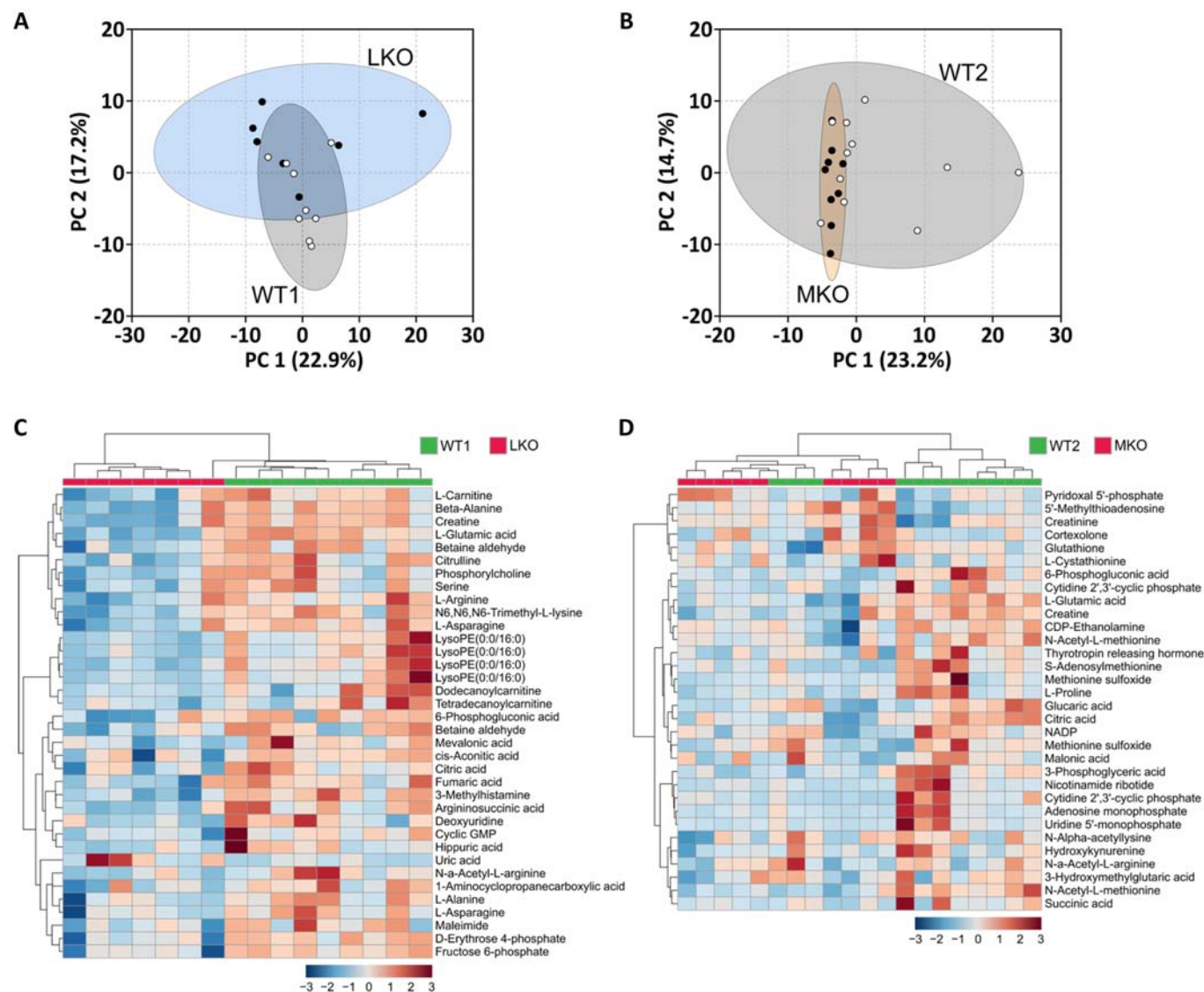
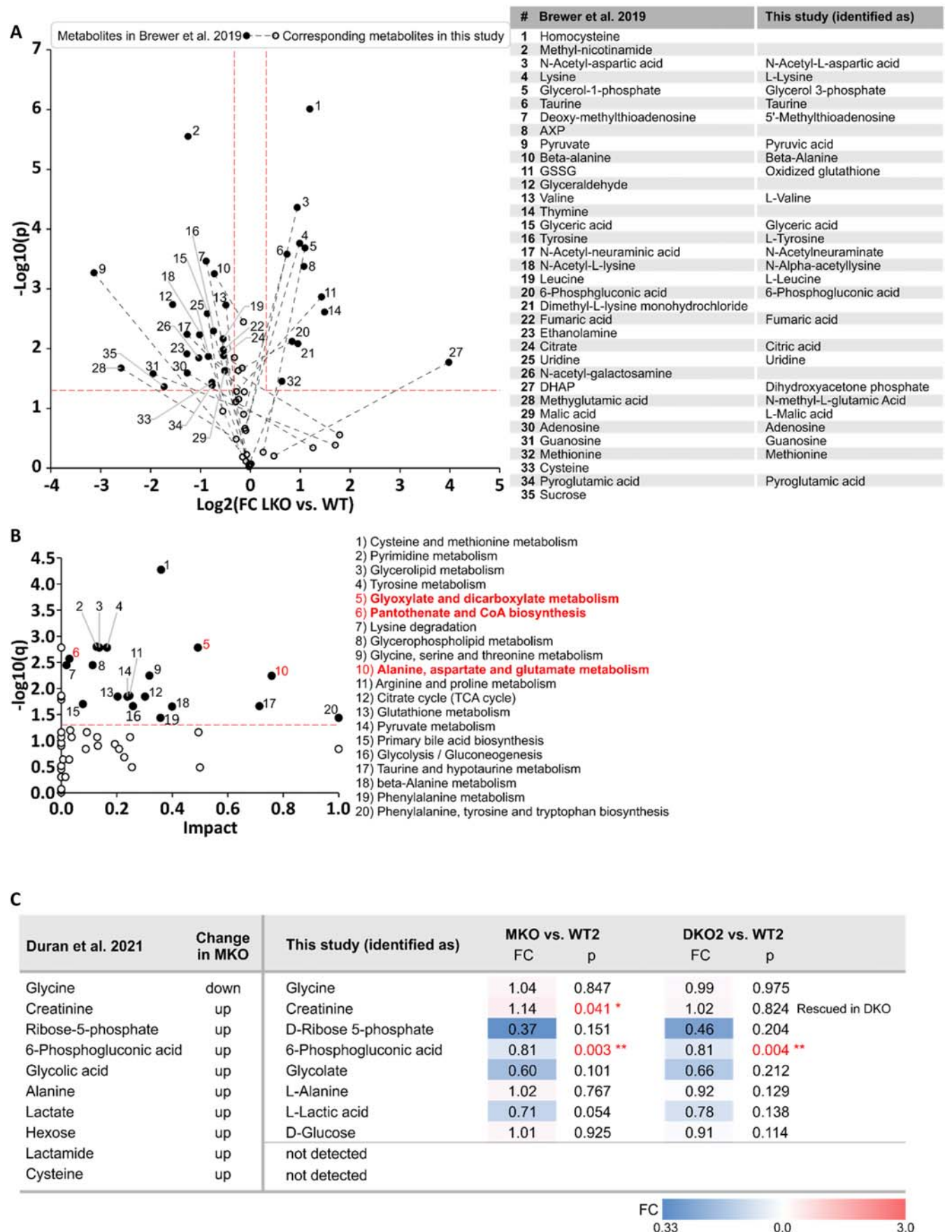


## Expanded View Figures



**Figure EV1. Cluster analyses using brain metabolite profiles from LD mice showing no or incomplete clustering.**

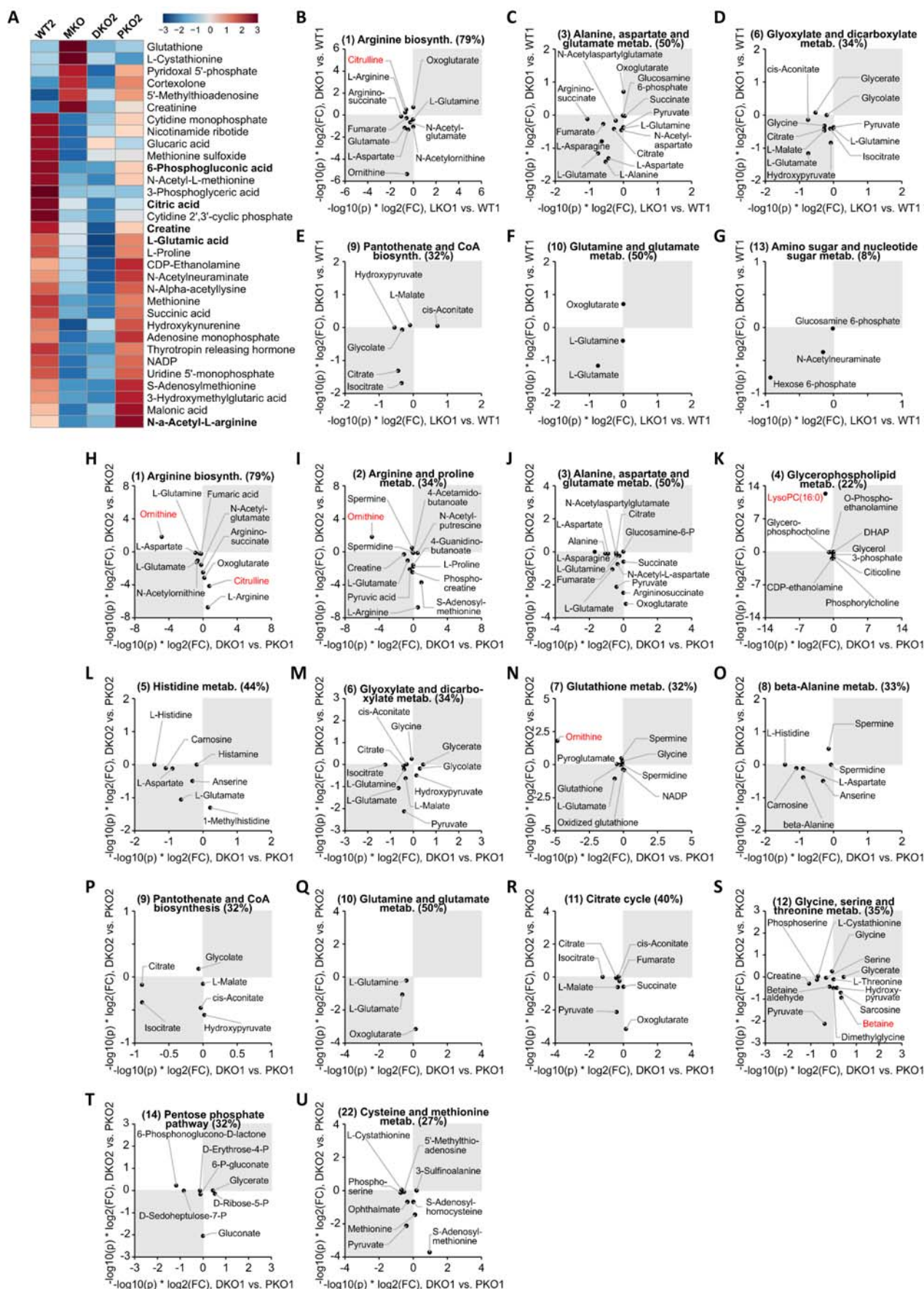
(A,B) PCA plots of metabolic profiles in LKO (A) and MKO (B) compared to respective WTs. (C, D) Heatmaps, comparing all significantly changed ( $p < 0.05$ ) brain metabolites in LKO (C) and MKO (D) to respective WTs in individual animals from unsupervised Ward cluster analysis with Euclidean distance measure based on unpaired two-tailed  $t$ -test. Data information: Significance levels determined by MetaboAnalyst 5.0 as follows:  $p < 0.05$  (C, D),  $n = 7-11$ . Corresponding to Fig. 3. Source data are available online for this figure.




