional contribution from glucose and glutamine to selected metabolites of the glycolysis, PPP, and the TCA cycle of DINCH- and MINCH-treated SGBS preadipocytes (ML – MINCH Low (10 nM) and MH – MINCH High (10  $\mu\text{M}$ )), SGBS cells treated with rosiglitazone (Rosi) and untreated control cells 3 and 24 h after labeling with  $[\text{U}-^{13}\text{C}]\text{glucose}$  and  $[\text{U}-^{13}\text{C}]\text{glutamine}$  on day 20, respectively ( $n=4$ ). Values are expressed as mean  $\pm$  SD ( $n = 4$ ). Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance and significant changes are shown compared to the untreated control ; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

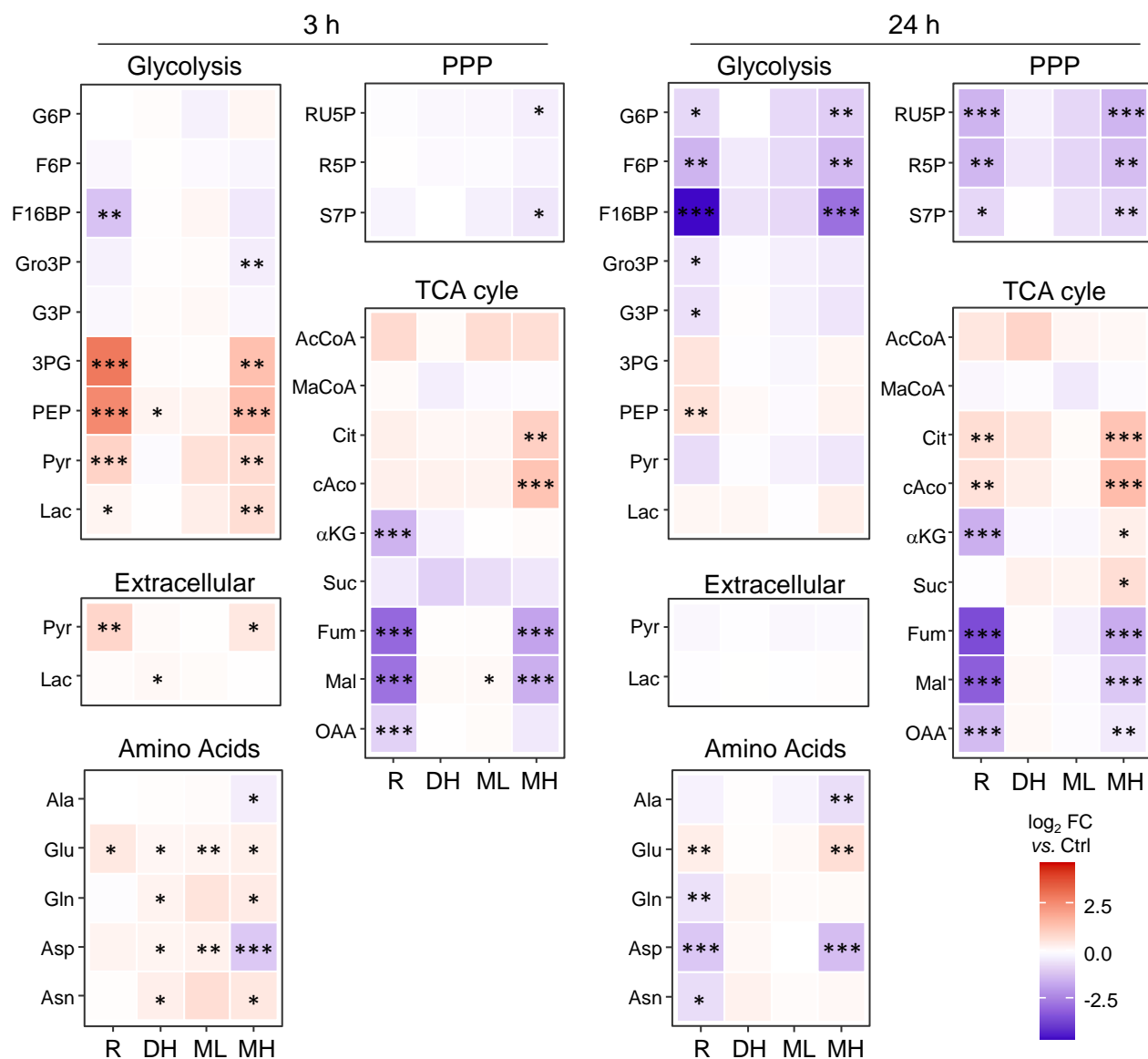


