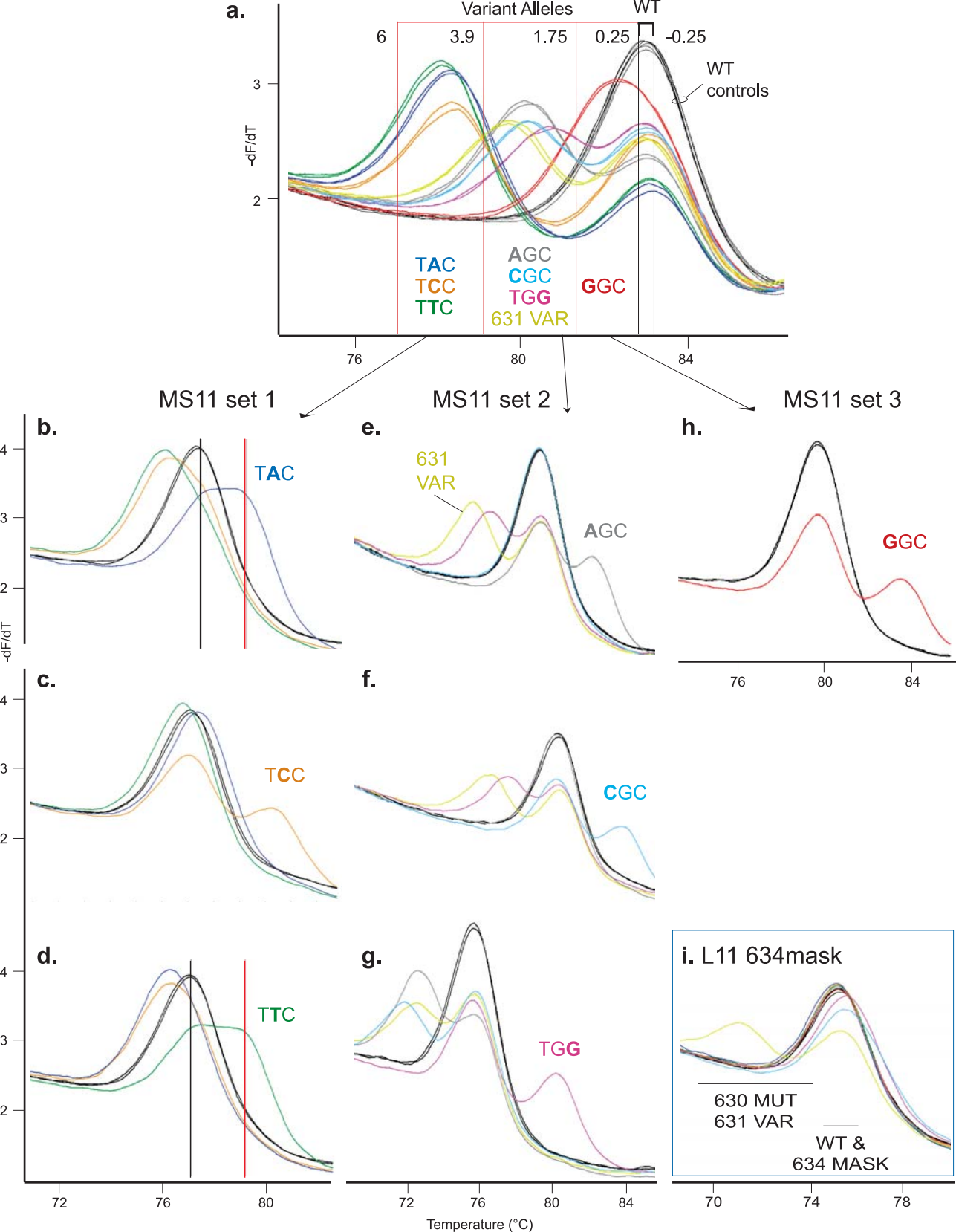


Data Supplement Figure 1. Genotyping mutations in *RET* exon 10 at codons 609 and 611.

