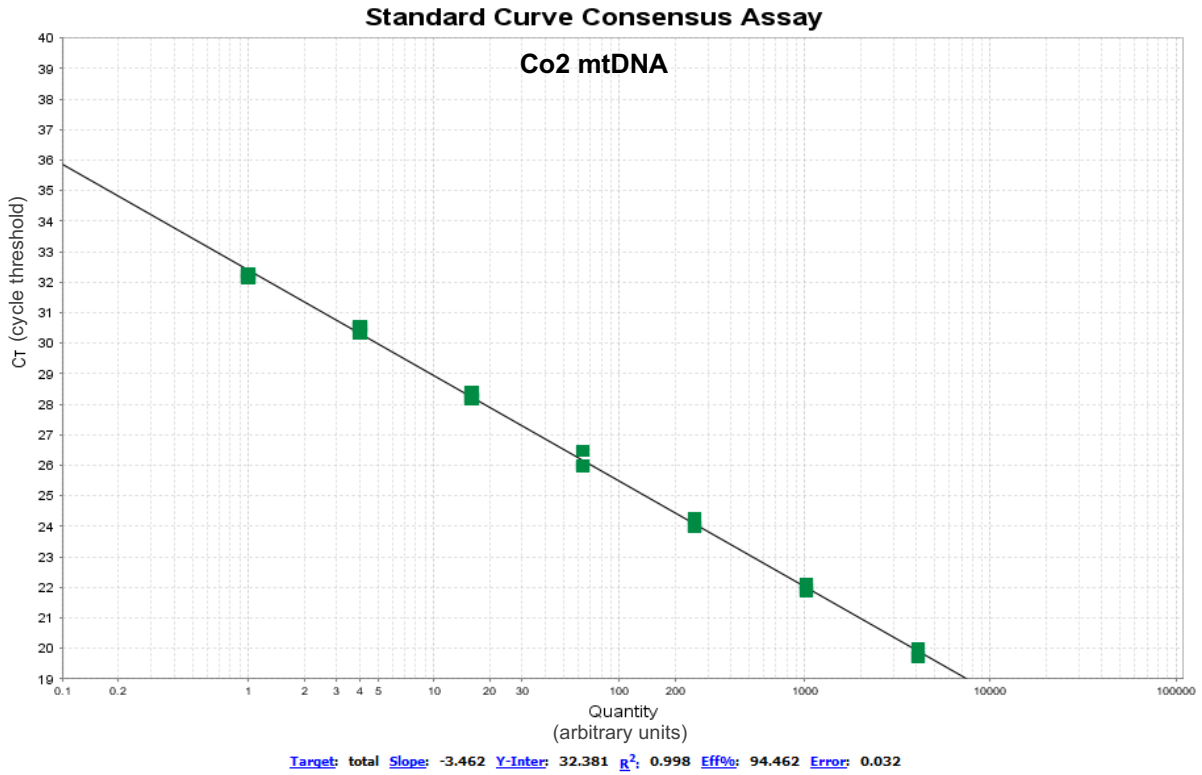
