Additional file 2

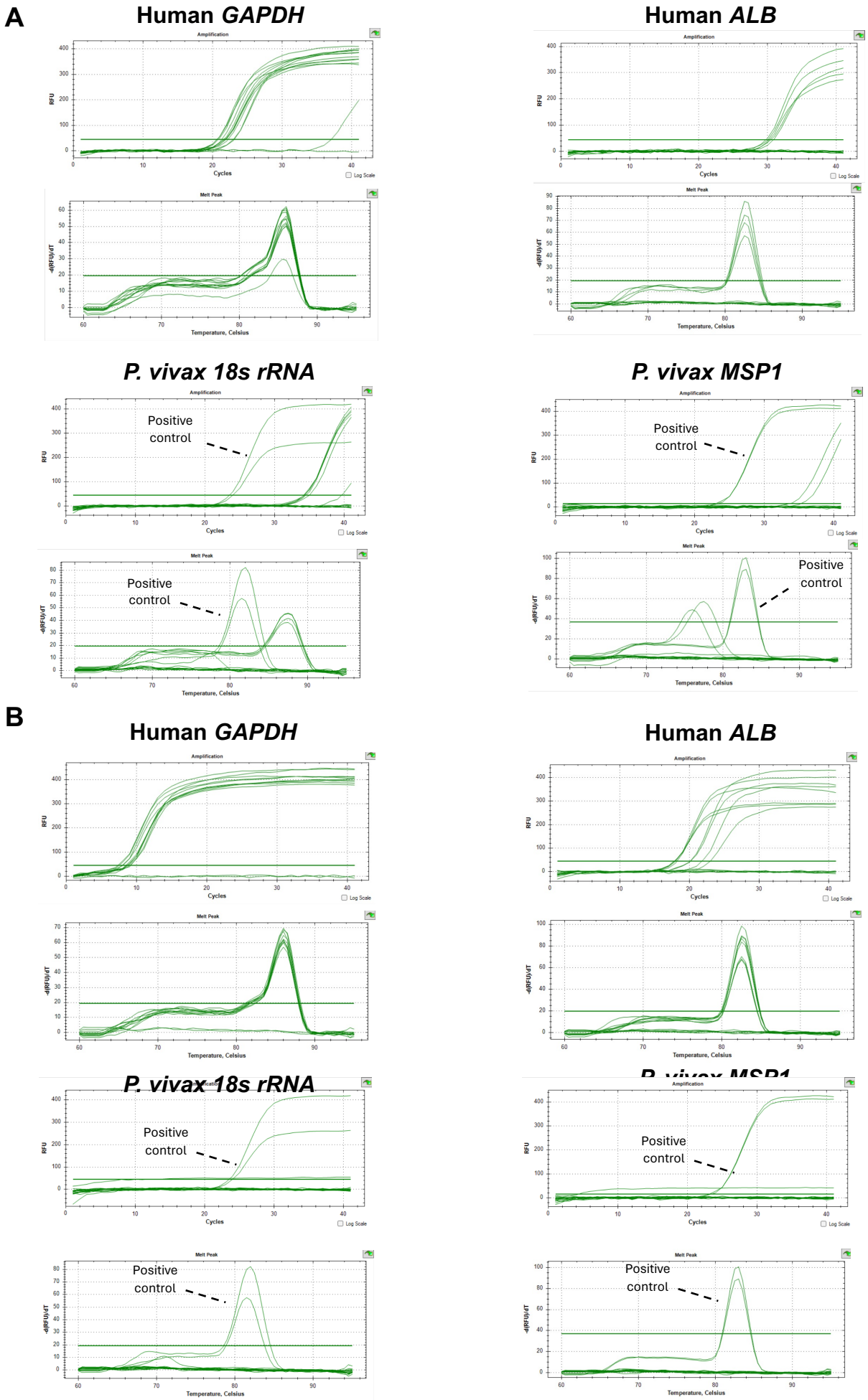

Figure S2. Quantitative reverse transcription-PCR (qRT-PCR) with and without preamplification of cDNA. Following RNA extraction and DNase treatment to remove gDNA, cDNA was synthesized and subjected to PCR for amplifying *Plasmodium 18s rRNA*, *MSP1*, human *GAPDH* and human albumin (*ALB*) transcript. (**A**) Amplification curves and melt peaks of amplicons obtained from qRT-PCR without preamplification of cDNA template. In the upper panels, the amplification curves show the thermal cycle numbers and the relative fluorescence unit of SYBR green on the x- and y-axis. *P. vivax*-infected liver organoid (LO) and non-infected LOs were subjected to qRT-PCR. Technical duplicate wells were performed. In the lower panels, the melt peaks indicate the melting temperature (x-axis) of the amplicons. The melting temperature is dependent on the DNA sequence. Thus, each melt peak is relatively specific to a given amplicon. (**B**) Amplification curves and melt peaks of amplicons obtained from qRT-PCR with preamplification of cDNA template. Following preamplification, the cDNA template of human *GAPDH* and *ALB* gene increased and could be detected at the early thermal cycle of amplification (a lower threshold cycle or Ct value). Preamplification of cDNA template did not cause non-specific amplification because the melting temperature of the amplicons remains unchanged (Lover panels in A and B). However, we failed to detect *Plasmodium 18s rRNA* and *MSP1* regardless of preamplification. Thus, the amount of *P. vivax* mRNA is relatively low in the LOs inoculated with sporozoites. gDNA of *P. vivax* was used as a positive control template for amplifying gene encoding *Plasmodium 18s rRNA* and *MSP1*.