

Fig S1

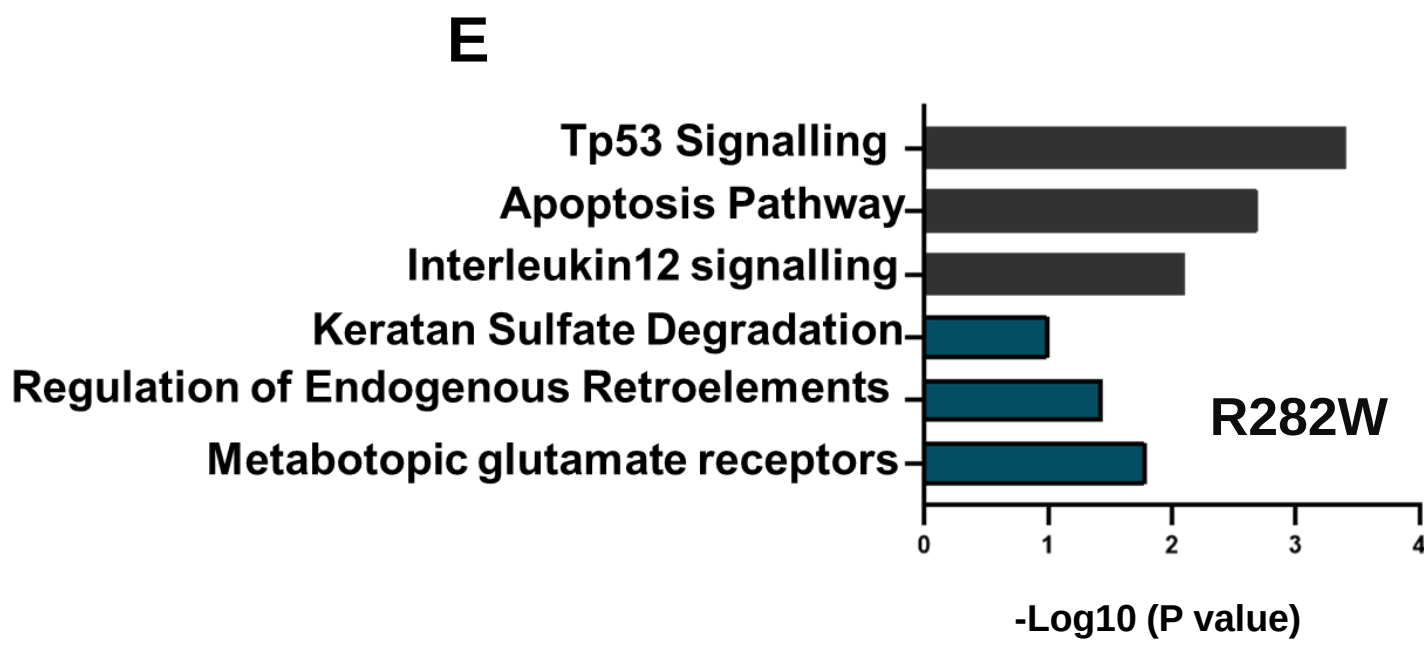
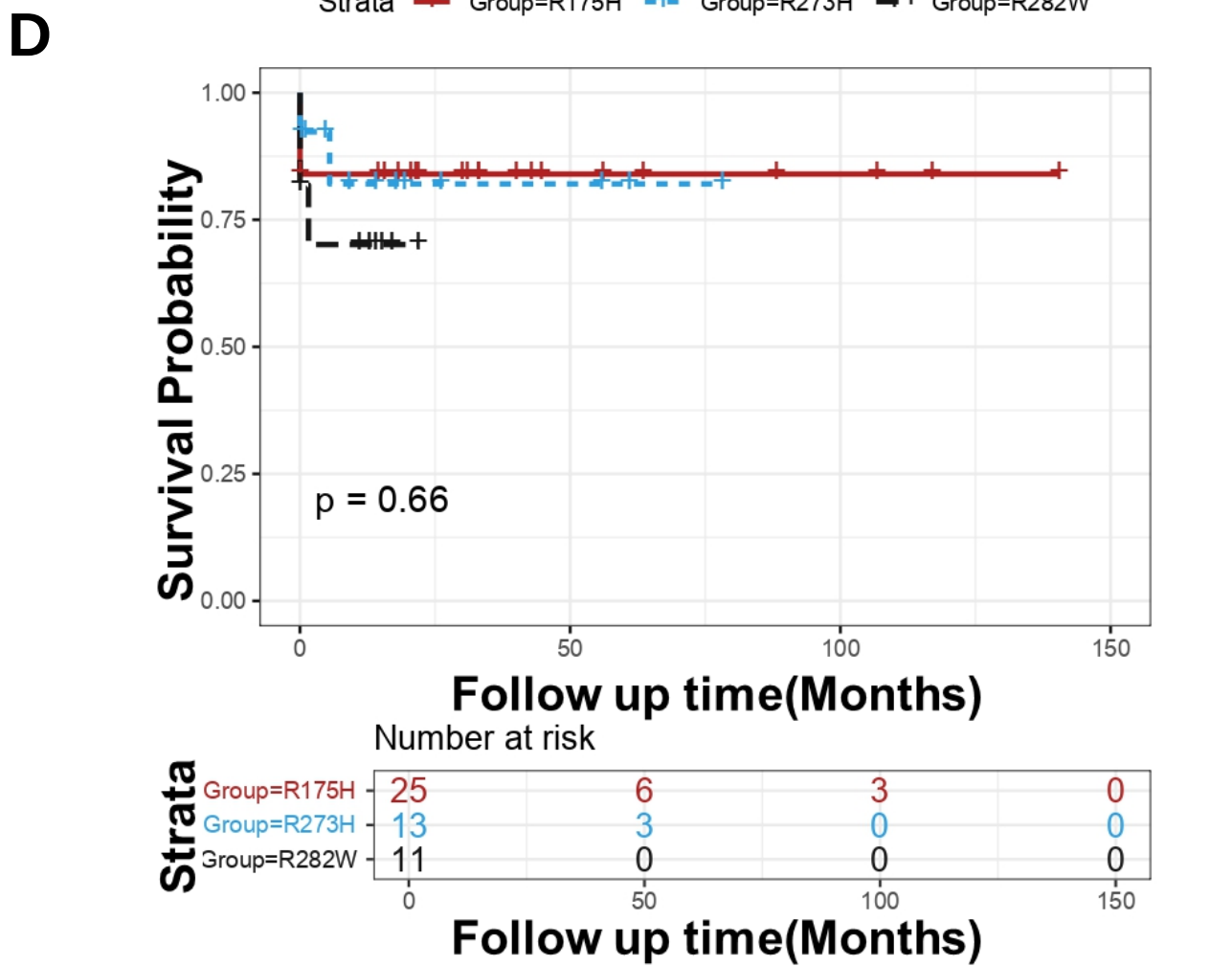
