

# Frequent BRCA2 Somatic Mutations in Colorectal Cancer Patients with Microsatellite Instability (MSI)

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## Abstract

Microsatellite instability (MSI) is a hallmark of mismatch repair (MMR)-deficient cancers including colorectal cancer (CRC). MSI represents 15% of CRCs as a result of either epigenetic silencing of MLH1 or mutations in one of the MMR genes: MLH1, MSH2, MSH6 and PMS2. MSI tumors have a better prognosis than microsatellite stable (MSS) tumors while responding differently to treatments, including relative resistance to 5-FU and high clinical benefit from immune checkpoint therapy. Deficient MMR can lead to deficient DNA double-strand-break repair. We recently reported on 26 MSI-High and 558 non-MSI-High CRCs profiled by Caris Life Sciences. MSI-High CRCs had a high mutation rate (50%) in BRCA2 (Shagisultanova et al., 2015 ASCO Annual Meeting Abstract No. e14684 "Association of increase in BRCA2 gene mutations in microsatellite instable (MSI-H) colorectal cancer (CRC) with increased c-MET expression"). We hypothesized there might be a pattern with specific BRCA2 mutations in MSI-H CRCs targeting the coding microsatellites in BRCA2. BRCA2 mutations could be potential targets in clinical therapy. We further investigated functional mutation patterns in BRCA2 in both MSI-H and MSS groups. Of 1104 profiled CRCs in the COSMIC v73 database, somatic BRCA2 mutations were mapped for 101 MSI-High versus 916 MSS CRCs. MSI-High CRCs showed a significantly higher mutation rate in BRCA2 as compared to MSS (38% vs 6%,  $P < 0.0000001$ ). A higher rate of damaging mutations (42% vs. 2%,  $P < 0.0001$ ) and a relatively distinct pattern of protein mutation distribution could be clearly mapped in MSI-High CRCs versus the MSS group. We found that specific mutations in coding microsatellites of BRCA2 in MSI-H CRCs not previously seen in either breast cancer or pancreatic cancer as reported in COSMIC v73. However, using the BIC database (<http://research.nhgri.nih.gov/bic/>) we detected 5 BRCA2 deleterious mutations that have been reported as germline mutations in breast cancer. We used the consensus result from five predictors and available 3-D structural information to predict deleterious properties of mutations including damaging BRCA2 protein structure and disruption of interactions with partner proteins including DSS1 and RAD51. Targeting BRCA2 mutations in MSI tumors and using the concept of synthetic lethality might be effective in BRCA-deficient CRCs with MSI.

## Frequent BRCA2 mutations in MSI-H CRCs

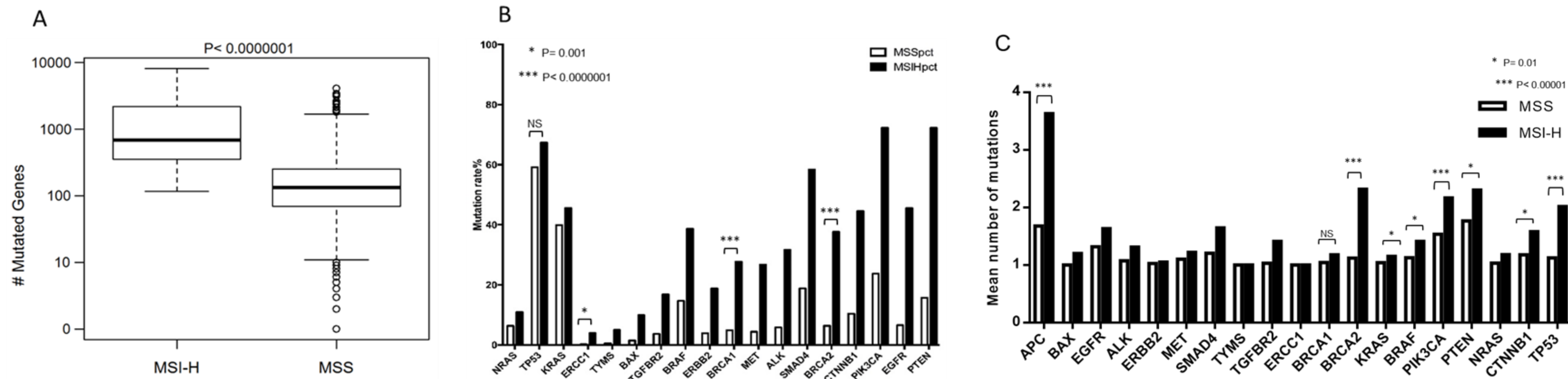


Figure 2. BRCA2 gene is among the highly mutated genes with higher mean number of mutations in MSI-H CRCs.

