



**Supplemental Figure S2** Specificity validation of reference gene amplifications. Melting curve analysis demonstrates single-peak profiles for all candidate reference genes, indicating specific amplification. The y-axis ( $-\Delta F/\Delta T$ ) represents the temperature-dependent fluorescence decay rate, with peak temperatures (T<sub>m</sub>) corresponding to product denaturation thresholds. The presence of single sharp peaks in melting curve profiles suggests primer specificity, while the absence of secondary peaks indicates no detectable formation of primer-dimers or other nonspecific amplifications.