a: Derivative melting curve plot for the primary WT10A probe data from Figure 1b. Three wild type controls in duplicate (WT, black traces) are shown with five heterozygous mutant samples in duplicate, two at codon 609 (thin traces) and three at codon 611 (thick traces). The colors used for mutant codon DNA sequences and traces are consistent throughout the figure. Graph traces for both the codon 609 and 611 mutation sequences are gray for **AGC**, light blue for **CGC**, red for **GGC**, dark blue for **TAC**, orange for **TCC**, green for **TTC**, and pink for **TGG**. The **AGC**, **GGC** and **TGG** mutations were not available for testing. The ΔT_m range values used to generate the T_m guidelines are listed next to each T_m guideline. Wild type allele T_m s are within the black T_m guidelines, $\pm 0.25^\circ\text{C}$ of control wild type sample. The mutant alleles detected by the primary probe were divided into two ΔT_m ranges by the red T_m guidelines. The mutation sequences that are predicted to be grouped into the each ΔT_m range are listed below the traces. The secondary assay data for the mutation-specific probe sets (MS10A set 1 or MS10A set 2) are displayed for each mutation group. **b-e:** The mutation group with ΔT_m values of 3.5 to 5°C was tested with the four probes in MS10A set 1: **(b)** MS 609/611 **TAC**, **(c)** MS 609/611 **TCC**, **(d)** MS 609/611 **TTC**, and **(e)** MS 609/611 **TGG**. **f-h:** The mutation group with ΔT_m values of 0.25 to 3.5°C was tested with the three probes in MS10A set 2: **(f)** MS 609/611 **AGC**, **(g)** MS 609/611 **CGC**, and **(h)** MS 609/611 **GGC**. On each mutation-specific probe graph (**b-h**), the codon mutation DNA sequence that complements the MS probe is listed. **i:** All mutant alleles (primary WT10A probe data, ΔT_m of 0.25 to 5°C) will also use the location probe, L10A 611mask, to locate codon position of the detected mutations. Two derivative melting temperature ranges are indicated, with the codon 609 mutant alleles (609 MUT) labeled as well as the wild type allele (WT) and masked codon 611 mutant alleles (611 MASK).



Data supplement Figure 2. Genotyping *RET* exon 11 mutations.

a: Derivative melting curve plot for the primary WT11 probe data. Three wild type controls in duplicate (WT, black traces) are shown with seven heterozygous codon 634 mutant samples in duplicate, as well as the heterozygous sequence variant at codon 631(GAC→GAT) (631 VAR, yellow traces). Codon 630 mutation samples were not available. The colors used for mutant codon DNA sequences and traces are consistent throughout the figure. Graph traces for the codon 634 mutations are gray for AGC, light blue CGC, red for GGC, dark blue for TAC, orange for TCC, green for TTC and pink for TGG. The ΔT_m range values used to generate the T_m guidelines are listed next to each T_m guideline. Wild type allele T_m s are within the black T_m guidelines, $\pm 0.25^\circ\text{C}$ of control wild type sample. Variant alleles detected by the primary probe were divided into three ΔT_m ranges by the red T_m guidelines. The sequence variations that are predicted to be grouped into the each ΔT_m range are listed below the traces. The secondary assay data for the mutation-specific probe sets (MS11 set 1, 2, or 3) are displayed for each of the three mutation groups. **b-d:** The mutation group with ΔT_m values of 3.9 to 6°C was tested with the three probes in MS11 set 1: **(b)** MS 630/634 TAC, **(c)** MS 630/634 TCC, and **(d)** MS 630/634 TTC. The cursors in part **b** and **d** show the T_m estimates for the mutant and wild type alleles, due to poorly differentiated peaks. **e-g:** The mutation group with ΔT_m values of 1.75 to 3.9°C was tested with the three probes in MS11 set 2: **(e)** MS 630/634 AGC, **(f)** MS 630/634 CGC, and **(g)** MS 630/634 TGG. The codon 631 sequence variant is in this ΔT_m group and was also tested with MS11 set 2. **h:** The mutation group with ΔT_m values of 0.25 to 1.75°C was tested with the one probe in MS11 set 3: MS 630/634 GGC. On each mutation-specific probe graph, the codon mutation DNA sequence that complements the MS probe is listed. **i:** All variant alleles (primary WT11 probe data, ΔT_m 0.25 to 6°C) will also use the location probe, L11 634mask, to locate

codon position of the detected mutations. Two derivative melting temperature ranges are indicated, with the expected alleles at each T_m range listed. The 631 sequence variant allele (631 VAR) and the 630 mutant alleles (630 MUT) are labeled, as well as the wild type allele (WT) and masked 634 mutant alleles (634 MASK).