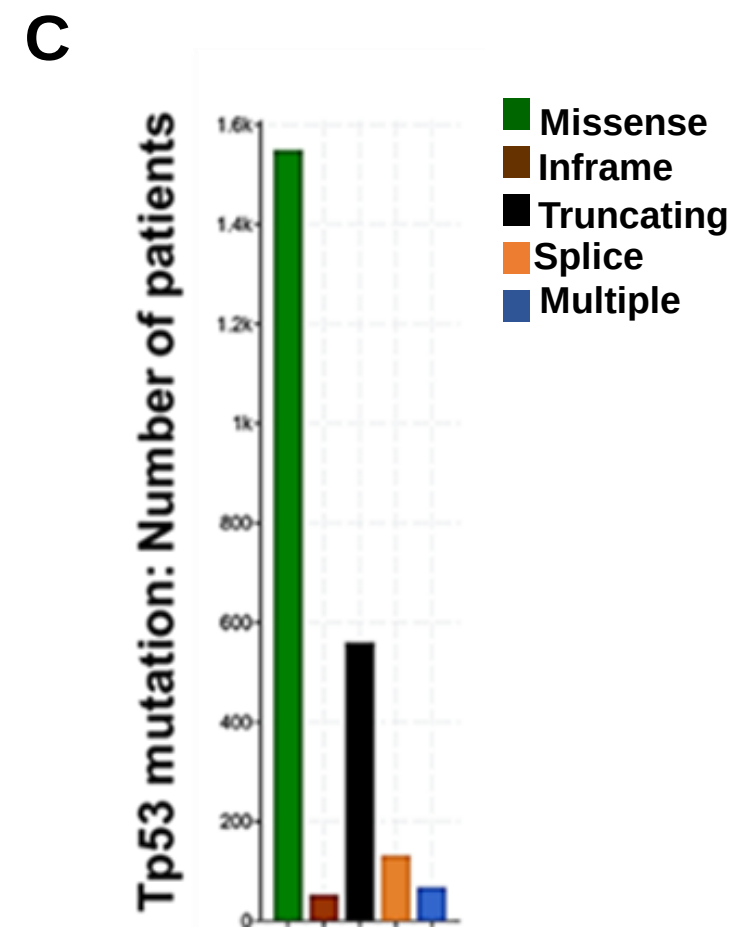
