

Figure 1S: PCR products from all primer pairs targeting FCGR3B, AQP9, FPR1, FPR2, NCF2, and β -ACTB were analyzed by 2% agarose gel electrophoresis following temperature gradient optimization. Each primer pair produced a single, distinct band corresponding to the expected amplicon size (table 1), without additional bands, smearing, or primer dimers. These clean gel patterns, combined with melt curve analysis, confirm the high specificity of all primers and validate their suitability for accurate quantitative gene expression analysis.

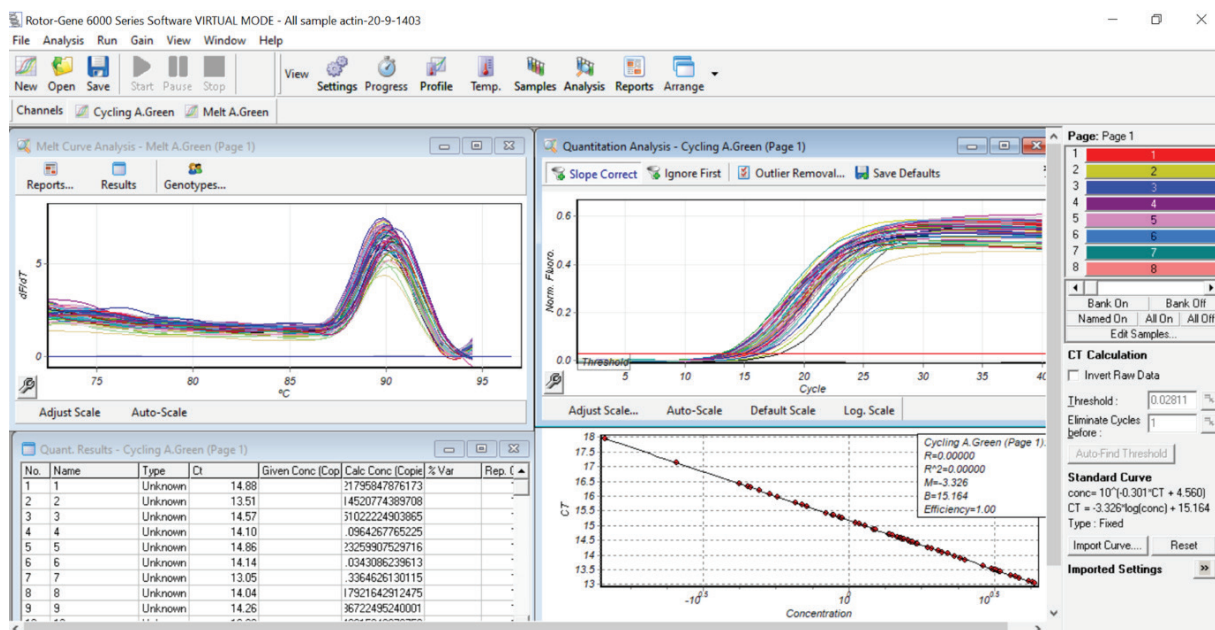


Figure 2S: This panel shows the validation of β -ACTB, which served as the reference gene for normalization in all qPCR assays. The amplification plot exhibits consistent exponential amplification across serial dilutions and technical replicates, indicating stable assay performance. The standard curve, constructed using a five-point 10-fold dilution series, demonstrates excellent linearity ($R^2 \geq 0.99$) and primer efficiency within the optimal range of 90-110%, ensuring accurate quantification. Melt curve analysis reveals a single, sharp peak, confirming the absence of nonspecific products or primer-dimer artifacts. Specificity is further supported by agarose gel electrophoresis, which displays a single band corresponding to the expected amplicon size. Collectively, these results validate β -ACTB as a reliable and robust reference gene for this study.