## Somatic BRCA2 mutations in MSI-H vs MSS CRC

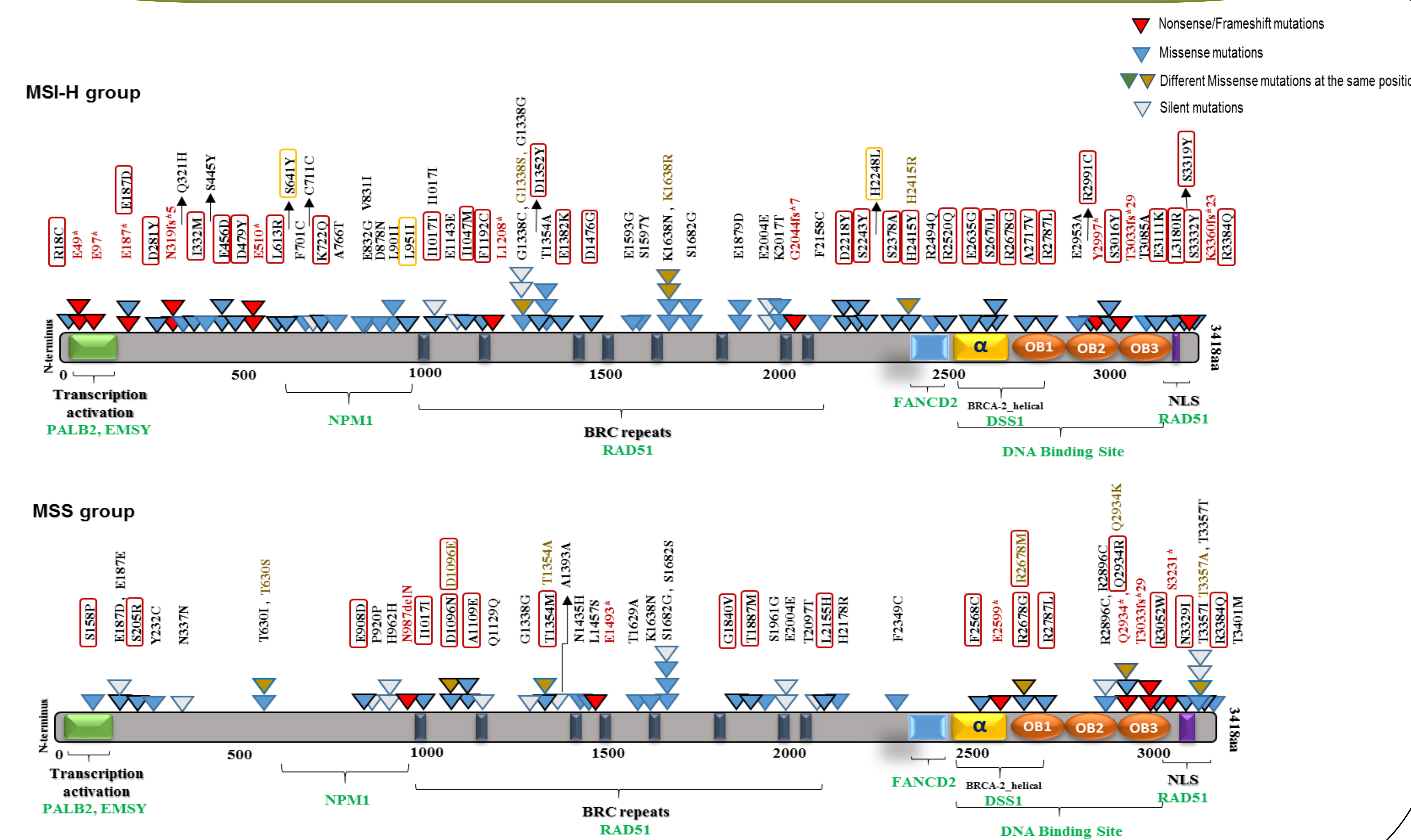


Figure 3. BRCA2 protein domain annotated with somatic alterations in MSI-H vs MSS CRCs.

