The whole transcriptome analysis

The gene expression was evaluated using the AmpliSeq™ Transcriptome Human Gene Expression Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. Pooled libraries were subjected to the Ion Chef™ System (Thermo Fisher Scientific) for template preparation. Libraries were then loaded onto an Ion 550™ chip and sequenced with the Ion S5™ sequencing system. The Ion Torrent Suite v5.10 software program was used to map read. Raw read-count data files were converted to reads per kilobase per million reads (RPKM) for read-count normalization. A differential gene expression (DGE) analysis was performed using the Transcriptome Analysis Console (TAC) software program (Thermo Fisher Scientific) with fold-change differences of > 4.0 or < -4.0. Distributed Stochastic Neighbor Embedding (t-SNE) and hierarchical clustering were performed in Orange and used for data visualization. The study was approved by the relevant institutional review boards (28-224).