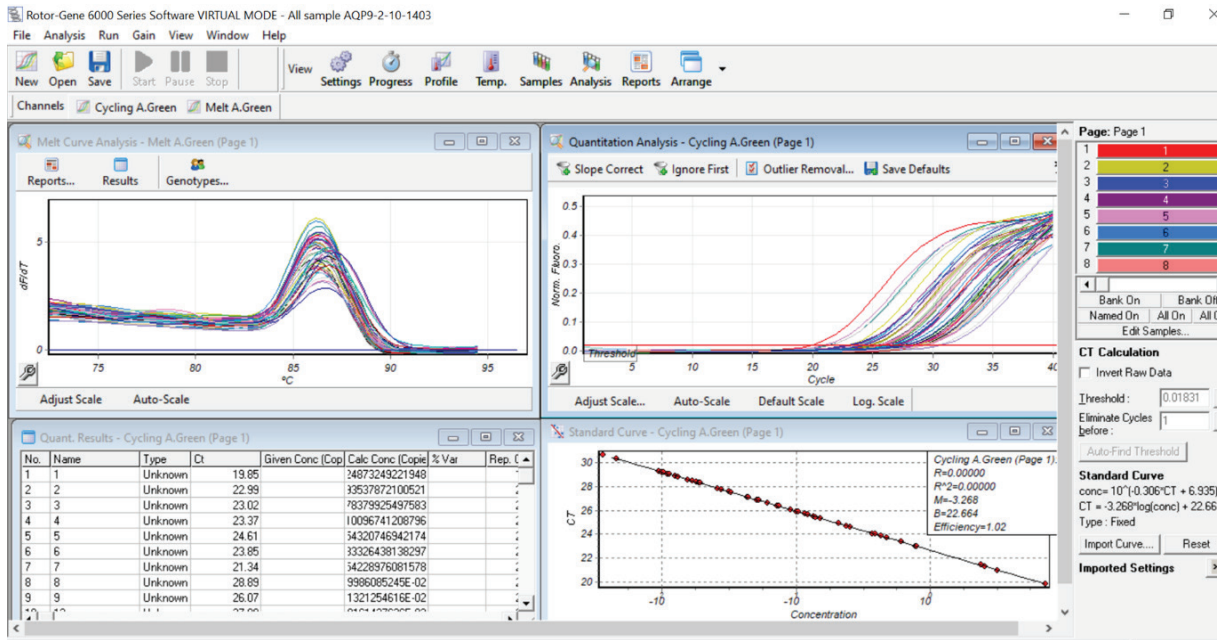


Figure 3S: This figure validates the AQP9 primer pair, demonstrating consistent exponential amplification across replicates, high linearity, and efficiency within the 90-110% range. Melt-curve and gel electrophoresis analyses confirm a single specific amplicon without nonspecific products, ensuring reliable and accurate quantification of AQP9 expression in this study.

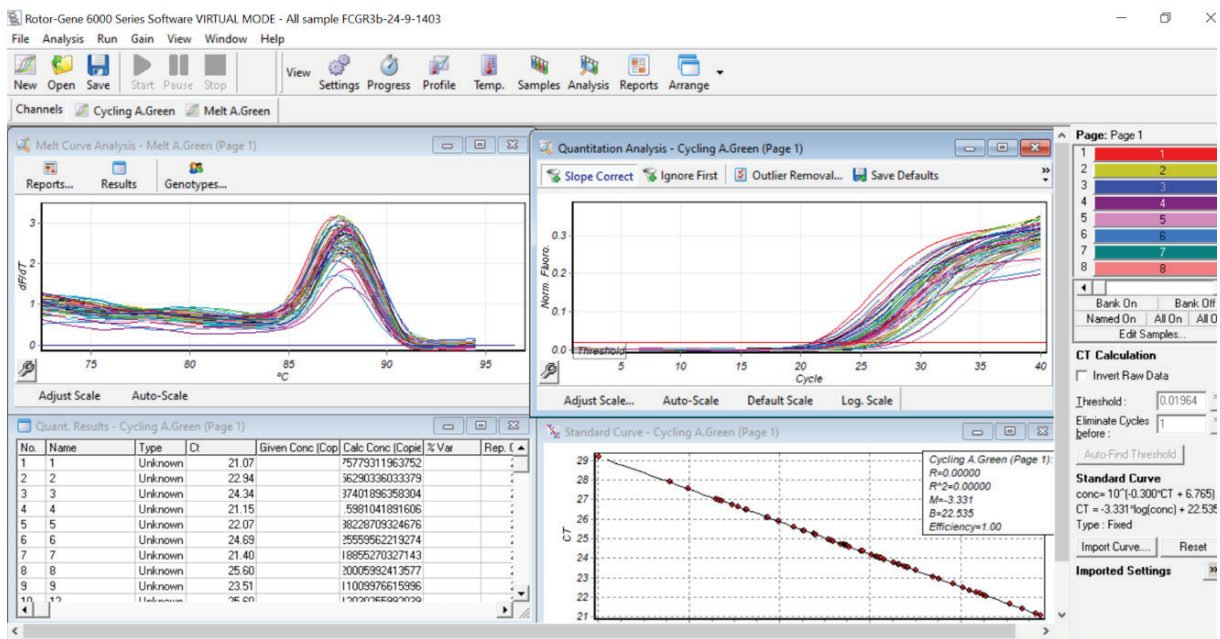


Figure 4S: This figure confirms the performance of the FCGR3B primer pair, showing reproducible exponential amplification, excellent linearity, and efficiency (90–110%). Melt curve and gel analyses verify a single specific product without nonspecific amplification, supporting accurate FCGR3B quantification throughout the study.