**Figure S6: Isotopologue enrichments after [1,2-<sup>13</sup>C]glucose labeling of treated SGBS preadipocytes.** Isotopologue enrichment from [1,2-<sup>13</sup>C]glucose labeling of selected metabolites of the glycolysis and PPP of MINCH-treated SGBS preadipocytes (ML – MINCH Low (10 nM) and MH – MINCH High (10 μM)) and SGBS cells treated with rosiglitazone (Rosi) 3 and 24 h after incubation with conditioned DMEM/F12 with <sup>13</sup>C label on day 12. Isotopologue enrichments are represented as relative fractional abundances after correction of natural isotope abundance (n = 4). Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance and significant changes are shown compared to the untreated control ; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.





**Figure S7: Lipid accumulation, DNA content and normalized lipid content of treated mature SGBS adipocytes.** (A) Visualization of lipid accumulation *via* Oil Red O staining and imaging at 10-fold magnification (size bar: 200  $\mu$ m) of DINCH- and MINCH-treated (DH: 10  $\mu$ M; ML: 10 nM and MH: 10  $\mu$ M), rosiglitazone-differentiated (Rosi), and untreated control (Ctrl) cells after 8 days of treatment of mature adipocytes and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. (B) Lipid accumulation assessed by measuring Nile red fluorescence of DINCH-, MINCH-, and rosiglitazone-treated, as well as untreated control cells after 8 days of mature adipocyte treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. (C) DNA content assessed by measuring DAPI fluorescence of DINCH-, MINCH- and rosiglitazone-treated, as well as untreated control cells after 8 days of mature adipocyte treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. (D) Lipid