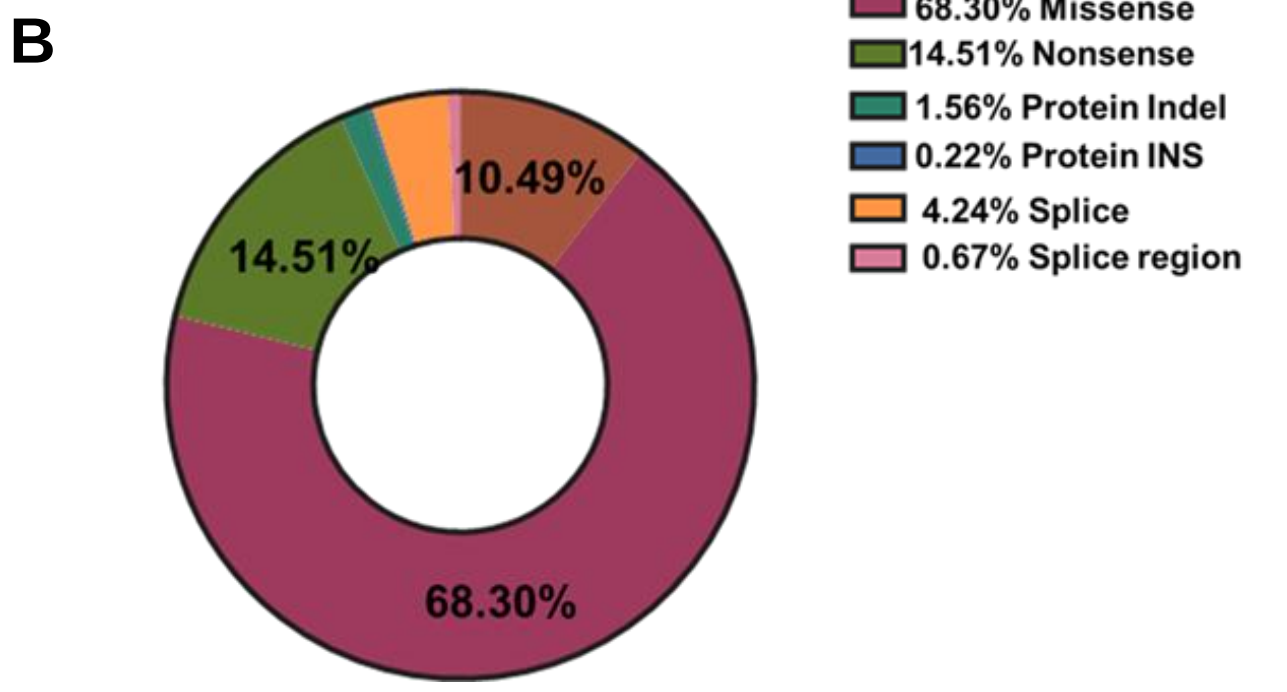
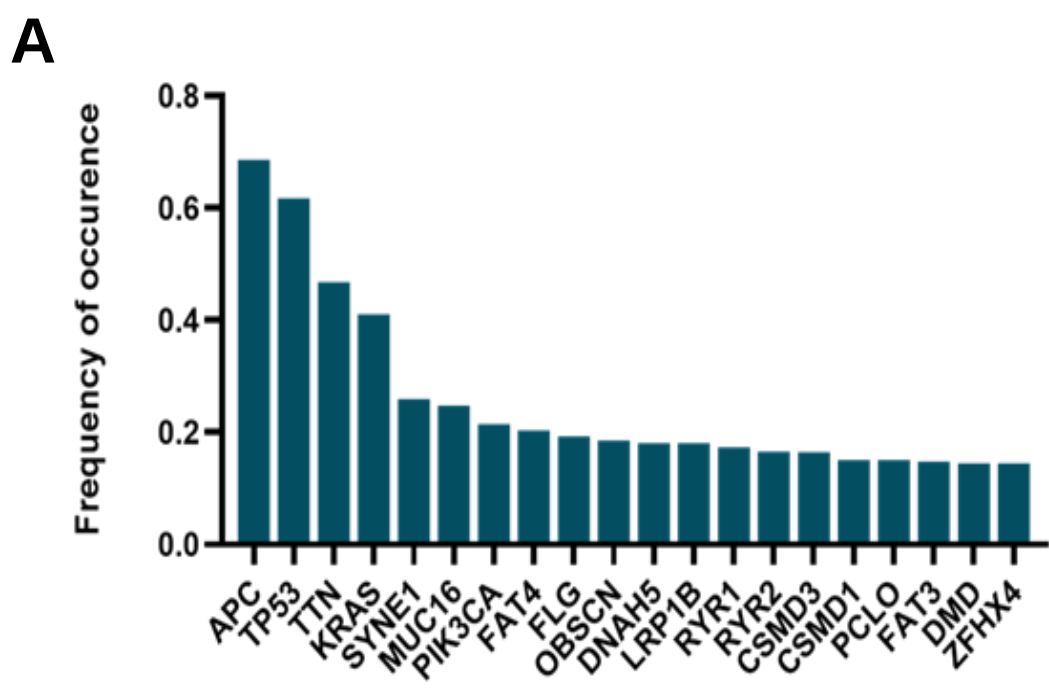