**Figure EV2. Meta-analysis of our metabolomic data and published data from CP-fixed LD brains shows no common metabolic signature.**

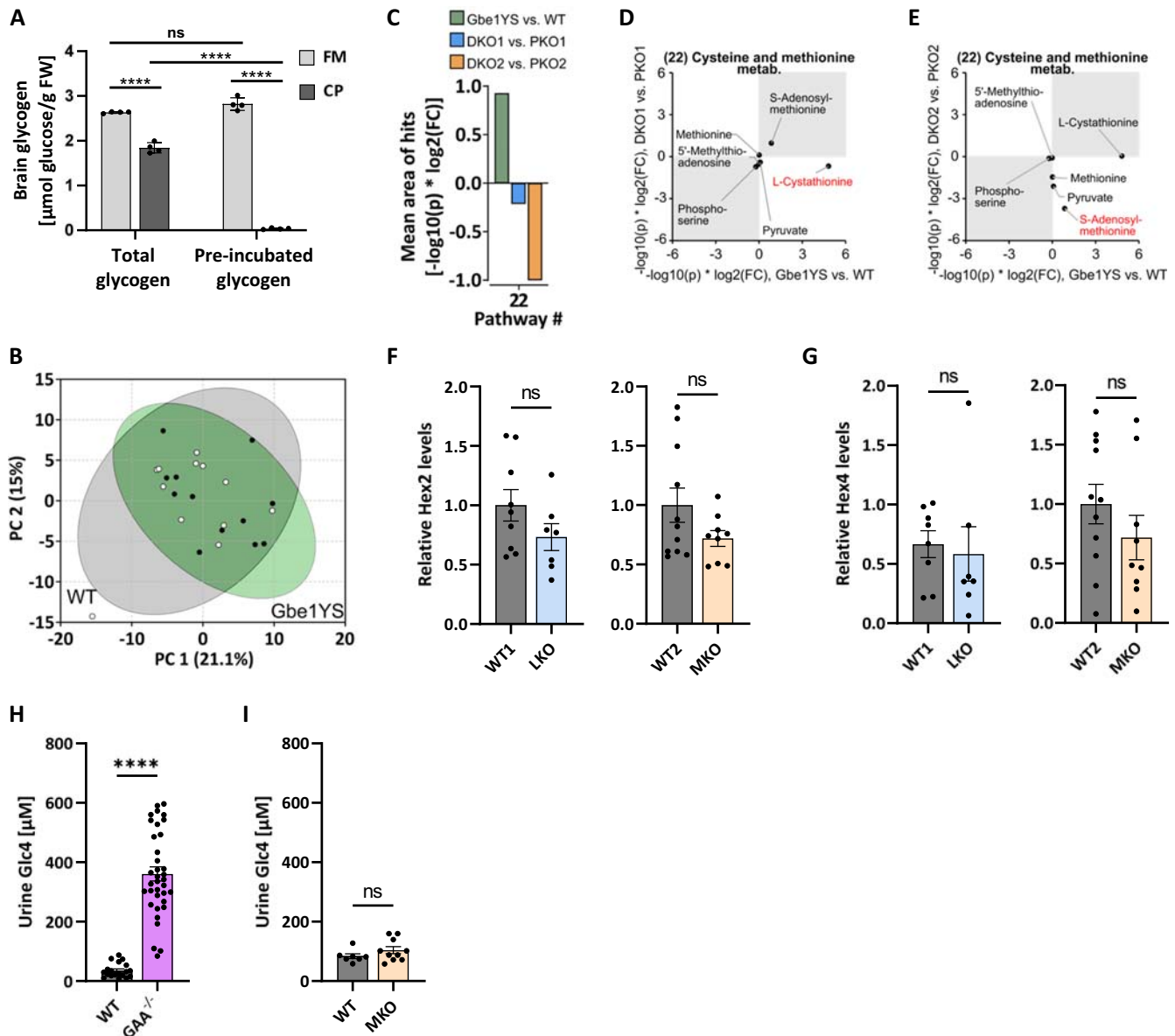
(A) Volcano plot of the 35 metabolites significantly changed ( $p < 0.05$ ,  $FC > 1.25$  or  $< 0.8$ ) in CP-fixed LKO brains in Brewer et al dataset (Brewer et al, 2019), displayed as filled circles and listed next to the plot. 25 of these metabolites were measured in the current study, are displayed as empty circles, and connected to the corresponding data point from Brewer et al using dashed lines.  $P$  values were calculated by unpaired two-tailed  $t$ -test. (B) Pathway analysis for published LKO dataset (Brewer et al, 2019). Changed pathways are displayed as filled circles and numbered according to the list next to the plot. Pathways 5, 6, and 10 (red) were also significantly changed in LKO in the current study (see Fig. 3E). (C) Table comparing ten metabolites found significantly changed in CP-fixed MKO brains in Duran et al (Duran et al, 2021) with identical metabolites detected in our study with fold changes (FC) and  $p$  values for MKO or DKO2, respectively, compared to WT2 ( $p > 0.05$  or  $FC$  of  $< 1.25 / > 0.8$ ).  $P$  values were calculated by unpaired two-tailed  $t$ -test. Data information: Significance levels determined by MetaboAnalyst 5.0 as follows:  $p < 0.05$  (A, B) \* $p < 0.05$ , \*\* $p < 0.01$  (C, red),  $n = 7$ –11. Corresponding to Fig. 3. Source data are available online for this figure.