content measured by Nile red fluorescence normalized to the DNA-content measured by DAPI fluorescence of DINCH- and MINCH-treated, rosiglitazone-treated, and untreated control cells after 8 days of mature adipocyte treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. All values are expressed as mean  $\pm$  SD (n = 4). One-way ANOVA followed by Dunnett's post-hoc test compared to the untreated control of the respective time point was performed to calculate statistical significance; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

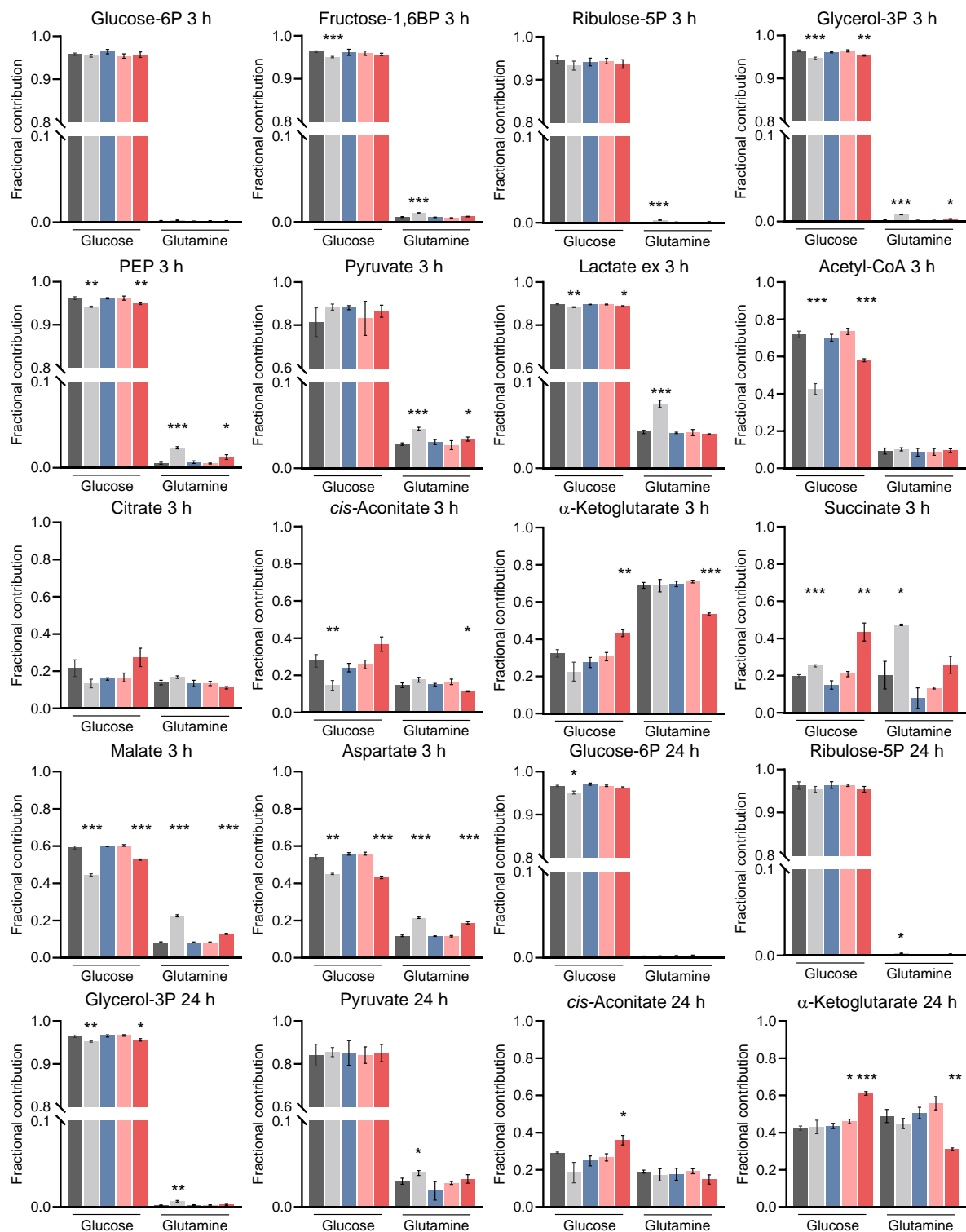


**Figure S8: Metabolite abundances of central carbon metabolism of treated mature SGSB adipocytes.**

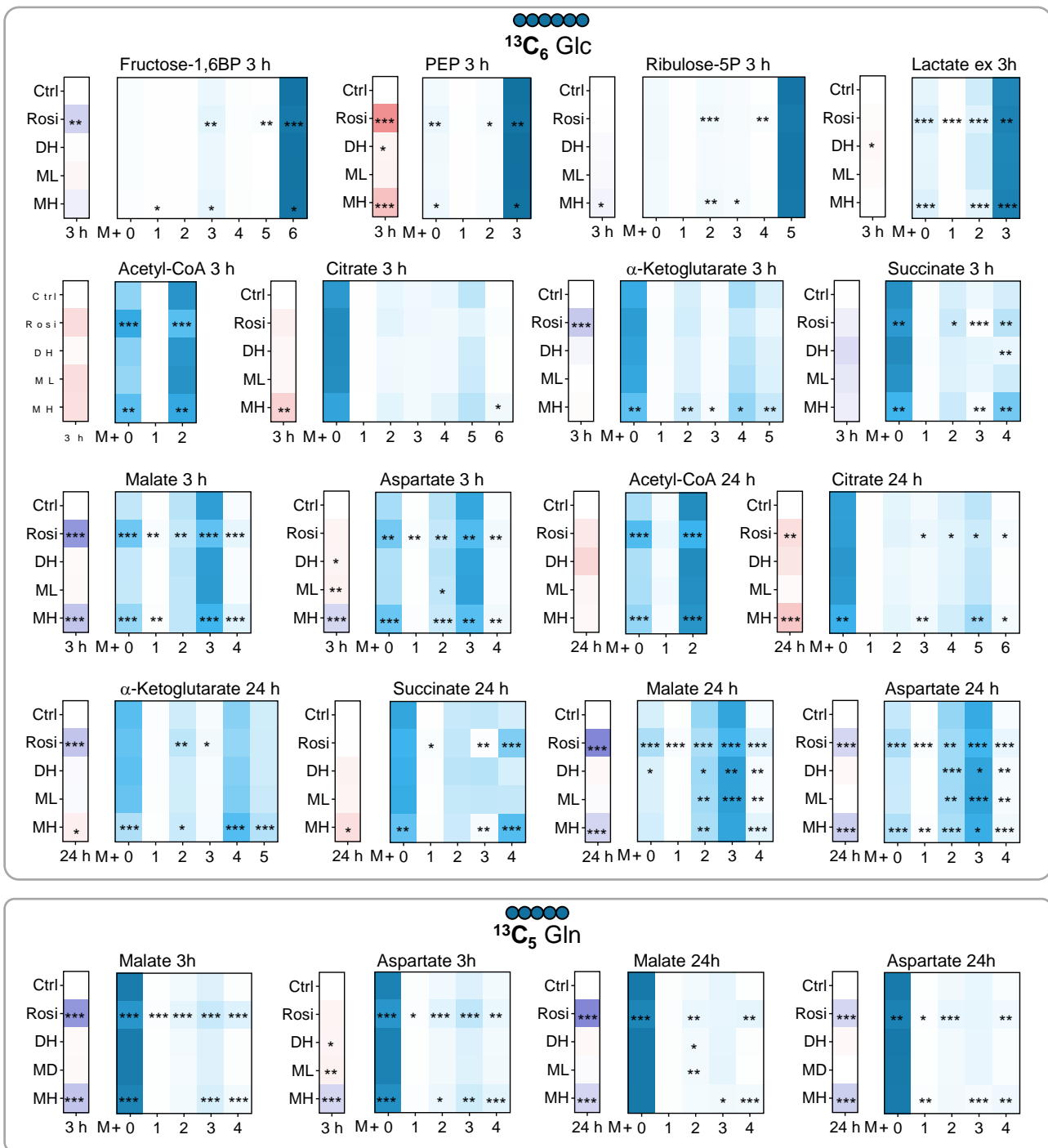
