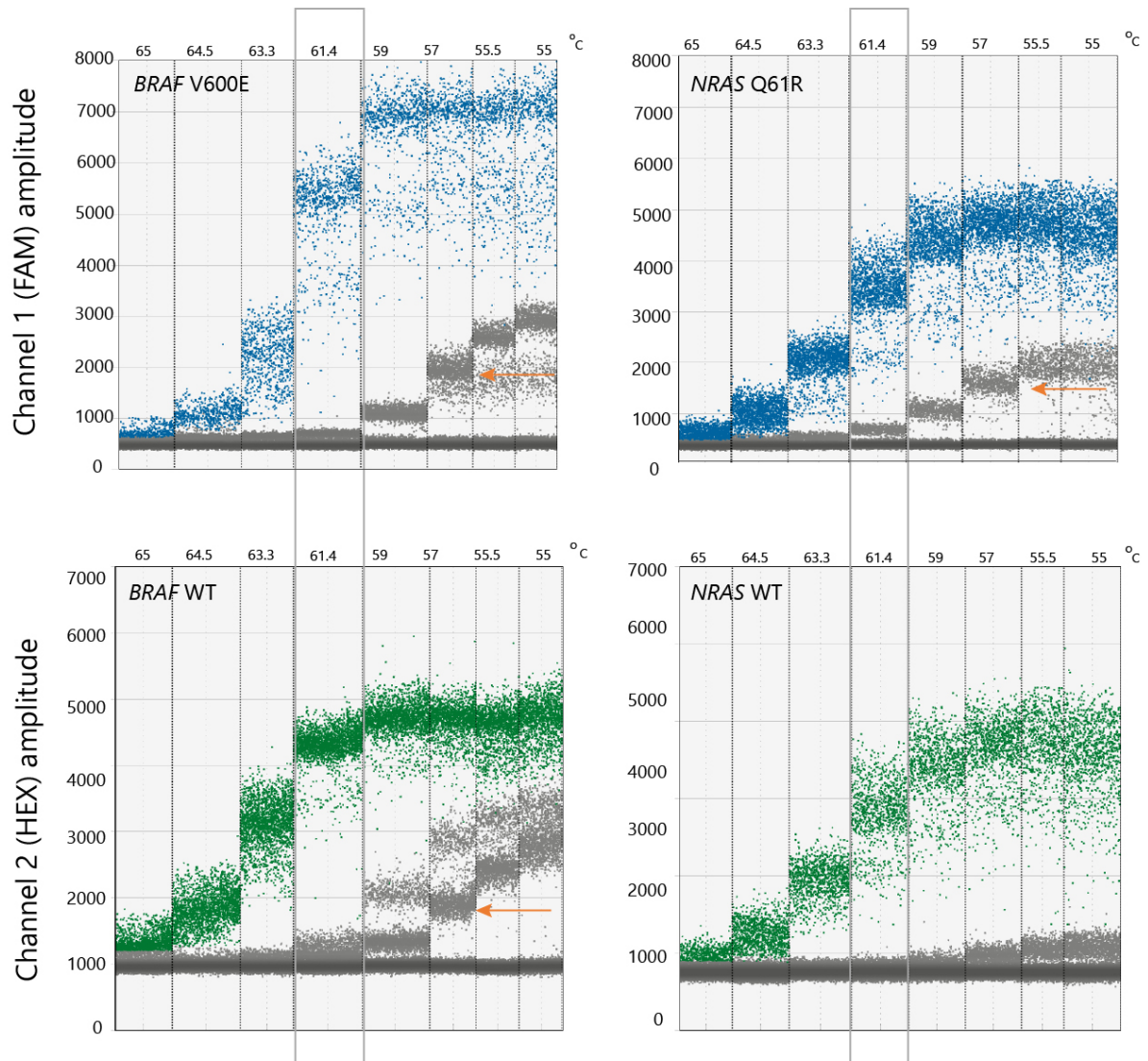




Supplementary Fig. 1 Optimisation of the annealing temperature for the *BRAF* V600E and *NRAS* Q61R ddPCR assays with a positive control template. Data visualized in a 1-D plot showing separation of the negative and positive droplets for *BRAF* V600E/*BRAF* WT and *NRAS* Q61R/*NRAS* WT ddPCR assays over the annealing/extension temperature gradient (55-65°C). Both fluorescent channels are shown separately. Blue droplets represent the detection of DNA with the mutant sequence. Green droplets represent the detection of DNA with the WT sequence. Grey droplets have no target DNA amplification. Optimal separation of the FAM and HEX positive droplets was achieved for both assays with the probe annealing temperature of 61.4°C, as indicated by the grey boxes. Non-specific hybridization by the probe is shown as additional clusters of negative (grey) droplets (examples indicated with orange arrows).