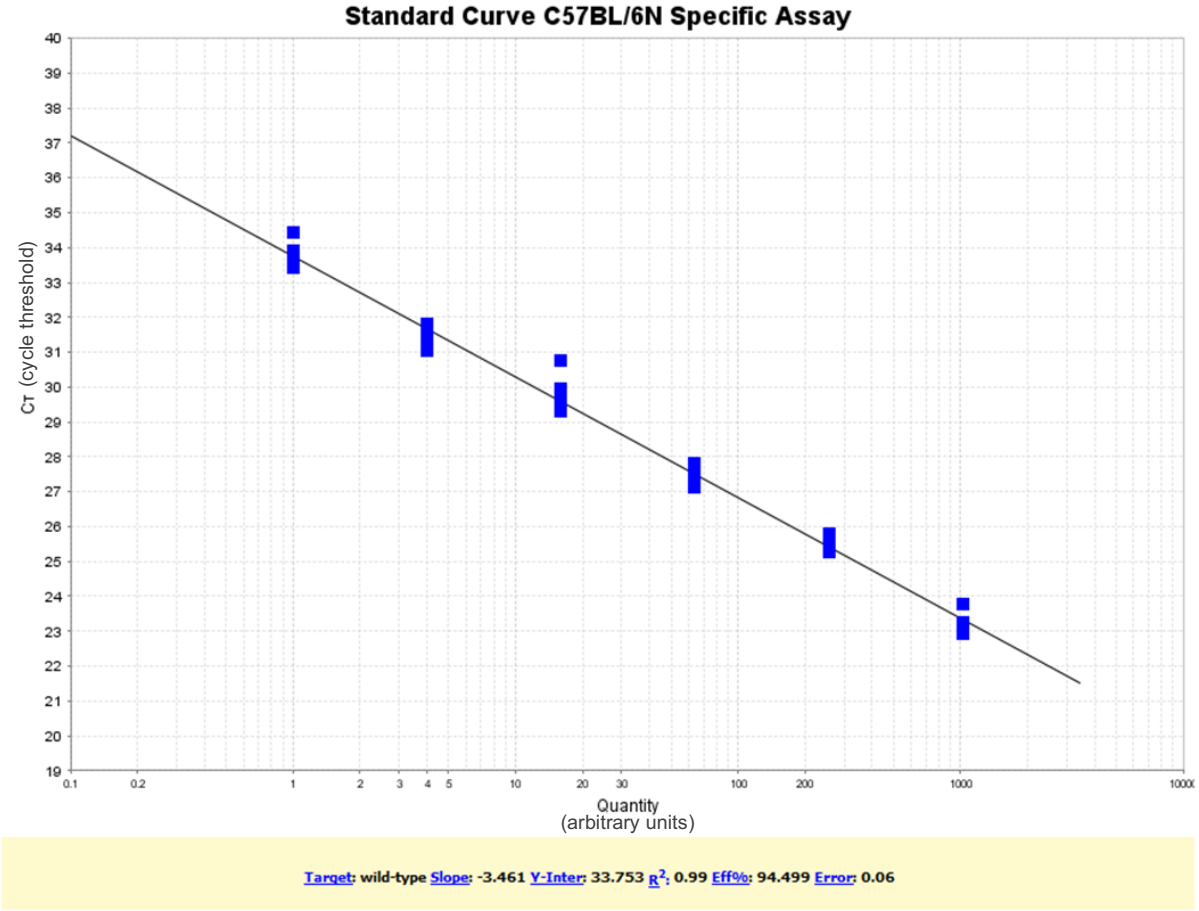