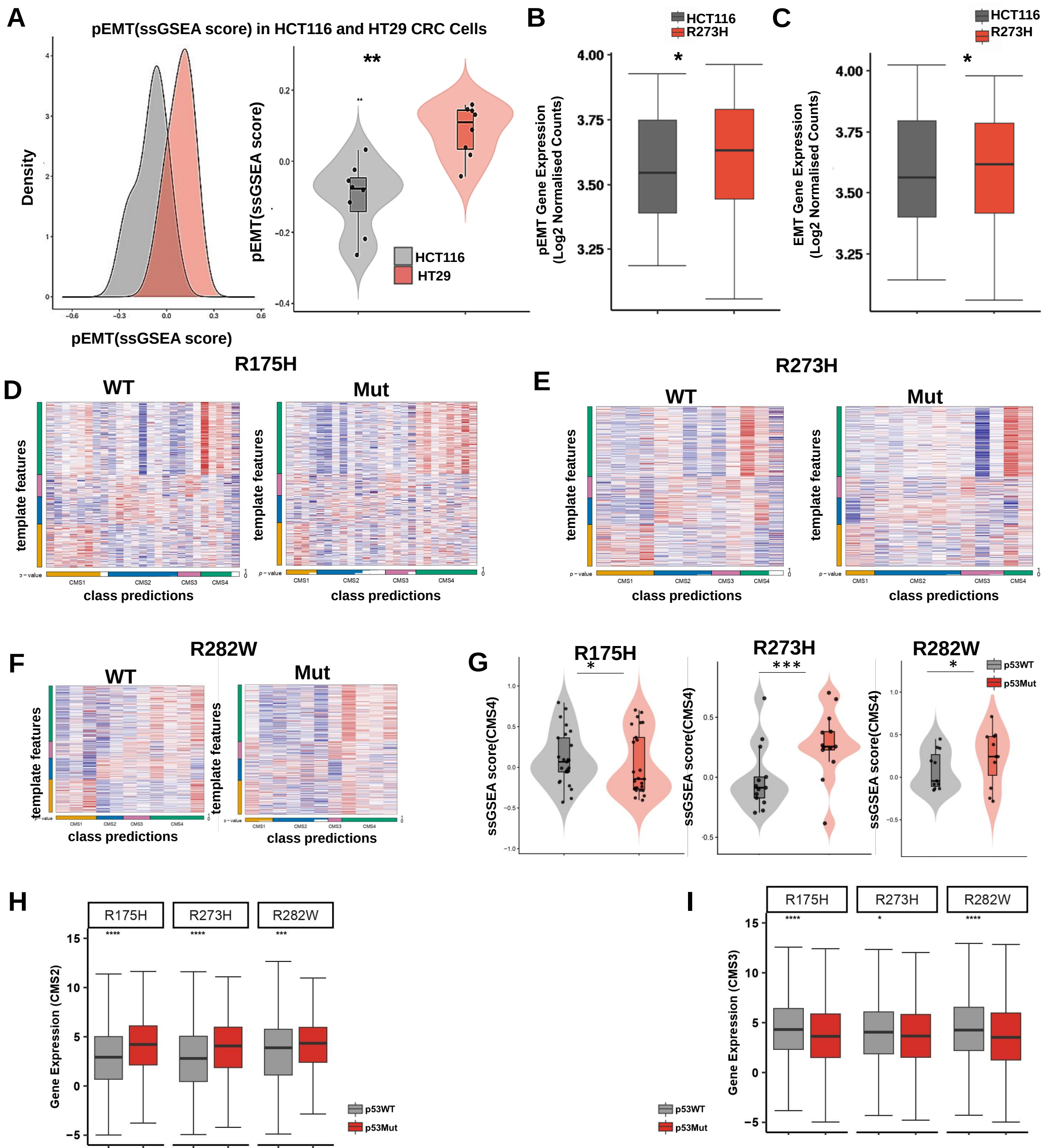
Fig S2

Fig S3

