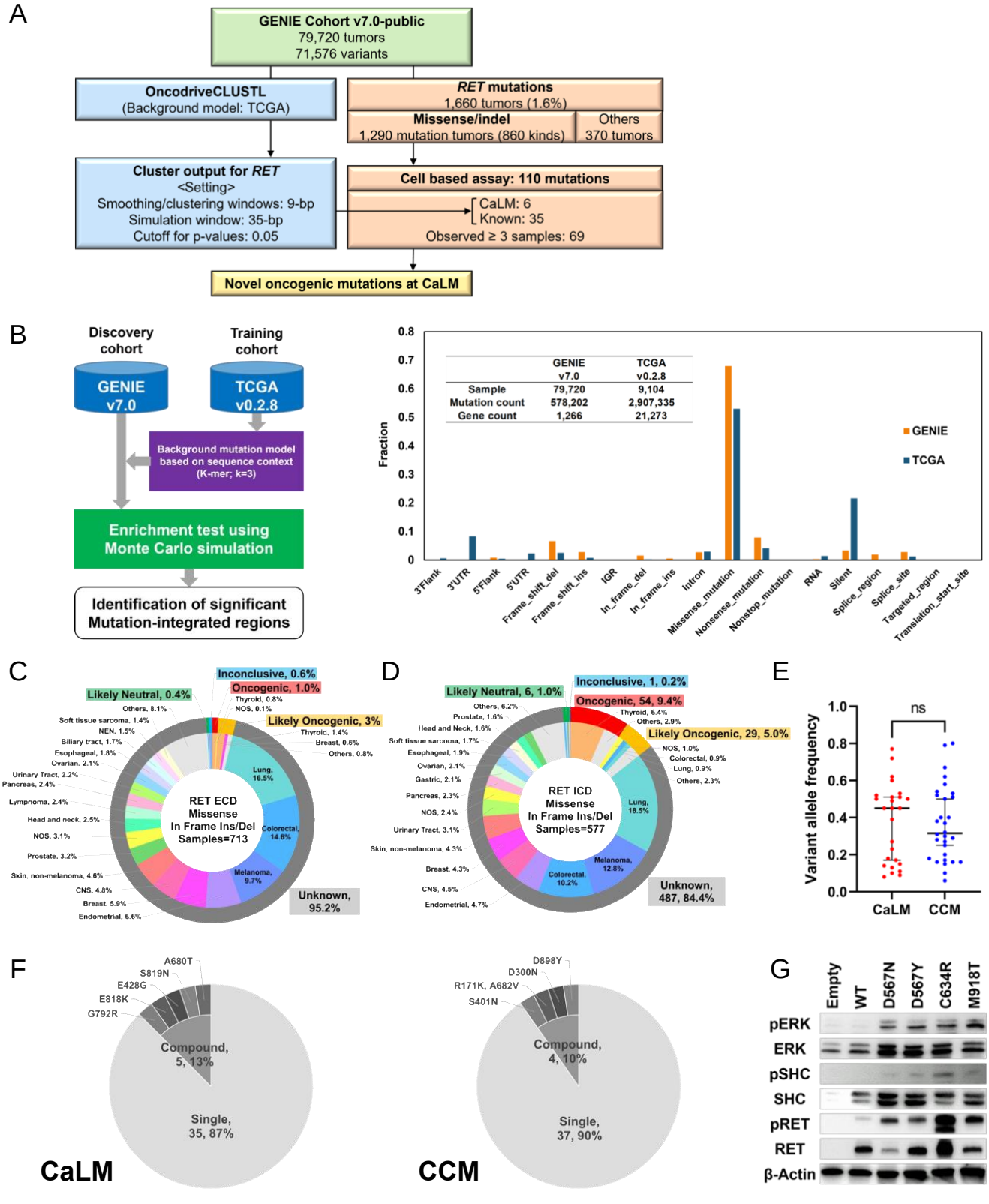
Supplementary Figure 1



Supplementary Figure S1. Identification of CaLM mutations among *RET* VUSs

A, *RET* mutant selection. The discovery cohort consisted of mutational data from 71,756 variants from 79,720 tumors, excluding gross alterations such as fusion and copy number alterations, which were subjected to OncodriveCLUSTL analysis. The modeling results of the *RET* gene were the output. Among 860 types of missense/in-frame indel *RET* mutations detected in the GENIE samples, 110 *RET* mutants selected using the following three criteria were subjected to gene-wide cell-based assays: i) mutations occurring in CaLM: six, ii) known mutations annotated as oncogenic in OncoKB or previously reported: 35 and OncoKB (5,6,9,10,12,13), and iii) mutants recurrently observed in three or more samples in the GENIE version 7.0 cohort: 69.

B, OncodriveCLUSTL analysis. A total of 1,290 *RET* variants obtained from GENIE cohorts were subjected to a trinucleotide enrichment test using a background mutation rate model based on a mutational trinucleotide context generated from TCGA (v0.2.8) samples (left). The TCGA dataset was used as the background because it contains similar fractions of diverse types of mutations to those of the GENIE dataset (version 7.0) (right). The table in the graph shows the number of samples, mutation counts, and gene counts of the two datasets.

C-D, *RET* mutations in the extracellular domain (ECD) and intracellular domain (ICD). Pie charts show the proportion of cancer types and OncoKB annotations (22) for the ECD (**B**) and ICD (**C**) mutations. The numbers in the pie charts refer to the total number of mutations in the ECD (**B**) and ICD (**C**).

E, The scatter plot shows the distributions of the variant allele frequencies (VAF) between the CaLM and CCM mutants from the samples obtained from the GENIE dataset. Thick and thin lines indicate each group's median and 95% confidence intervals, respectively. n.s.: not significant by two-tailed paired and unpaired t tests.

F, Single and compound mutations in the CaLM and CCM segments. Both are mostly detected as a single mutation in each tumor.

G, Biological significance of CaLM mutants. HEK293H cells transfected with empty vector, WT RET, and D567N, D567Y, C634R and M918T RET mutants were subjected to immunoblot analysis of pERK, ERK, pSHC, SHC, pRET, RET, and β-actin.