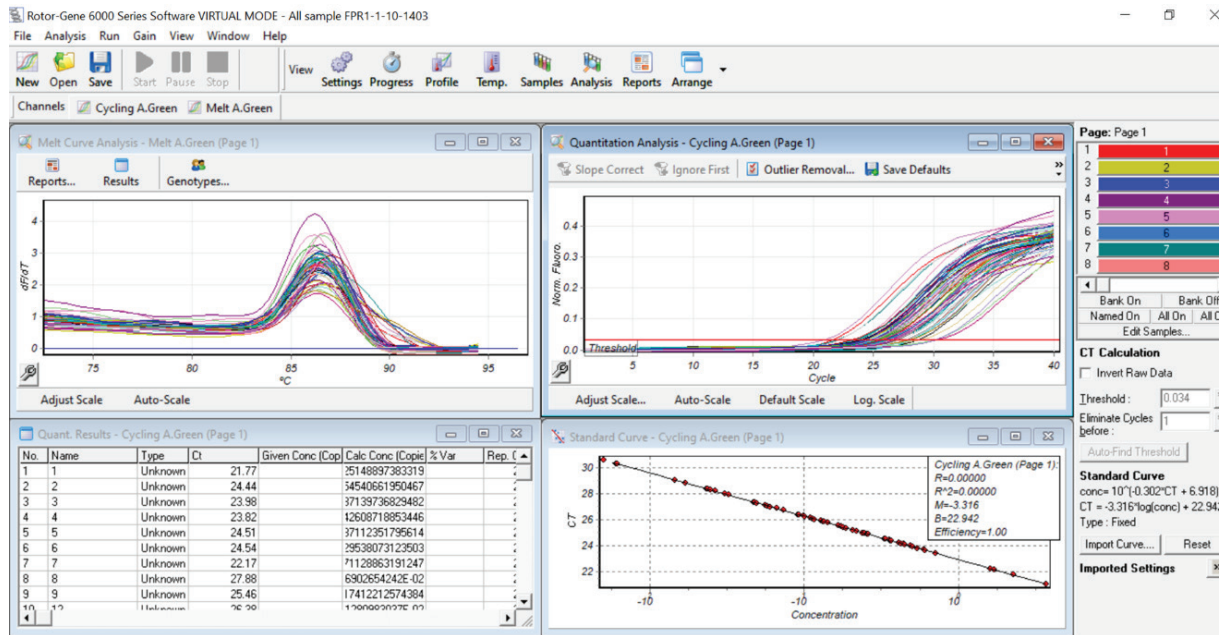


Figure 5S: This panel validates the performance of the FPR1 primer, demonstrating consistent amplification kinetics, excellent linearity, and optimal efficiency. Melt-curve and gel analyses confirm a single specific product, ensuring reliable FPR1 quantification.



Figure 6S: This figure validates FPR2 primers, showing reproducible amplification, optimal efficiency, and high linearity across replicates. Melt-curve and gel analyses confirm the presence of a single specific product without artifacts, ensuring accurate quantification of FPR2 expression.

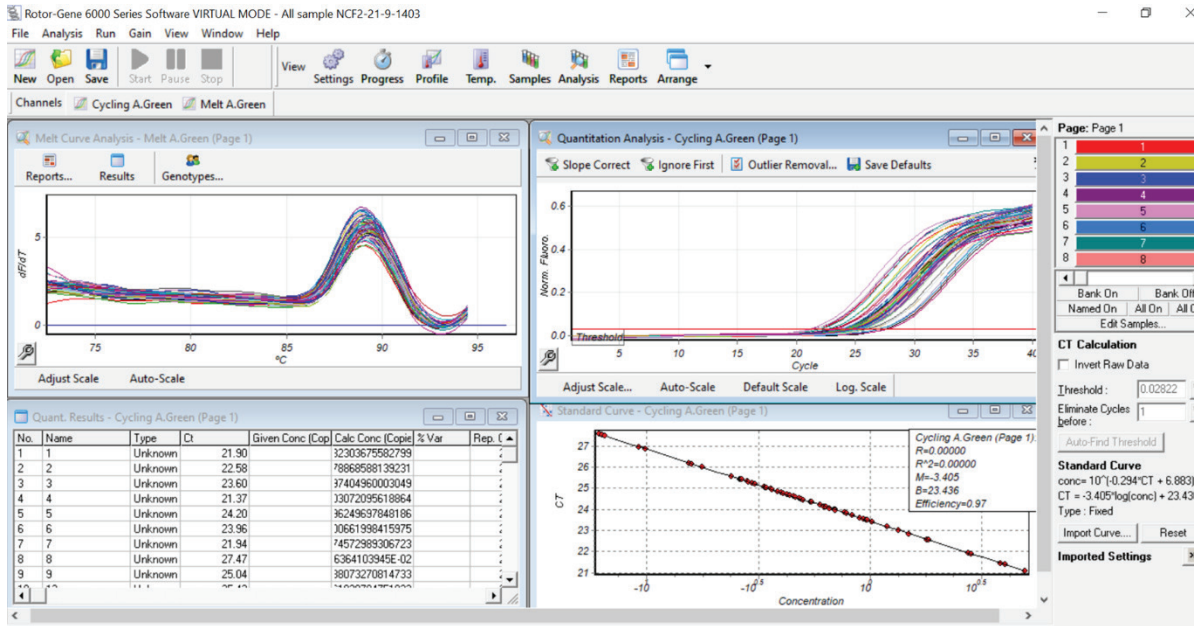


Figure 7S: This figure confirms the validation of NCF2 primers, demonstrating consistent amplification, efficient performance, and strong linearity across replicates. Melt curve and gel electrophoresis analyses verify the presence of a single, specific amplicon, supporting precise quantification of NCF2 expression.