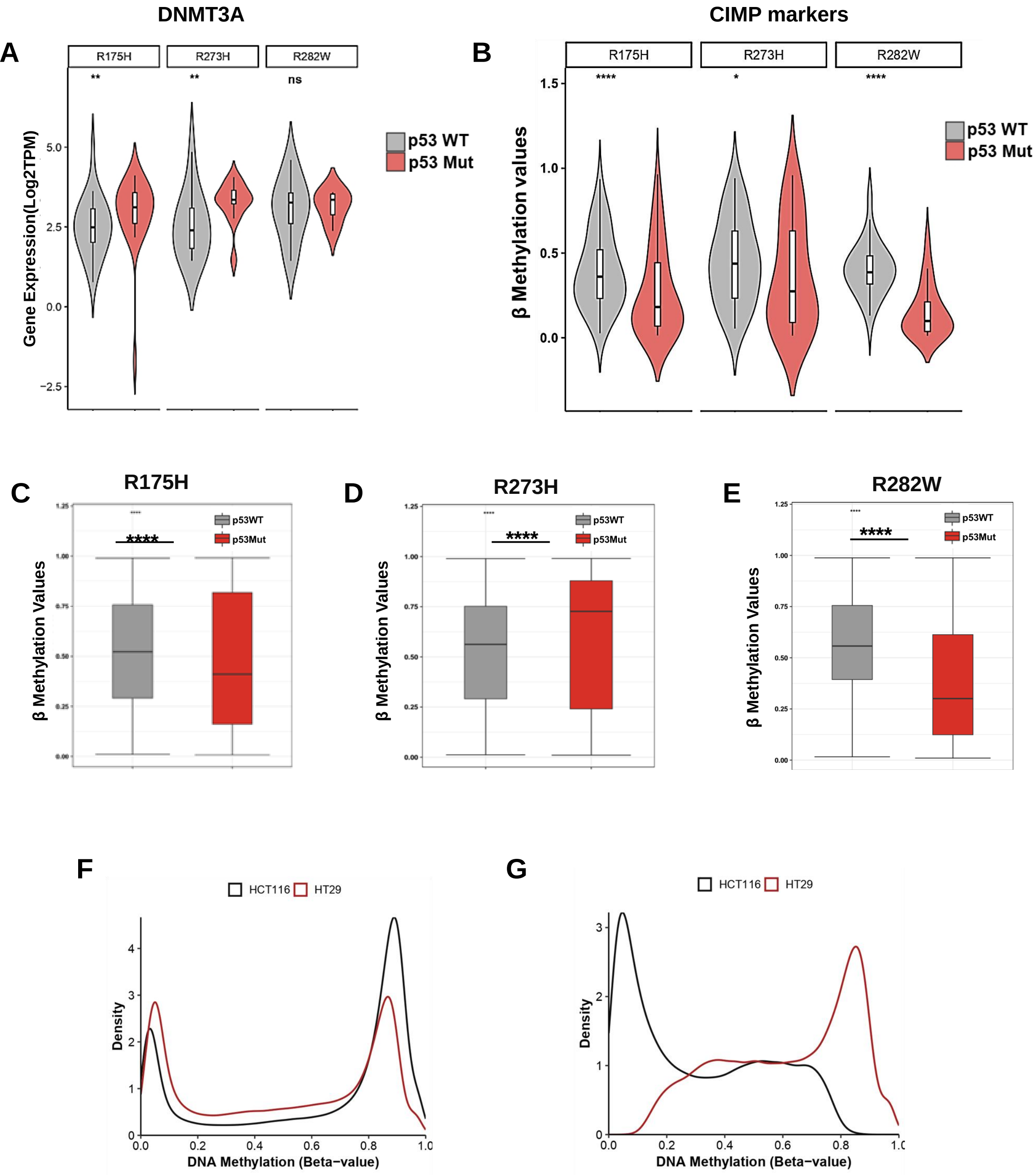


Fig S4