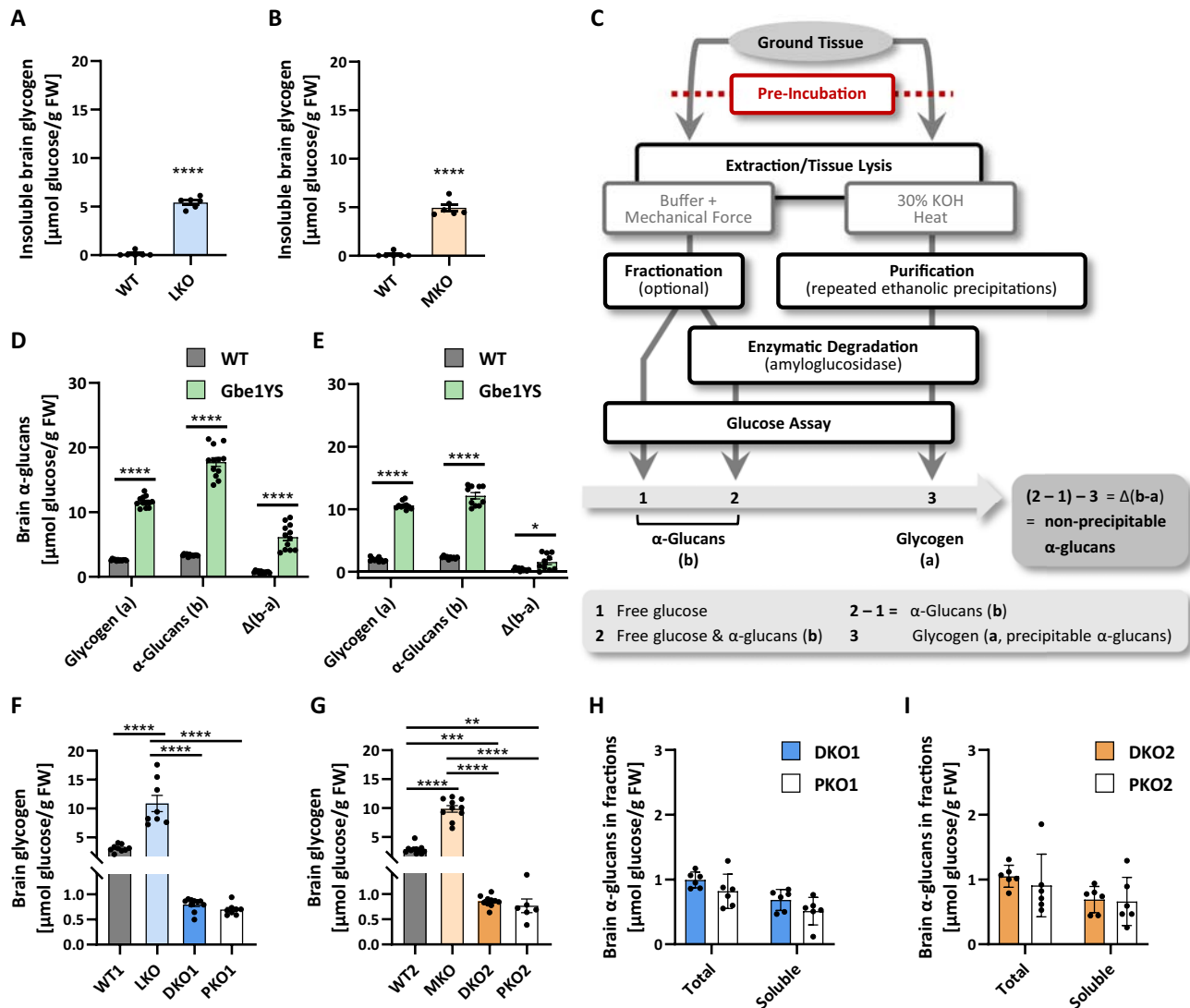
◀ **Figure EV3. Metabolite and pathway changes in LKO and MKO not rescued by PTG knockout.**

(A) Heatmap for malin cohort, comparing all significantly changed metabolites in MKO between the four genotypes. Metabolites significantly changed in both LD mice in bold. (B–G) Volcano plot areas of all detected metabolites from pathways #1 (B), #3 (C), #6 (D), #9 (E), #10 (F), and #13 (G) used for calculation of mean areas in Fig. 4J plotted for LKO vs. WT1 comparison with DKO1 vs. WT1. *P* values for each metabolite were calculated by unpaired two-tailed *t*-test. (H–U) Volcano plot areas of all detected metabolites from pathways #1 (H), #2 (I), #3 (J), #4 (K), #5 (L), #6 (M), #7 (N), #8 (O), #9 (P), #10 (Q), #11 (R), #12 (S), #14 (T), and #22 (U) used for calculation of mean areas in Fig. 4K plotted for DKO1 vs. PKO1 comparison with DKO2 vs. PKO2. *P* values for each metabolite were calculated by unpaired two-tailed *t*-tests. Data information: Off-centered dots in B–U correspond to metabolites with increased fold-change (FC) and/or significance. Red-font metabolites were significantly