Metabolite abundances of measured metabolites of the glycolysis, PPP, TCA-cycle, and associated amino acids of DINCH and MINCH-treated mature SGSB adipocytes (DH: DINCH 10  $\mu$ M; ML: MINCH 10 nM and MH: MINCH 10  $\mu$ M), SGSB cells treated with rosiglitazone (R) compared to untreated control cells after 20 days of treatment and additional incubation with conditioned DMEM/F12 without  $^{13}$ C label for 3 and 24 h. Abundances are expressed as log<sub>2</sub> fold change (FC) compared to the untreated control of the respective time point (n = 4) and Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance;

\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

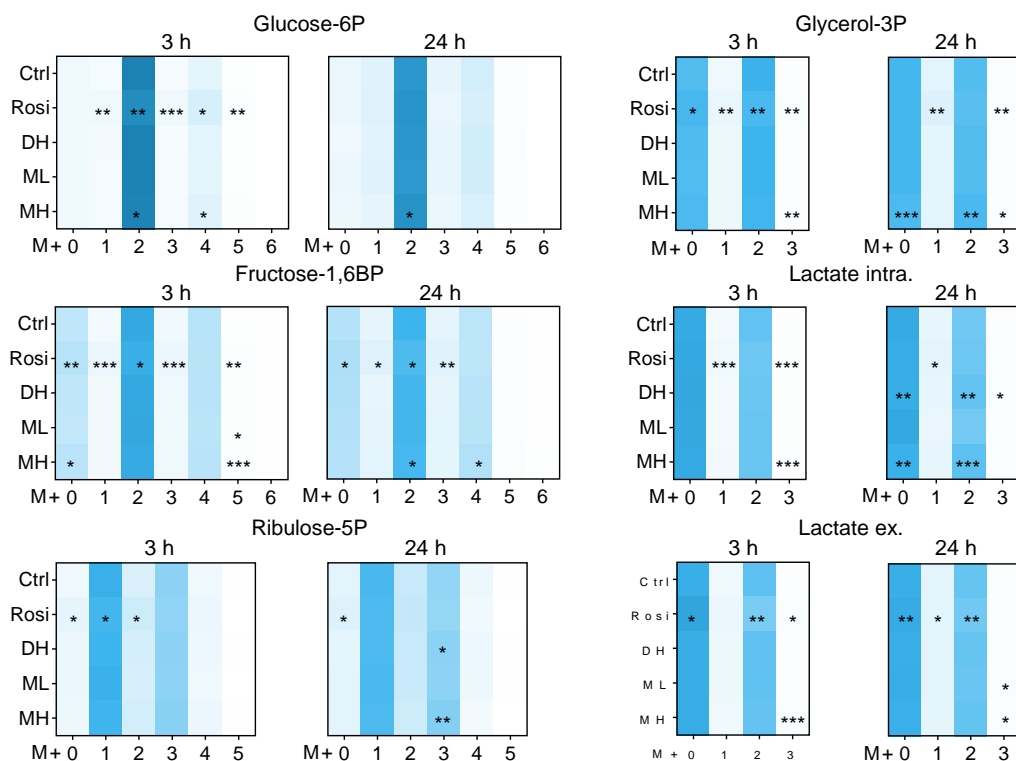




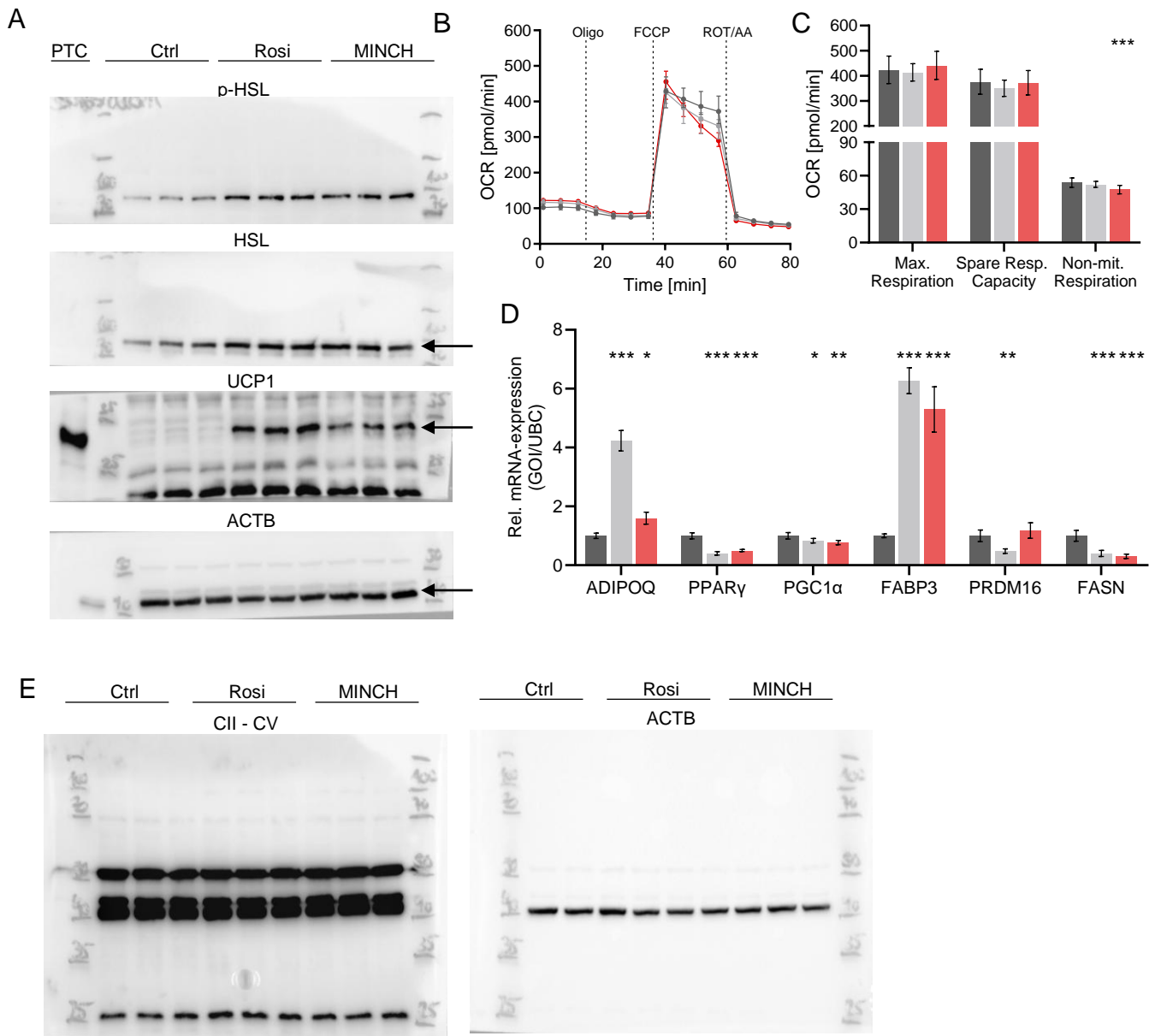
**Figure S9: Fractional contribution from glucose and glutamine to metabolites of treated mature SGBS adipocytes.**  $^{13}\text{C}$  fractional contribution from glucose and glutamine to measured metabolites of the glycolysis, PPP, and the TCA cycle of DINCH- and MINCH-treated mature SGBS adipocytes (DH – DINCH High (10  $\mu\text{M}$ ; blue); ML – MINCH Low (10 nM; light red) and MH – MINCH High (10  $\mu\text{M}$ ; red)), SGBS cells treated with rosiglitazone (Rosi; grey) and untreated control cells (dark grey) 3 and 24 h after insulin stimulation on day 20 and labeling with  $[\text{U-}^{13}\text{C}]\text{glucose}$  and  $[\text{U-}^{13}\text{C}]\text{glutamine}$ , respectively ( $n = 4$ ). Values are expressed as mean  $\pm$  SD. Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance and significant changes are shown compared to the untreated control; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .



