**RNA isolation, quantitative PCR, and RNA sequencing**

All RNA was isolated using Direct-zol RNA Miniprep Kit using manufactures protocol from samples collected in Trizol reagent. RNA concentration and purity were quantified using a Nanodrop Microvolume Spectrophotometer. 500ng RNA was used for RT-PCR using qScriptä cDNA SuperMix kit and manufacturers protocol. cDNA was diluted 1:5 with ddH2O and qPCR was carried out using 2X PowerUpäSYBRä Green Master Mix (primer sequences listed in table). The cycle threshold (Ct) of target genes was normalized to 18s housekeeping gene, relative expression of mRNA was calculated using the DDCt method, and results expressed as fold-change.

For determining which genes were differentially expressed by RNAseq, transcript-level abundance of paired-end RNA-seq data was estimated by Salmon (1.1.0)1 using the mouse transcriptome from Genecode (vM24). Transcript-level quantification was aggregated to the gene-level with Tximport (1.14.2)2. The resulting gene counts were used as an input to DESeq2 (1.26.0)3. Differentially expressed genes below a BH adjusted p-value of 0.05 were considered significant. Volcano plots were created using the Enhanced Volcano R package (1.4.0)4. Gene symbols were added by BiomaRt (2.42.1)5. Snakemake (5.10.0)6 was used as a workflow management system for processing fastq files with salmon.

References

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