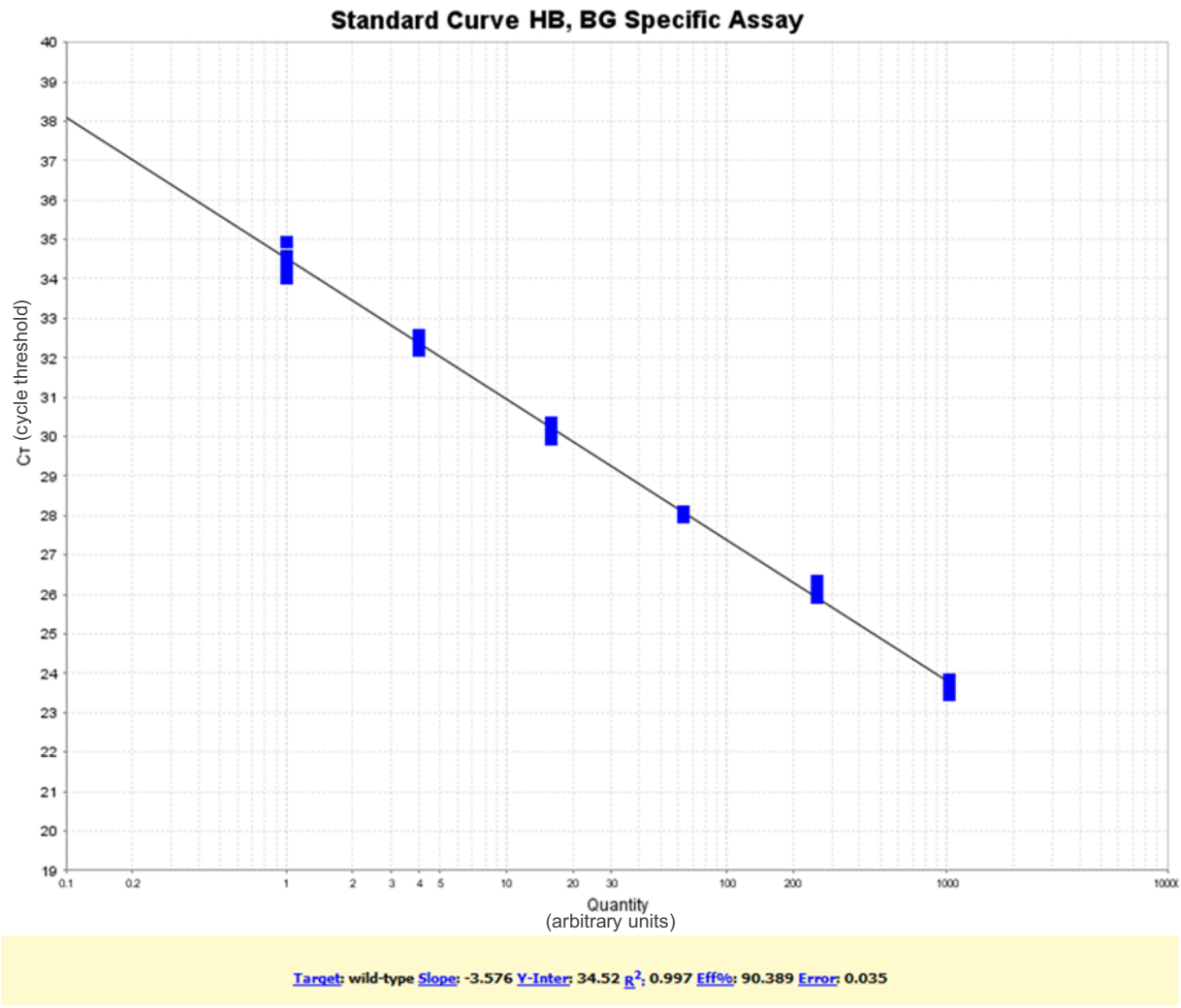