changed in both genotype comparisons but with different directionality. Pathway numbers as in Fig. 4A. Corresponding to Fig. 4. Percentages indicate detected metabolites of all metabolites in the pathway. Source data are available online for this figure.



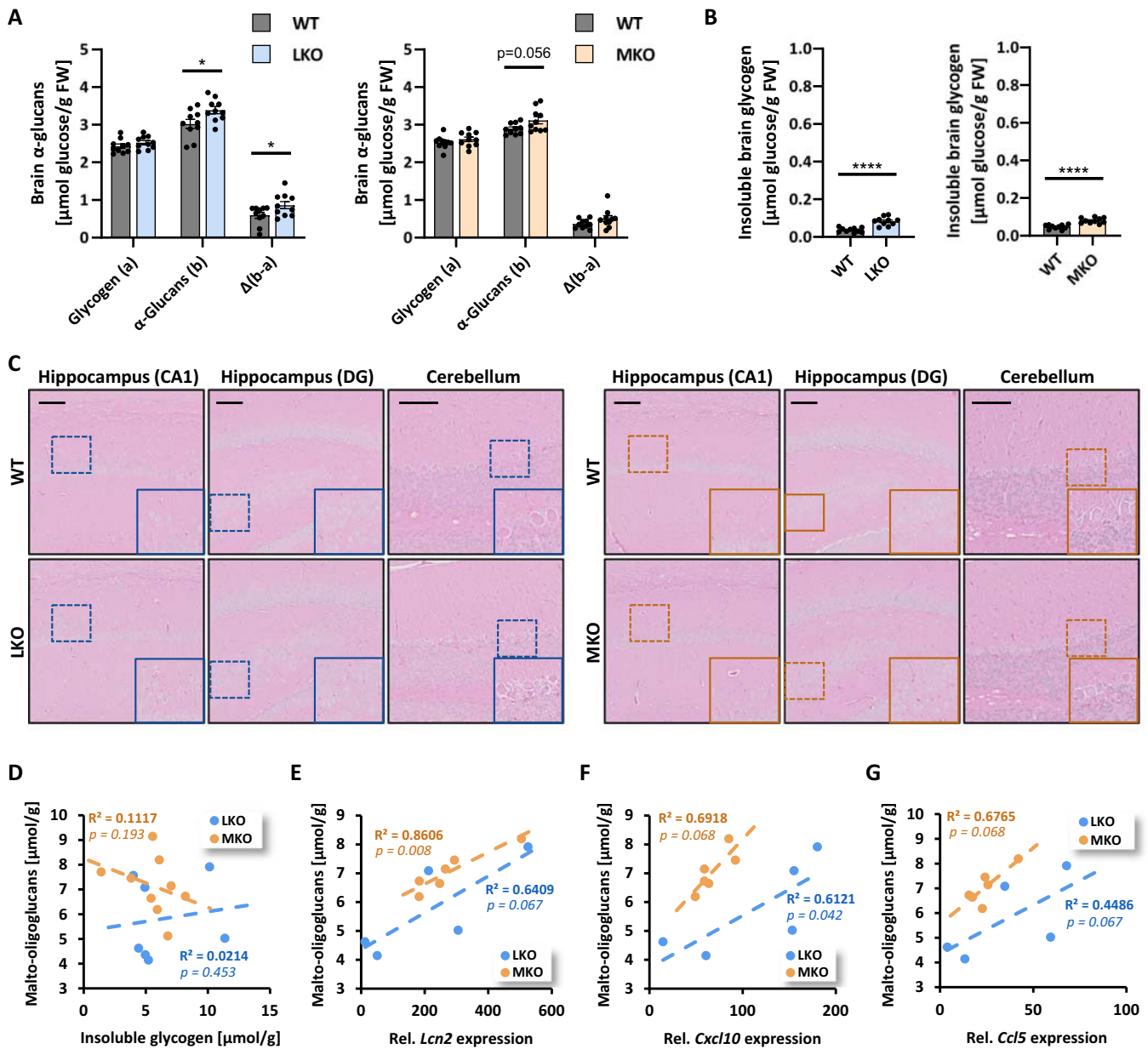
**Figure EV4. Increased Hex4/Glc4 levels in Gbe1YS, but not LD mice are comparable to changes in Pompe disease.**

(A) Brain total and preincubated glycogen in CP- and FM-fixed brains of WT mice from Gbe1YS cohort.  $n = 4$ . (B) PCA plot of metabolic profile in Gbe1YS compared to respective WT.  $n = 12$ . (C) The mean area of hits to show directionality of change (calculations as shown in Fig. 4I) displayed for pathway #22 that was significantly changed in all three indicated genotype comparisons.  $P$  values for each metabolite in the pathway were calculated by unpaired two-tailed  $t$ -tests. (D, E) Volcano plot areas of metabolites from pathway #22 used for calculation of mean areas in G plotted for Gbe1YS ( $n = 12$ ) vs. WT ( $n = 12$ ) comparison with DKO1 ( $n = 10$ ) vs. PKO1 ( $n = 8$ ) (D) or DKO2 ( $n = 10$ ) vs. PKO2 ( $n = 6$ ) (E), respectively. Off-centered dots correspond to metabolites with increased fold-change (FC) and/or significance. Red-font metabolites were significantly changed in both genotype comparisons but with different directionality.  $P$  values for each metabolite were calculated by unpaired two-tailed  $t$ -tests. (F, G) Relative metabolite levels of hexose disaccharide Hex2 (F) and hexose tetrasaccharide Hex4 (G) in LKO and MKO mice compared to their respective WT.  $n = 7$ –11. (H, I) Urine Glc4 levels in Pompe disease mice GAA<sup>-/-</sup> (H) and MKO mice (I).  $n = 19$ –36 (H),  $n = 7$ –10 (I). Data information: Data in (A) and (F–I) are presented as mean  $\pm$  SEM. \*\*\*\* $p < 0.0001$  by two-way ANOVA with Tukey post hoc analysis (A) or by Welch's  $t$ -test (F–I); ns not significant, CP cryopreservation, FM focused microwave. Corresponding to Fig. 5. Source data are available online for this figure.  $P$  values: (A) Tot. (FM vs. CP) 2.2E-07, Pre. (FM vs. CP) 2.4E-13, CP (Tot. vs. Pre.) 7.6E-12, FM (Tot. vs. Pre.) 0.058; (H)  $< 1.0E-15$ . Source data are available online for this figure.