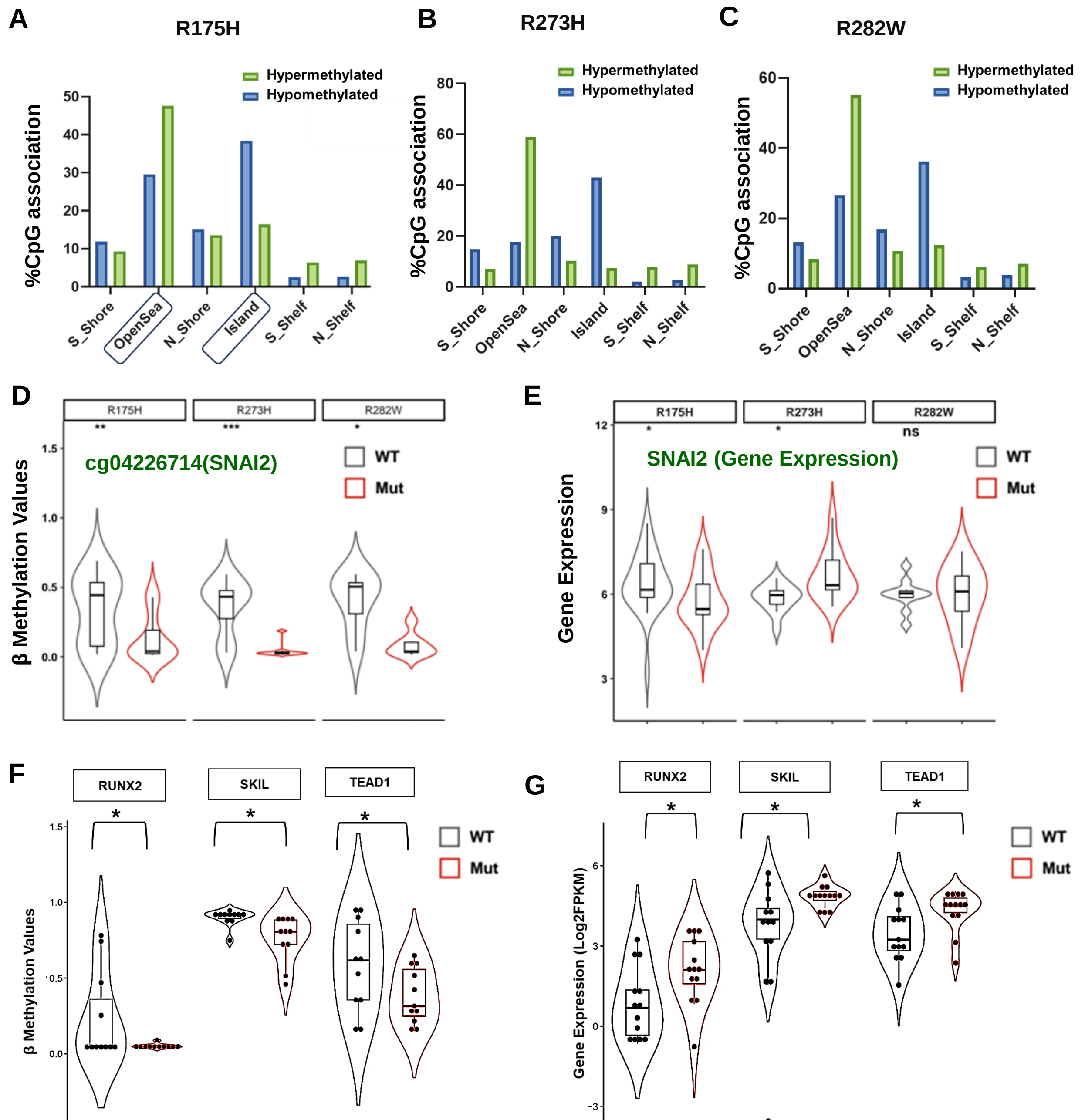
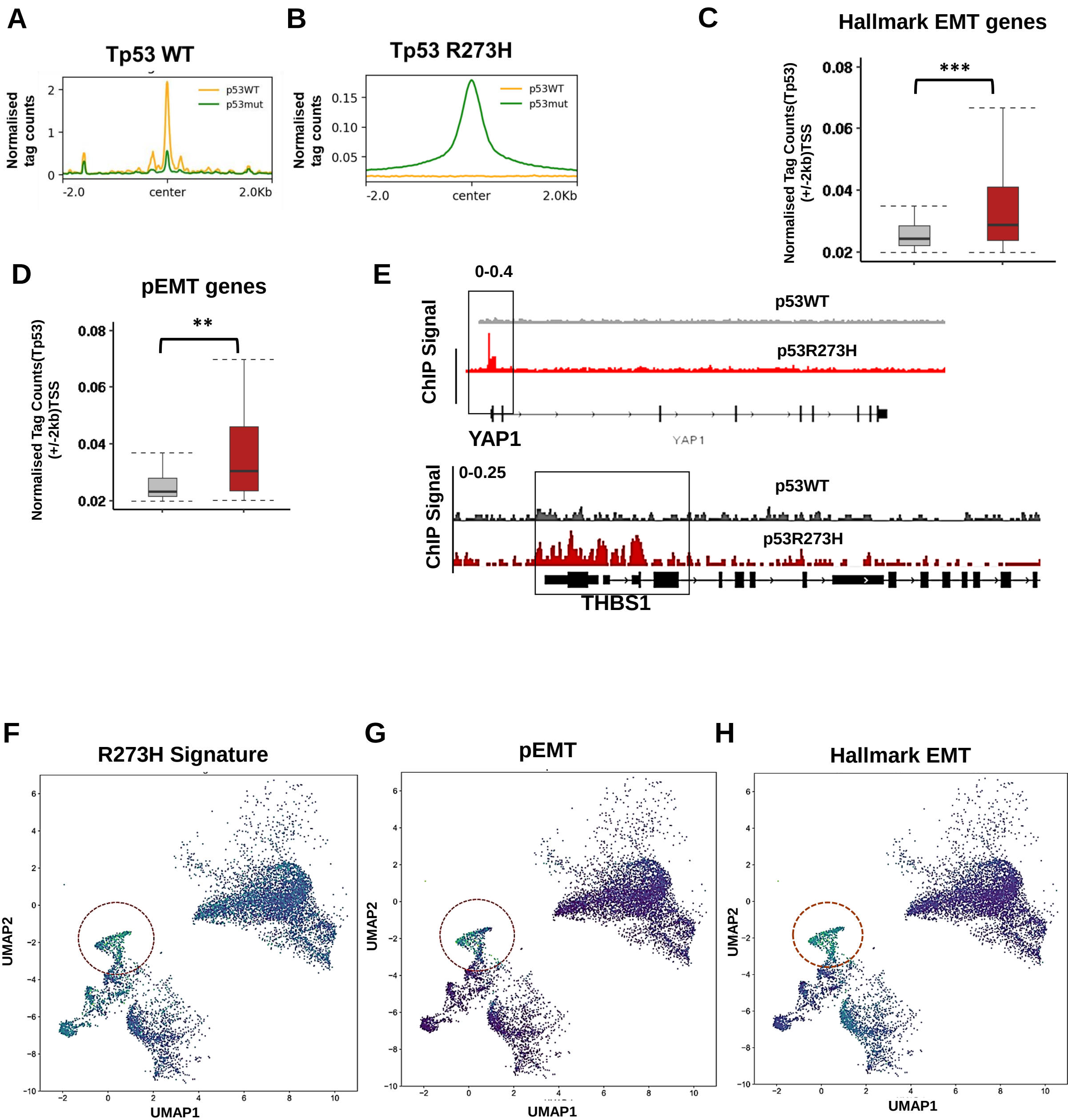