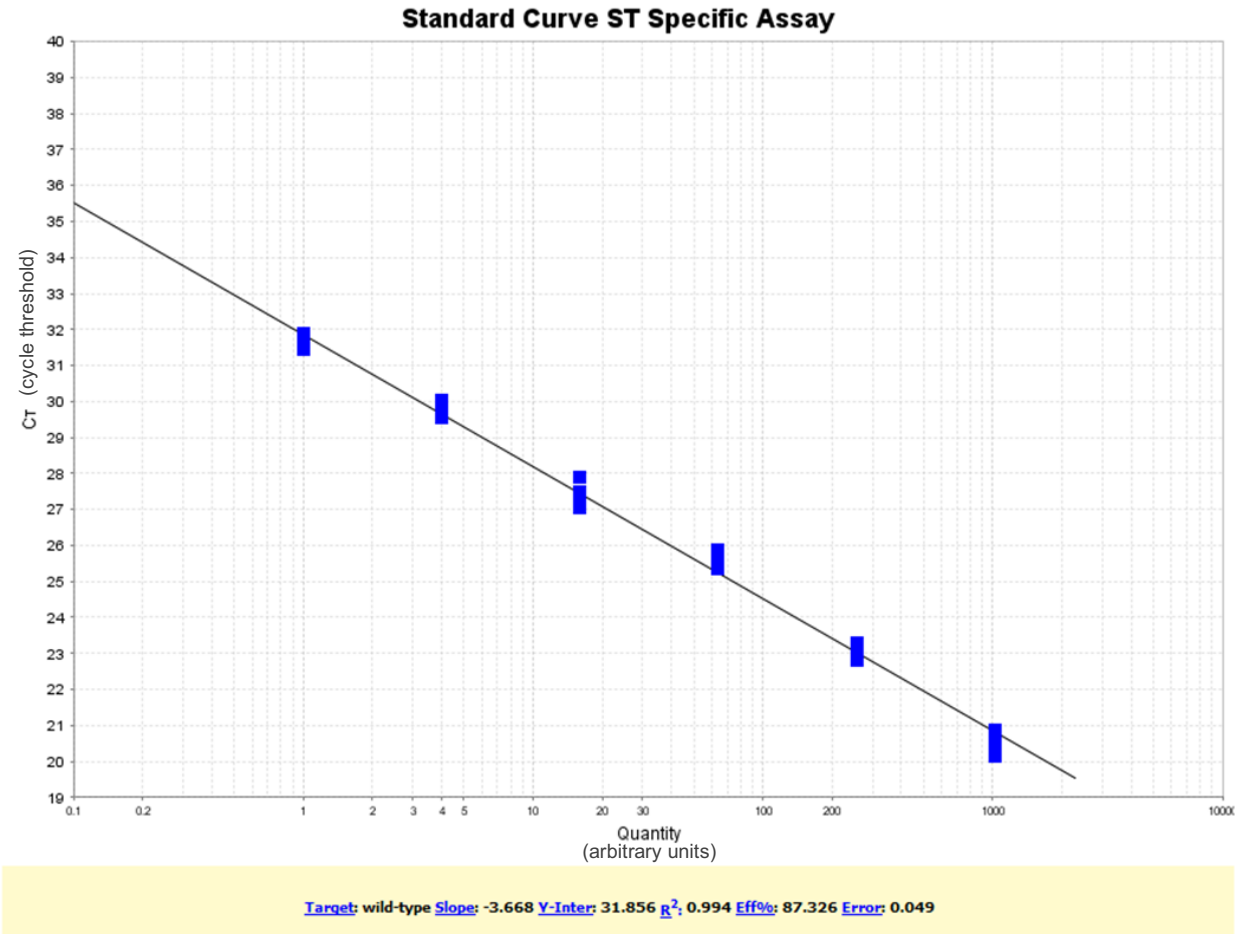
Supplementary Figure 1 | Standard curve of the Co2 mtDNA consensus assay. This assay was used to normalise changes in the input mtDNA;qPCR was performed in triplicates. x-axis: Quantity of input DNA (arbitrary units); y-axis: qPCR Ct-value (cycle threshold).