## BRCA2 mutations in coding microsatellites

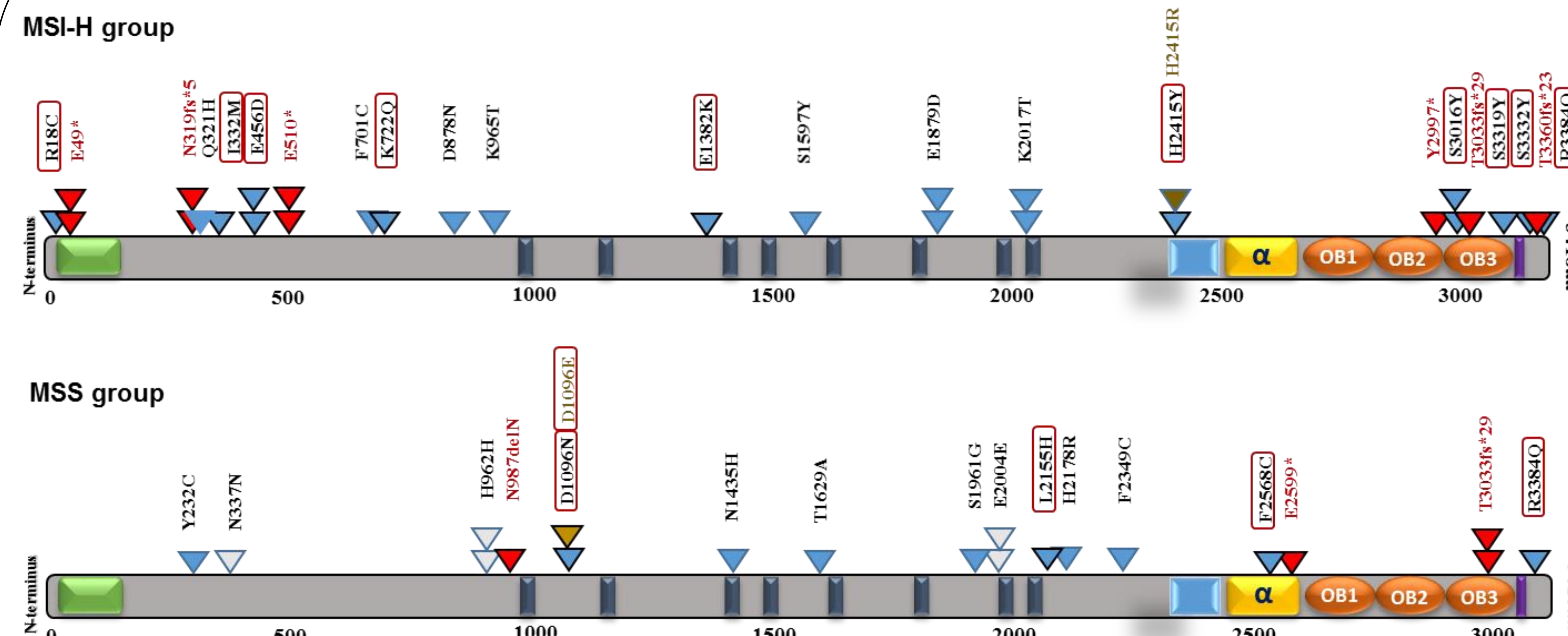


Figure 4. BRCA2 protein domain annotated with somatic alterations in coding microsatellites. BRCA2 mutations in MSI-H patients vs. in MSS group.

