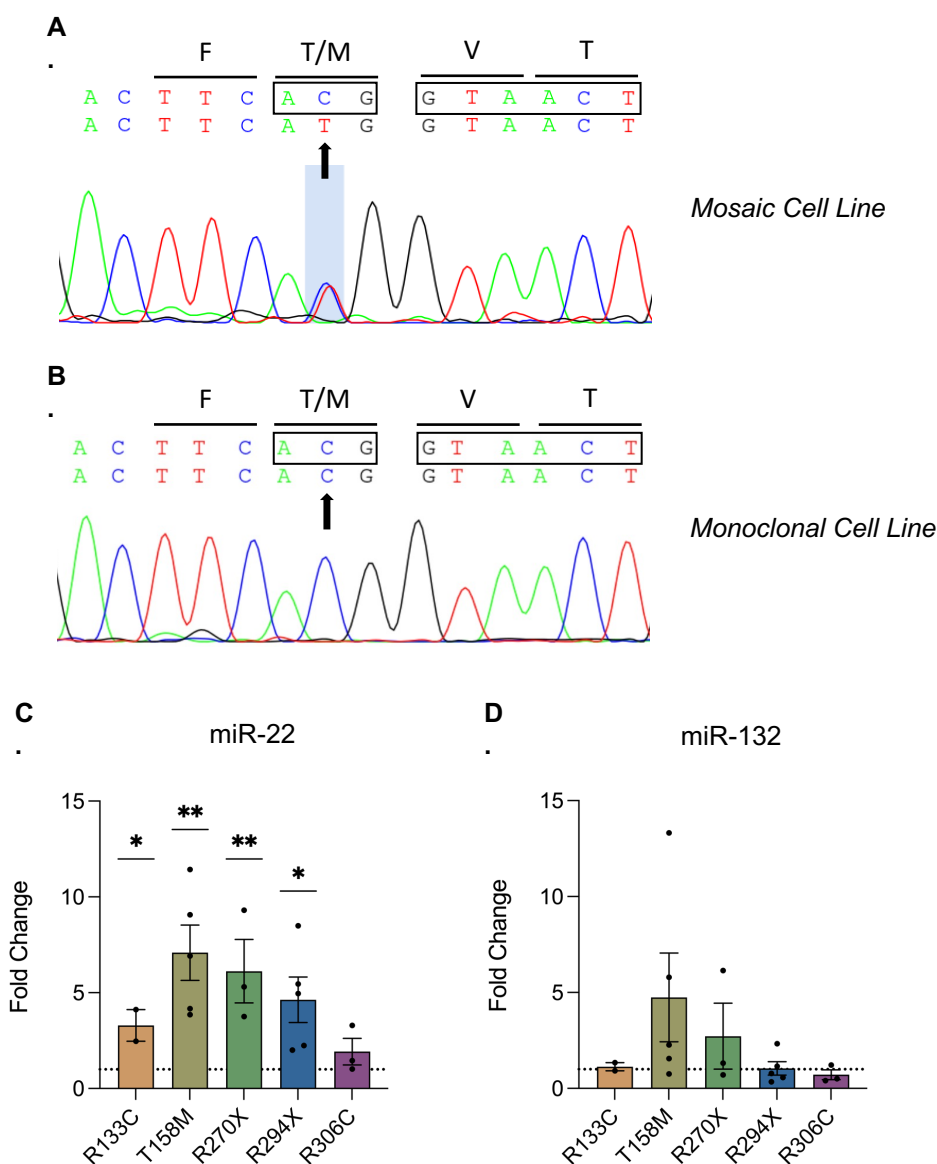




Supplemental Figure 5. Sequencing and miRNA Analysis of T158M Fibroblast Cell Lines. Sequencing chromatograms of RT-PCR products from T158M fibroblast cell lines. The analysis confirms the successful isolation of a monoclonal cell culture from a mosaic cell culture for use as a control. **(A)** The mosaic cell line exhibits both the mutated codon 158 (ACG to ATG, threonine to methionine) and the non-mutated ACG codon. **(B)** The monoclonal T158M cell line shows only the non-mutated ACG codon, verifying the absence of the T158M mutation. **(C-D)** Quantification of miRNA levels in total miRNA samples isolated from R133C, T158M, R270X, R294X, and R306C mutant fibroblasts, compared to isogenic wild-type (WT) controls from heterozygous T158M fibroblasts. **(C)** miR-22 levels did not show significant changes across the samples, although an upward trend was noted in T158M and R270X mutants. **(D)** miR-132 levels were significantly increased in all mutations except R306C, with p-values of 0.0155, 0.0033, 0.0067, and 0.0178 for R133C, T158M, R270X, and R294X, respectively. Notably, miR-132 was highly upregulated in the T158M line (7.09-fold increase). Data represent mean $\pm$ SEM from $n = 3-5$ mutant fibroblast lines per condition. Statistical significance was determined using Student's t-tests, with significance levels denoted as follows: * $p < 0.05$, ** $p < 0.01$.