Supplementary Figure 2 | Standard curve of the C57BL/6N laboratory mouse mtDNA specific assay. This assay was used to quantify C57BL/6N laboratory mouse mtDNA; qPCR was performed in triplicates. x-axis: Quantity of input DNA (arbitrary units); y-axis: qPCR Ct-value (cycle threshold).

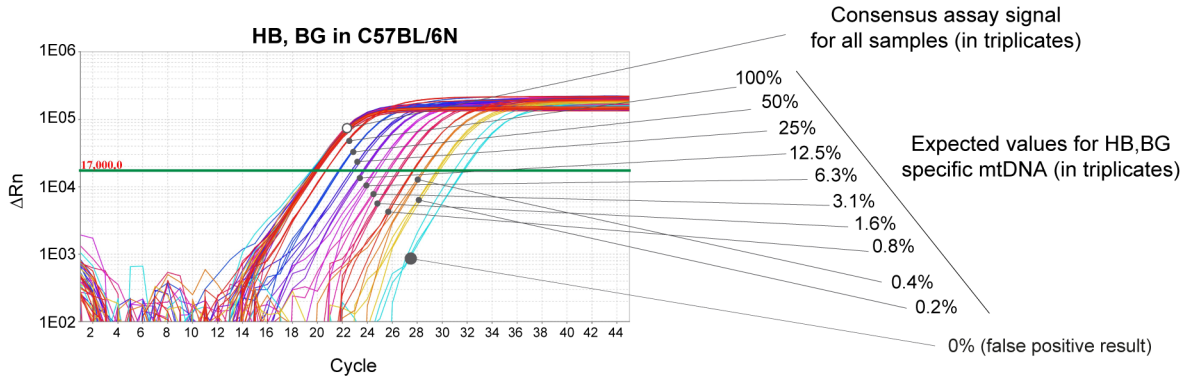


Supplementary Figure 3 | Standard curve of the BG and HB wild-derived mouse mtDNA specific assay. This assay was used to quantify BG and HB wild-mouse mtDNA; qPCR was performed in triplicates. x-axis: Quantity of input DNA (arbitrary units); y-axis: qPCR Ct-value (cycle threshold).

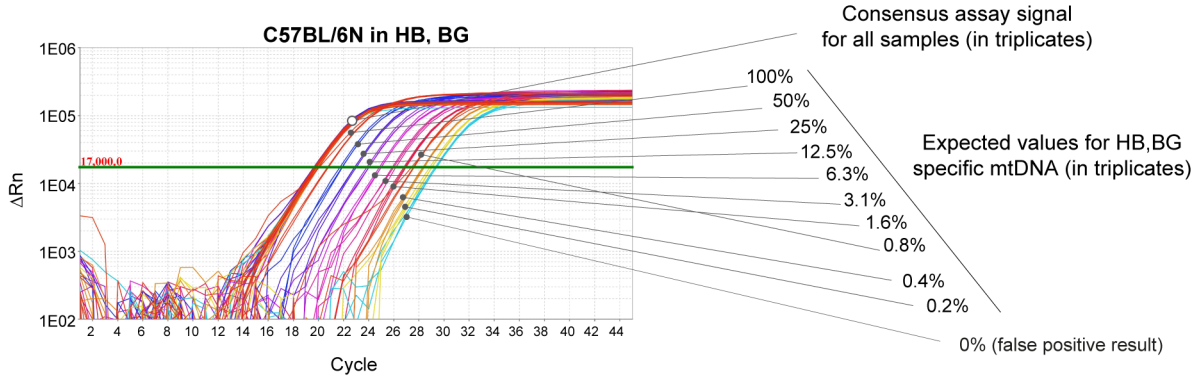


Supplementary Figure 4 |Standard curve of the shows the standard curve of the ST wild-derived mouse mtDNA specific assay. This was used to quantify ST wild-mouse mtDNA; qPCR was performed in triplicates. x-axis: Quantity of input DNA (arbitrary units); y-axis: qPCR Ct-value (cycle threshold).