Fig S5



Supplementary Figure Legends

Fig S1: Stratification of colon tumours based on p53 mutational status

- A. Barplot representing the status of the top 20 mutated genes in colon tumours. Tp53 is the second most mutated gene after APC.
- B. Pie Chart representing prevalence of different Tp53 mutation in colon tumours as observed through TCGA-COAD. Missense mutations are the most prevalent mutations in Tp53.
- C. Barplot representing the frequency of occurrence of different types of mutations in Tp53. Missense mutations are the most prevalent mutation followed by truncating mutation
- D. Survival probabilities of three different p53 mutation in colon cancer. Kaplan Meier (KM) plot representing the survival profiles of top 3 prevalent Tp53 missense mutations. R273H tumours shows lower survivability as compared to R282W and R175H tumours. The X axis represents the survival time in months while the Y axis represents the survival probability
- E. Column chart representing Gene Set Enrichment Analysis (GSEA) of the differentially expressed genes in p53R282W tumours (blue) and p53 WT (grey) (through reactome). R282W tumours show positive enrichment of metabotropic glutamate receptors.

Fig S2: Different p53 missense mutations show varied proportion of CMS subtypes based on associated gene expression

- A. Higher partial EMT signature observed in HT29 CRC cells(red) as compared to HCT116(grey). Kernel density plots estimate of pEMT score in HCT116 and HT 29 CRC cells(left) and box and violin plot representing enrichment of pEMT score in HT29 CRC cells as compared to HCT116. (t-test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)
- B. Box plot representing the distribution of pEMT signature genes in HCT116 (grey) and HCT116 p53 KO overexpressing R273H (red). Higher expression of pEMT signature genes observed in HCT116 p53 KO overexpressing R273H. (t-test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)
- C. Box plot representing the distribution of Hallmark EMT signature genes in HCT116 (grey) and HCT116 p53 KO overexpressing R273H (red). Higher expression of Hallmark EMT signature genes observed in HCT116 p53 KO overexpressing R273H. (t-test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)
- D. The consensus molecular subtypes (CMS) classification of CRC samples using CMScaller in p53 WT (left panel) and R175H (right panel). p53R175H associates with higher CMS4 proportion
- E. The consensus molecular subtypes (CMS) classification of CRC samples using CMScaller in p53 WT (left panel) and R273H (right panel). p53R273H associates with higher CMS2 proportion
- F. The consensus molecular subtypes (CMS) classification of CRC samples using CMScaller in p53 WT (left panel) and R282W (right panel). p53R282W associates with decrease in CMS1 proportion
- G. Box and Violin plot representing ssGSEA score of CMS4 gene signatures in p53 WT (black) and p53 mutation (red) across all three mutational cohorts. R273H mutation shows significant

enrichment of CMS4 gene signatures. (t-test was performed with p value > 0.05, ns; p value ≤ 0.05, *; p value ≤ 0.01, **; p value ≤ 0.001, ***)

H. Box plot representing the distribution of CMS2 gene expression in p53WT (grey) and p53mut(red). All three different p53 mutant tumours associate with significant increase in expression of CMS2 genes. (t-test was performed with p value > 0.05, ns; p value ≤ 0.05, *; p value ≤ 0.01, **; p value ≤ 0.001, ***)

I. Box plot representing the distribution of CMS3 gene expression in p53WT (grey) and p53mut(red). All three different p53 mutant tumours associate with significant decrease in expression of CMS3 genes. (t-test was performed with p value > 0.05, ns; p value ≤ 0.05, *; p value ≤ 0.01, **; p value ≤ 0.001, ***)

Fig S3: Distinct methylation profiles were observed around diverse genomic regions of CRC patients with p53 mutations

A. Box and violin plots representing the expression profile of the DNA methyl transferase DNMT3A in p53 WT (grey) and p53 mutant(red) tumours. R175H and R273H show significant higher expression of DNMT3A but not R282W tumors as compared to p53WT tumours. (t-test was performed for the statistical analysis, p value > 0.05, ns; p value ≤ 0.05, *; p value ≤ 0.01, **; p value ≤ 0.001, ***)

