Reads were mapped to the mouse genome (mm10) using GMAP/GSNAP (???) (v. XXX) [ref]. Only unique and concordant mapped reads were subsequently used for feature quantification. Refseq (v. XXX) gene counts were obtained using featureCounts (v. XXX) [ref].

All statistical analyses were performed using open source software freely available through the R/Bioconductor project [1]. Gene counts were filtered using a proportion test (counts per million cutoff of 1), as implemented in the NOISeq package (v.2.22.1) [2]. RUV normalization was performed using RUVSeq (v. 1.12.0) [3] after the data was normalized by Trimmed Mean of M-values (TMM) [4] using edgeR (v. 3.20.9) [5]. Specifically, RUVs (with k=5) was used after defining groups based on both genotype and treatment and using a list of 2677 negative control genes obtained as genes with an adjusted *P*-value >0.9 in the comparison between sleep deprivation and controls in microarray data available through GEO (GSE78215) (obtained from Additional File 4 of [6]). Analogously, a list of 579 positive control genes to evaluate the effect of normalization was obtained as …. Differential expression analysis was performed using edgeR (v. 3.20.9) with a factorial design that included genotype (wild-type or Shank3 mutant) and treatment (sleep deprivation or control). We specified the following contrasts: wild-type sleep deprived vs wild-type controls (WTSD vs WTHC); Shank3 mutant sleep deprived vs Shank3 mutant controls (S3SD vs S3HC); Shank3 mutant controls vs wild-type controls (S3HC vs WTHC); and Shank3 sleep deprived vs wild-type sleep deprived (S3HC vs WTHC). A model that included an interaction term between genotype and treatment was also considered, but the interaction effect was significant for only two genes. To gain insight on the different effects in sleep deprivation between wild-type and mutant, we considered the union of the differentially expressed genes between genotypes both in controls and sleep deprived mice. We then clustered the resulting gene list using hierarchical clustering and looked for gene set enrichment for each cluster using ….

The R code to reproduce all the main figures and tables of the article is available on GitHub (github.com/drighelli/peixoto).

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