a

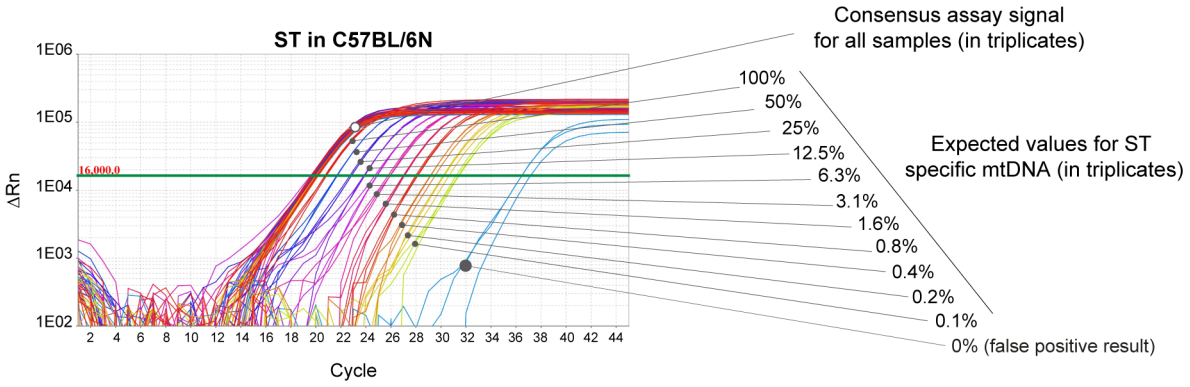


b

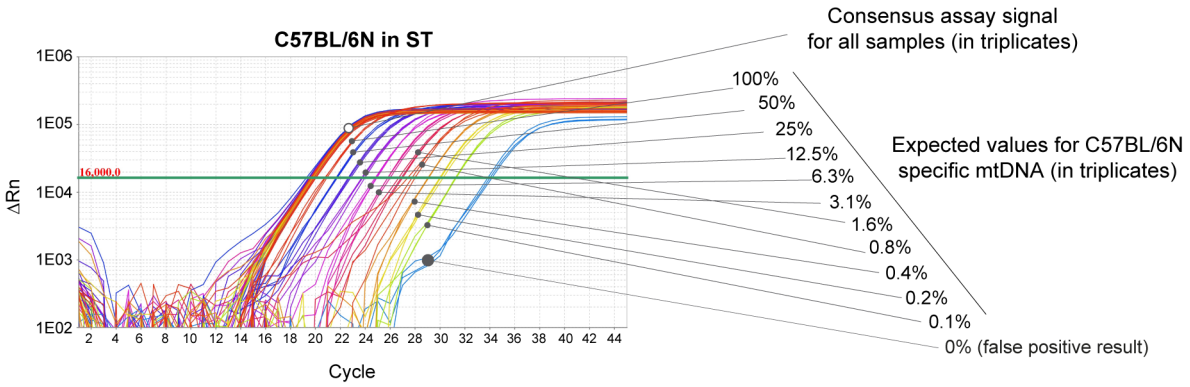


Supplementary Figure 5 | Discrimination between BG, HB wild-mouse mtDNA and C57BL/6N mtDNA. ARMS-qPCR amplification plots from triplicate samples with mtDNA mixtures of match and mismatch mtDNA. All samples were also subjected to consensus assay for total mtDNA detection. **a,** dilution series of BG or HB wild-derived mtDNA detected in a mixture containing also C57BL/6N mtDNA; **b,** dilution series of C57BL/6N mtDNA detected in a mixture containing also BG or HB wild-derived mtDNA. See also Supplementary Table 8 for respective result values (average of triplicates).