## Mutations in BRCA2-DSS1 model structure

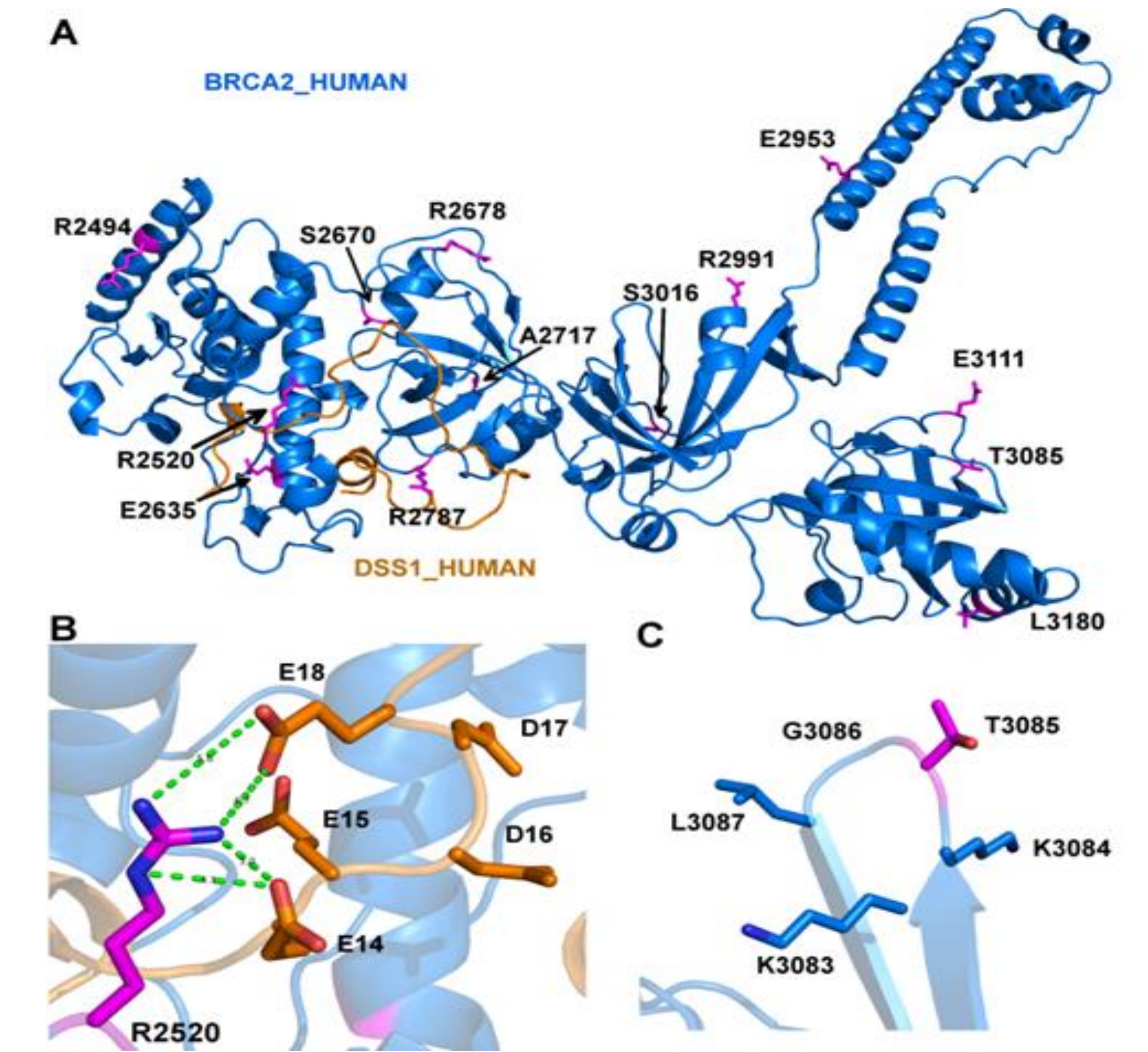


Figure 5. The mutations in the human BRCA2-DSS1 model structure.

