## SUPPLEMENTAL METHODS

### Sample Analysis and Data processing

*Standard Operational Procedures (SOP)*

To maximize quality of the output all samples were analyzed by a series of steps defined by Standard Operational Procedures (SOP). Reproducibility was monitored and ensured within the system with procedures implemented to identify any changes and shifts in the platform during sample analyses. Procedure including injection of blank (extraction solvent without sample) to characterize chromatographic background, running lipid standards to calibrate retention times specific to individual lipid classes and pooled sample (Mix of all samples analyzed) after every $10^{th}$ sample. This technique is well-established and suitable for long-term metabolic studies to monitor instrument performance and enable comparison of data acquired in different times. With limited number of samples included in the study, pool sample was injected at the beginning, in the middle, and in the end of the analysis and used to confirm acceptable retention time and mass accuracy shifts. As retention time calibration standards, the SPLASH LIPIDOMIX mass spec standard was used (Avanti Polar Lipids, Inc.). For sample randomization, a random number was assigned to each sample using the “RAND()” function in Microsoft Excel; these numbers were then sorted in ascending order.

Data Pre-Processing

For overall feature description, features that were not detected in 60% of a sample group were deemed as a “false positive” and subsequently deleted. In other words, if the feature was present in less than 40% of the sample of the specific group, it was considered as the absent feature. Before tables were put through Metaboanalyst for statistical analysis, additional trimming of the data was done. Retention times before 9 mins and after 37 mins were deleted due to these signals being attributed to overall noise.

Data Analysis

Multivariate, univariate and partition analyses were done using a combination of Metaboanalyst and Microsoft Excel. For the partition analysis, a ratio of the hippocampus intensity divided by the cortex intensity of the same animal was calculated for each feature. This resulted in five ratios for wild type and five ratios for the $Kv1.1^{-/-}$ genotype which were then used for statistical analysis. Ratios then represent a distribution of the individual metabolites in various brain sections and may more accurately reflect metabolic disbalance between these tissues. The p-values were calculated using Metaboanalyst software. Here, any features missing 50% of values were removed and remaining missing values were imputed with minimum values. Then, auto scaling was used for normalization due to having the best result. After this, raw p-values ($p<0.05$) and a fold-change (FC) threshold of >2 from the volcano plot feature were used to determine significance.

For multivariate analyses (comparing both genotypes and tissues), IQR filtering was used for variable filtering. No missing value imputation was performed; however, this did not affect overall results. After this, Pareto scaling was used to normalize the data. For the dendrogram, average algorithm with Euclidean distance measure was used for hierarchical clustering. Average clustering was chosen here because it is suggested to be less affected by outliers (Manning et al., 2009). PLS-DA score plots have been reported. One-way ANOVA analysis was used to determine significant features in multivariate analysis (ANOVA $p<0.05$). However, most of this difference appeared to be due to tissue. Because of this, the tissues were analyzed separately in univariate analyses.

For univariate analysis of hippocampal and cortical tissue, data scaling and normalization in followed the same steps described above for multivariate analysis in Metaboanalyst. Again, raw p-values and a FC threshold of >2 were obtained from the volcano plot were used for statistical significance (t-test $p<0.05$). Only the significant features were used

to construct the heatmaps. Here, average clustering with Euclidean distance measure was used for hierarchical clustering on the heatmap as well. In general, average clustering seemed to performed better than other types of clustering.

Finally, calculation of FDR values for univariate analyses was performed in Microsoft Excel using normalized data files from Metaboanalyst. Upon evaluation, it was found that Metaboanalyst uses this file to provide the raw p.values in the Volcano Plot feature. Further, it was also found this software makes certain assumptions on normality for statistical tests, which the authors were unable to replicate by hand, thus indicating a need to calculate FDR values in Excel. FDR was calculated using the modified adjusted p-value equation previously described by Benjamini et al. (Benjamini et al., 2009). In cases where the FDR was larger than 1, it was reported as 1 per Benjamini et al.

*Pathway Enrichment Analysis:*

KEGG IDs were uploaded to Reactome and Metaboanalyst for pathway enrichment analyses. Reactome provided more detailed pathway analysis so this has been reported. Pathways with an adjusted $p<0.05$ have been reported in Supplemental Table 3.

*Data Availability*

Raw data files have been uploaded to Metabolights under the identifier MTBLS1432.

References:

Benjamini, Y., Heller, R., & Yekutieli, D. (2009). Selective inference in complex research. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, *367*(1906), 4255–4271. https://doi.org/10.1098/rsta.2009.0127

Manning, C. D., Raghavan, P., & Schutze, H. (2009). 17 Hierarchical Clustering. In *An Introduction to Information Retrieval* (Online Edition, pp. 377–388). essay, Cambridge University Press.