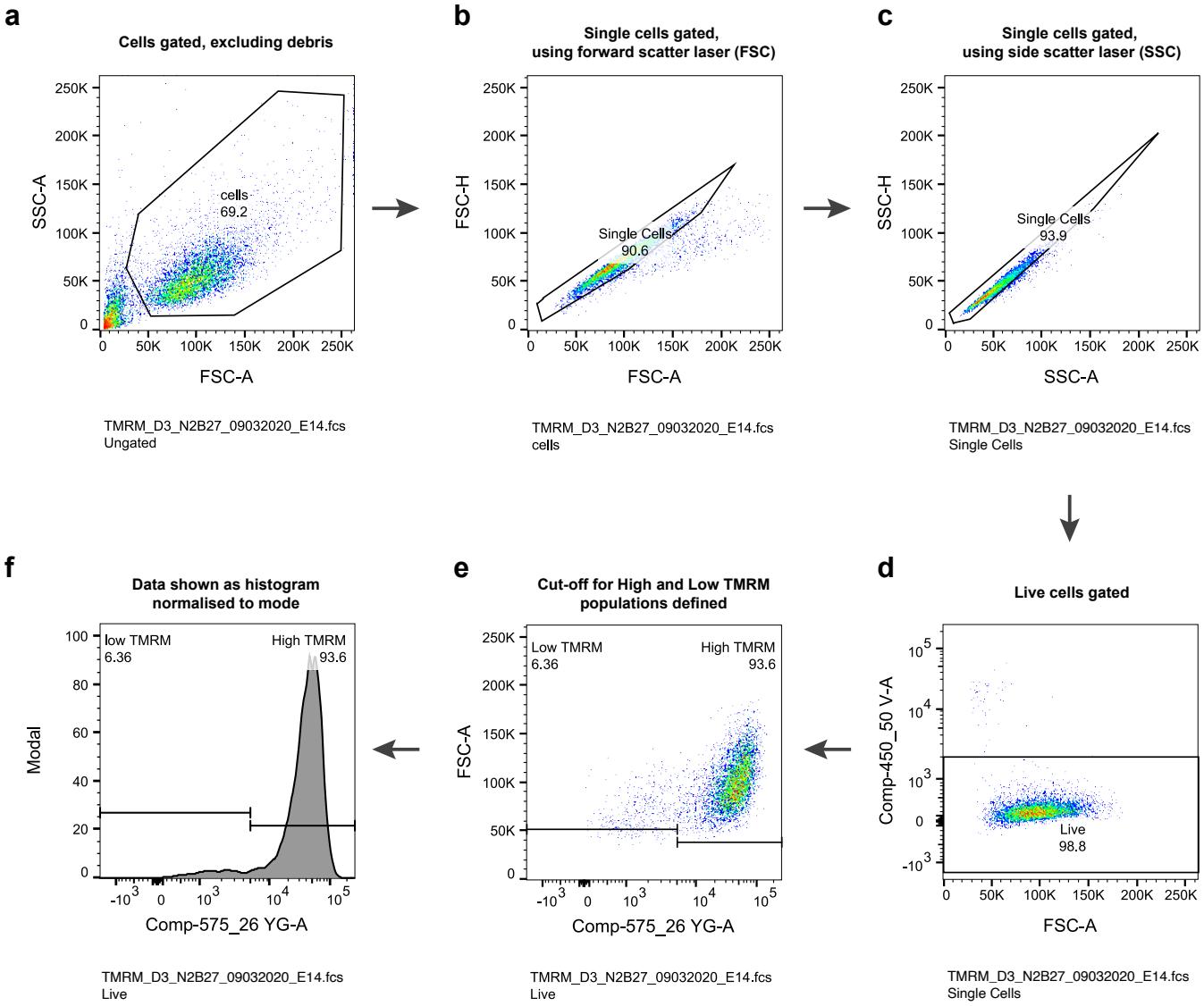
a



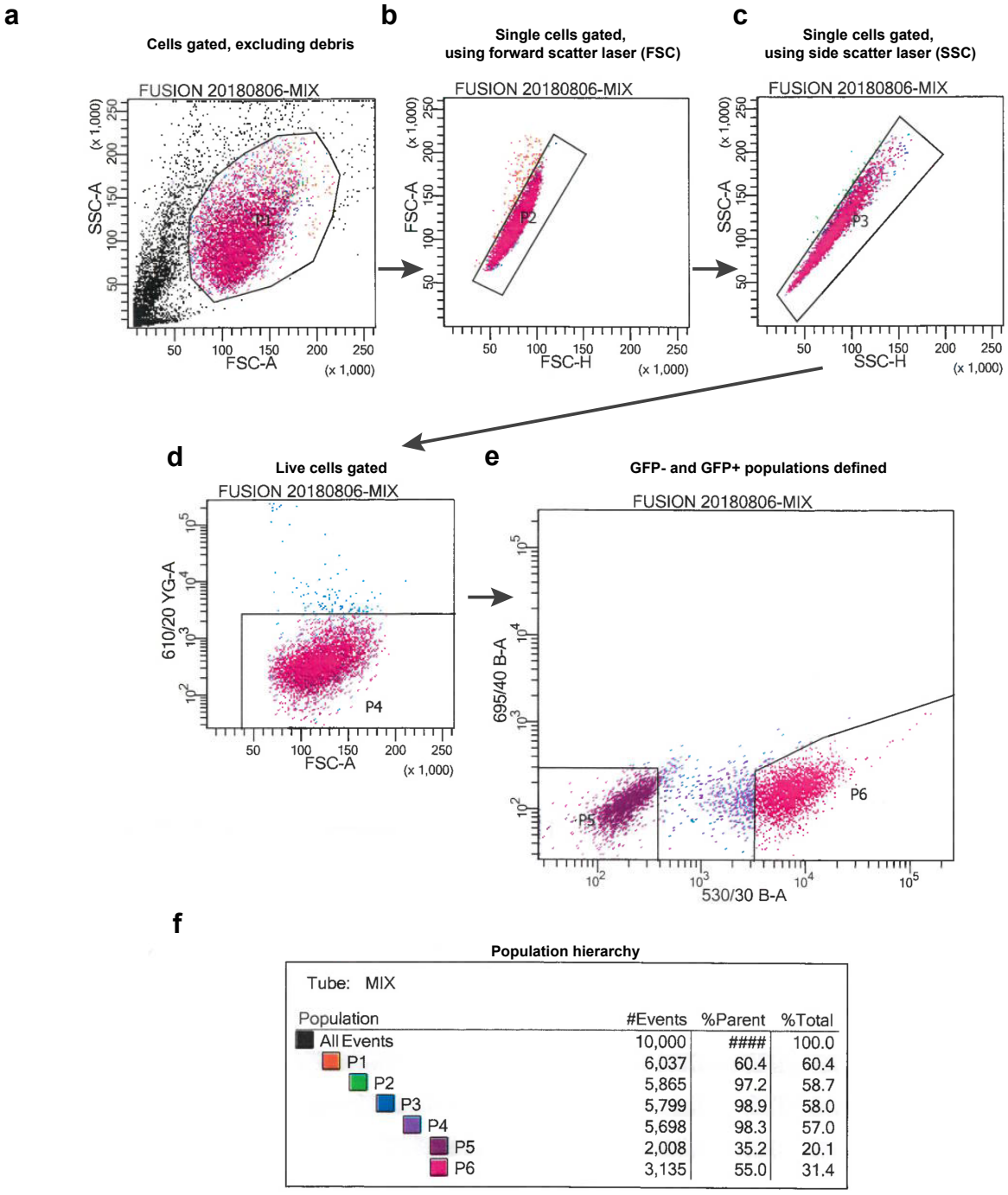
b



Supplementary Figure 6 | Discrimination between ST wild-mouse mtDNA and C57BL/6N mtDNA. ARMS-qPCR amplification plots from triplicate samples with mtDNA mixtures of match and mismatch mtDNA. All samples were also subjected to consensus assay for total mtDNA detection. **a,** dilution series of ST wild-derived mtDNA detected in a mixture containing also C57BL/6N mtDNA; **b,** dilution series of C57BL/6N mtDNA detected in a mixture containing also ST wild-derived mtDNA. See also Supplementary Table 9 for respective result values (average of triplicates).



Supplementary Figure 7 | FACS gating strategy adopted for experiments with mitochondrial dyes, here exemplified with TMRM. a, SSC-A vs FSC-A plot. **b-f**, Arrows indicate that the population defined was drilled down for subsequent gating/analysis, expect for the transition from (**e**) to (**f**), where only the chart changes from dot plot do histogram. Sytox blue (1:1000, S34857, ThermoFisher Scientific, UK). Relates to Figure 3g, Figure 4f,g,k, Figure 5d,f, Extended Data Figure 5e-g, Extended Data Figure 8b-d,f.



Supplementary Figure 8 | FACS gating strategy adopted for the cell sorting experiments. This approach was taken for all the FACS experiments preceding RNA isolation for bulk RNA-Seq of cells in competitive assay, according to the GFP label of one of the populations. **a-e**, Populations were drilled down as shown in the hierarchy table until live unlabeled (GFP-, P5) and GFP-labeled (GFP+, P6) cells were isolated (**e**). Propidium iodine (1:1000, 81845, Sigma, UK) were used as viability staining. Relates to Figure 7g-h and Extended Data Figure 8j.