## Somatic mutations of MLH1 & MSH2 in CRCs

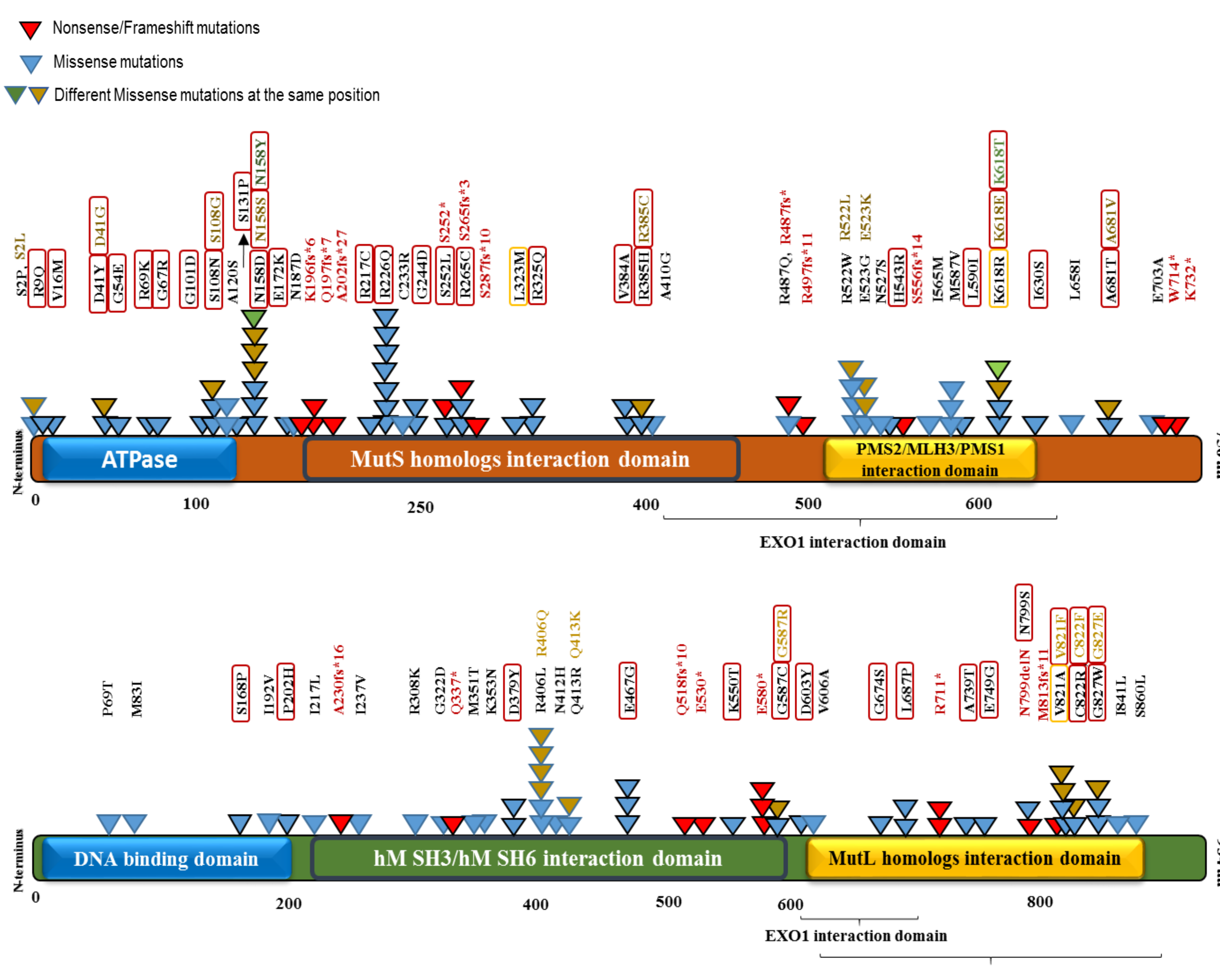


Figure 1. MLH1 and MSH2 protein domain annotated with somatic non-synonymous alterations observed in CRCs.

## Conclusions

- Significant enrichment of mutations in BRCA genes is observed in MSI-H tumors.
- Colorectal cancer patients with MSI-H display a distinct pattern of BRCA2 mutations in frequency, diversity and position in comparison to MSS patients.
- More frameshift and/or nonsense point mutations of BRCA2 are observed to be distributed in N-terminal, BRC repeats and C-terminal in MSI-H group
- Coding microsatellites in BRCA2 are more mutated with higher potential damaging mutations in MSI-H patients than MSS group.
- Functionally damaging mutations predicted in BRCA2 were not detected to necessarily be frequent, however, have significant effect to disrupt the protein-protein interactions
- Only few BRCA2 mutations in CRCs have been reported in breast/ovarian cancer suggests that these mutations are CRC specific.
- BRCA2, as a HR element with high recorded microsatellite frequency, could be targeted in MMR deficient system.

## Future Directions

- Functional analysis of mutations in the BRC domains or DSS1-interacting domain in BRCA2
- Drug sensitivity assays on BRCA2 mutant
- Discriminate environmental influenced mutations from tumor-specific ones