**Figure EV5. Metabolically volatile  $\alpha$ -glucan is present in Gbe1Y5 but absent in DKO mice showing rescue compared to LD mice.**

(A, B) Insoluble (preincubated) glycogen in LKO (A) and MKO (B) mice after CP fixation.  $n = 6$ . (C) Schematic showing methodology of glycogen determination as precipitable glycogen (a) or  $\alpha$ -glucan (b) which additionally contains lower molecular weight glucans ( $\geq 2$  glucose units). (D, E) Brain  $\alpha$ -glucans in Gbe1Y5 mice after FM (D) or CP (E) fixation. Terminology is explained in (C).  $n = 10$ –12. (F, G) Brain total glycogen following FM fixation in laforin (F) and malin (G) cohort as in Fig. 1C but with different y-axis scale.  $n = 6$ –11. (H, I) Total and soluble brain  $\alpha$ -glucan in PKO and DKO from the laforin (H) and malin (I) cohort after FM fixation.  $n = 6$ . Data information: All data were presented as mean  $\pm$  SEM. \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; \*\*\*\* $p < 0.0001$  by two-way ANOVA with Tukey post hoc analysis (F, G) or by Welch's t-test (A–E, H, I). Corresponding to Fig. 6. P values: (A)  $1.7\text{E-}07$ ; (B)  $1.03\text{E-}05$ ; (D) Glyc.  $3.4\text{E-}13$ ,  $\alpha$ -Gluc.  $2.6\text{E-}10$ ,  $\Delta(a-b)$   $9.7\text{E-}07$ ; (E) Glyc.  $<1.0\text{E-}15$ ,  $\alpha$ -Gluc.  $7.5\text{E-}09$ ,  $\Delta(a-b)$   $0.0147$ ; (F) WT1 vs. LKO  $1.3\text{E-}08$ , LKO vs. DKO1  $1.7\text{E-}11$ , LKO vs. PKO1  $4.9\text{E-}11$ ; (G) WT2 vs. MKO  $5.6\text{E-}14$ , MKO vs. DKO2  $5.5\text{E-}14$ , MKO vs. PKO2  $5.5\text{E-}14$ , WT2 vs. DKO2  $0.00063$ , WT2 vs. PKO2  $0.0022$ . Source data are available online for this figure.



**Figure EV6. Malto-oligoglucan accumulation only found in aged LD mice correlates well with neuroinflammatory marker expression.**

(A) Brain  $\alpha$ -glucans in 5-week-old LKO and MKO, respectively, compared to the respective WT after FM fixation. Terminology explained in Fig. EV5C.  $n = 10$ . (B) Insoluble (preincubated) glycogen in 5-week-old LKO and MKO mice, respectively after CP fixation.  $n = 10$ . (C) Representative PASD images of hippocampus and cerebellum in LKO and MKO mice, respectively. Scale bar, 100  $\mu$ m. The region highlighted by small square is shown enlarged at the right bottom of each image (bigger squares outlined in blue or orange). (D–G) Correlation coefficients ( $R^2$ ) between malto-oligoglucans and insoluble glycogen (D) or neuroinflammatory markers *Lcn2* (E), *Cxcl10* (F), and *Ccl5* (G), respectively, in aged LKO and MKO.  $n = 5$ –9. Data information: Data were presented as mean  $\pm$  SEM (A, B) or individual data points (D–G). \* $p < 0.05$ ; \*\*\*\* $p < 0.0001$  by Welch's  $t$ -test. Corresponding to Fig. 6.  $P$  values: (A)  $\alpha$ -Gluc. 0.0265,  $\Delta(a-b)$  0.0439; (B) WT vs. LKO 5.0E-05, WT vs. MKO 2.5E-05. Source data are available online for this figure.