**Figure S10: Metabolite abundances and isotopologue enrichments after [U- $^{13}\text{C}$ ]glucose and [U- $^{13}\text{C}$ ]glutamine labeling of treated mature SGBS adipocytes.** Metabolite abundances and isotopologue enrichment from [U- $^{13}\text{C}$ ]glucose and [U- $^{13}\text{C}$ ]glutamine labeling of selected metabolites of the glycolysis, PPP, TCA cycle, associated amino acids and acetyl-CoA of DINCH- and MINCH-treated mature SGBS adipocytes (DH – DINCH High (10  $\mu\text{M}$ ); ML – MINCH Low (10 nM) and MH – MINCH High (10  $\mu\text{M}$ )), SGBS cells treated with rosiglitazone (Rosi) 3 and 24 h after incubation with conditioned DMEM/F12 with (isotopologue enrichment) or without (abundances)  $^{13}\text{C}$  label on day 20. Metabolite abundances were normalized to the DNA content determined by DAPI fluorescence and are presented as log<sub>2</sub> fold changes compared to the control (n = 4). Isotopologue enrichments are represented as relative fractional abundances after correction of natural isotope abundance (n = 4). Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance and significant changes are shown compared to the untreated control; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Figure S11: Isotopologue enrichments after [1,2-<sup>13</sup>C]glucose labeling of treated mature SGBS adipocytes.** Isotopologue enrichment from [1,2-<sup>13</sup>C]glucose labeling of selected metabolites of the glycolysis and PPP of DINCH- and MINCH-treated mature SGBS adipocytes (DH – DINCH High (10 μM); ML – MINCH Low (10 nM) and MH – MINCH High (10 μM)), SGBS cells treated with rosiglitazone (Rosi) and untreated control cells (Ctrl) 3 and 24 h after incubation with conditioned DMEM/F12 with <sup>13</sup>C label on day 12. Isotopologue enrichments are represented as relative fractional abundances after correction of natural isotope abundance (n = 4). Welch ANOVA followed by Games-Howell post-hoc test was performed to calculate statistical significance and significant changes are shown compared to the untreated control; \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Figure S12: Analysis of brown/brite adipocyte markers and metabolic genes of treated mature adipocytes.** (A) Original Western blot exposure images of hormone-sensitive lipase (HSL), Ser660 phosphorylated HSL (pHSL), uncoupling protein 1 (UCP1) and  $\beta$ -actin (ACTB) in 10  $\mu$ M MINCH-treated, rosiglitazone-treated, and untreated control cells 24 h after incubation with conditioned DMEM/F12 without  $^{13}\text{C}$  label ( $n = 3$ ). Arrows indicate the “band of interest”. (B) Time resolved oxygen consumption rate (OCR) in 10  $\mu$ M MINCH-treated, rosiglitazone-treated, and untreated mature adipocytes on day 20 after stimulation with 2  $\mu$ M oligomycin (Oligo), 2.5  $\mu$ M carbonylcyanide-p-trifluoromethoxyphenylhydrazine (FCCP), and 0.5  $\mu$ M rotenone/antimycin A (ROT/AA) measured by Seahorse XF Pro Analyzer, Agilent ( $n=18-20$ ). (C) Maximal respiration, spare respiratory capacity and non-mitochondrial capacity calculated based on the measurement of the OCR (panel B) in 10  $\mu$ M MINCH-treated, rosiglitazone-treated and untreated control cells on day 20 ( $n=18-20$ ). (D) mRNA-expression of PPAR $\gamma$ , brown/brite adipocyte marker (adiponectin (ADIPOQ)), peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 $\alpha$ ), fatty acid binding protein 3 (FABP3), PR domain containing 16 (PRDM16)) and fatty acid synthase (FASN) in 10  $\mu$ M MINCH-treated and rosiglitazone-treated cells relative to the expression in untreated control cells 24 h after incubation with conditioned DMEM/F12 without  $^{13}\text{C}$  label, calculated by the  $\Delta\Delta\text{CT}$  method and normalized to ubiquitin C (UBC) expression ( $n = 4$ ). Values of the normalized expression of the respective gene of interest (GOI/UBC) are expressed as mean  $\pm$  SD. One-way ANOVA followed by Dunnett's post-hoc test against the untreated control was performed to calculate statistical significance; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . GOI – gene of interest (E) Original Western blot exposure images of complexes II-V of oxidative phosphorylation (SDHB, UQCRC2, MTCO1, and ATP5F1A) and ACTB in 10  $\mu$ M MINCH-treated, rosiglitazone-treated, and untreated control cells 24 h after incubation with conditioned DMEM/F12 without  $^{13}\text{C}$  label ( $n = 3$ ). CII – complex II: succinate dehydrogenase [ubiquinone] iron-sulfur subunit (SDHB), CIII – complex III: cytochrome b-c1 complex subunit 2 (UQCRC2), CIV – complex IV: cytochrome c oxidase subunit 1 (MTCO1), and CV – complex V: ATP synthase subunit alpha (ATP5F1A).