B. Box and violin plot representing methylation values of CIMP markers in p53 WT (grey) and p53 mutant(red) tumours. All p53 mutant tumours associate with lower methylation values as compared to p53 WT tumours. (t-test was performed for the statistical analysis, p value > 0.05, ns; p value ≤ 0.05, *; p value ≤ 0.01, **; p value ≤ 0.001, ***)

C-E. Box and density plots representing distribution of methylation values (β values) of significant probes in Tp53 WT (grey) and Tp53 mut tumours (red) randomly chosen population of 5 CRC tumours in each cohort. C. Tumours with R175H mutation show significantly lower methylation values as compared to p53 WT tumours. D. Tumours with R273H mutation show significantly higher methylation values as compared to p53 WT tumours. E. Tumours with R282W mutation shows lower methylation values as compared to p53 WT tumours. (Wilcoxon-rank was performed with p value > 0.05, ns; p value ≤ 0.05, *; p value ≤ 0.01, **; p value ≤ 0.001, ***)

F. Density plot depicting overall distribution of beta methylation values in HCT116 (grey) and HT 29(red) CRC cells.

G. Density plot depicting distribution of hypermethylated regions of R273H CRC tumours in HCT116 (grey) and HT29 (red)CRC cells.

Fig S4: Hypomethylated YAP/TAZ genes and EMT-TFs correlate with increased gene expression profiles in patients with R273H mutation

A. Column bar chart representing distribution of hypermethylated(green) and hypomethylated (blue) genomic regions with R175H mutation. Open sea regions correspond to hypermethylation profile, while CpG islands correspond to hypomethylation.

B. Column bar chart representing distribution of hypermethylated(green) and hypomethylated (blue) genomic regions with R273H mutation. Open sea regions correspond to hypermethylation profile, while CpG islands correspond to hypomethylation.

C. Column bar chart representing distribution of hypermethylated(green) and hypomethylated (blue) genomic regions with R273H mutation. Open sea regions correspond to hypermethylation profile, while CpG islands correspond to hypomethylation.

D. Box and Violin plot representing the distribution of methylation levels of cg04226714 (SNAI2) probes across all three mutation cohorts (p53 WT (black) and p53 mutants (red)). R273H mutation shows hypomethylation at SNAI2 promoters. (Wilcoxon-rank test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)

E. Box and violin plot representing the distribution of SNAI2 expression profile across all three mutation cohorts (p53 WT (black) and p53 mutants (red) tumours). R273H tumours show significantly higher expression of SNAI2. (t-test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)

F. Box and violin plot representing the methylation levels of probes associated with RUNX2, SKIL and TEAD1 in p53WT tumours (black) and p53R273H tumours (red). R273H tumours associates with lower levels of methylation of probes associated with RUNX2, SKIL and TEAD1 as compared to p53 WT tumours. (Wilcoxon-rank test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)

G. Box and violin plot representing the expression profile of genes involved in YAP/TAZ signalling including RUNX2 and TEAD1 in p53WT tumours (black) and p53R273H tumours (red). R273H tumours shows significant upregulation of RUNX2, SKIL and TEAD1 as compared to p53WT tumours. (t-test was performed with p value> 0.05, ns; p value<=0.05, *; p value<=0.01, **; p value<=0.001, ***)

Fig S5: Mutant p53 R273H enriches at partial/mesenchymal EMT states in colorectal cancer

A. Plot Profile representing p53WT signal (yellow) and p53 R273H signal (green) at WT enriched regions (centered at +/-2kb peaks). WTp53 enriched regions correlate with higher WTp53 signal

B. Plot Profile representing p53WT signal (yellow) and p53 R273H signal (green) at R273H enriched regions (centered at +/-2kb peaks). R273H enriched regions correlate with higher R273H signal

C. Box plot representing normalized tag counts of WTp53(grey) and p53 R273H(red) around the TSS of hallmark EMT genes (+/-2kb TSS). R273H shows significantly higher enrichment at the promoters of hallmark EMT gene sets.

D. Box plot representing normalized tag counts of WTp53(grey) and p53 R273H (red) around the pEMT genes (+/-2kb TSS). R273H shows significantly higher enrichment at the promoters of pEMT gene sets.

E. IGV screenshot representing higher enrichment of p53R273H (red) at the promoters of YAP1 and THBS1(pEMT gene) as compared to p53WT (grey).

F-H. Signature enrichment visualized across tumour clusters: UMAP projections coloured by percell enrichment scores for (F) R273H transcriptional signature, (G) partial EMT (pEMT), and (H) Hallmark EMT. Colour scale (viridis): green = high enrichment, blue = low enrichment. The red dotted circle highlights a representative cluster (e.g., Tu1) where R273H enrichment co-occurs with elevated partial and full EMT programs, indicating mutant p53-associated phenotypic reprogramming.