# SUPPLEMENTARY MATERIAL

## Supplementary Methods

### Clinical Comparison

EO-CRC vs. AO-CRC: EO-CRC patients treated at MSKCC during a similar timeframe with identically annotated clinical and genomic information were compared to AO-CRC patients. In metastatic patients, first-line chemotherapy exposure, response and duration of therapy, as well as survival were compared between EO-CRC and a previously published metastatic AO-CRC MSKCC cohort.[22] For most analyses, the EO-CRC cohort was further stratified based on age at CRC diagnosis, age 36-49 versus age ≤35, in line with SEER data demonstrating ages 52 and 36 years being one and two standard deviations below the mean age at CRC diagnosis, respectively. Tumor grading and clinical staging were performed based on the 2021 NCCN Clinical Practice Guidelines in Oncology [1,2].

### Germline Analyses

Variants were interpreted based on the American College of Medical Genetics and Genomics criteria.[29] All pathogenic (P) or likely pathogenic (LP) variants were included in the report; variants of unknown significance were not reported. Germline variants were classified as having high- (relative risk >4), moderate- (relative risk 2-4), or low-penetrance (relative risk <2) and/or as being recessive or of uncertain clinical actionability as previously published.[28] In contrast to the somatic analysis which was limited to patients with microsatellite stable (MSS) CRC, the germline analysis included patients with MSI tumors. For individuals with germline pathogenic variants, the tumor was subsequently interrogated to assess somatic variants and/or loss of heterozygosity in the gene(s) corresponding to the germline findings. Individuals with P/LP variants were offered genetic counseling.

### Statistical Analysis

To investigate the assumption of proportionality within our Cox model, we assessed the Schoenfield residuals and found that the global result did not violate the assumption of proportionality ($P$>0.05). We did, however, observe that 4 covariates had $P$-values less than 0.05 (WNT Pathway, APC, BRAF, and HAI PUMP).

### References


1. Benson AB *et al.* 2021 NCCN Clinical Practice Guidelines in Oncology: Colon Cancer Version 2.2021. *NCCN Guidelines Version 2.2021* (2021).
2. Benson AB *et al.* 2021 NCCN Clinical Practice Guidelines in Oncology: Rectal Cancer Version 1.2021. *NCCN Guidelines Version 1.2021* (2021).

# Supplementary Tables

**Supplementary Table 1.** List of somatic mutations identified by MSK-IMPACT (mutation annotation format) (*available for separate download*).

**Supplementary Table 2.** MSK-IMPACT Germline Gene List

| **Genes_88** | **Genes_76** |
|---|---|
| *ALK* | *ALK* |
| *APC* | *APC* |
| *ATM* | *ATM* |
| *BAP1* | *BAP1* |
| *BARD1* | *BARD1* |
| *BLM* | *BLM* |
| *BMPR1A* | *BMPR1A* |
| *BRCA1* | *BRCA1* |
| *BRCA2* | *BRCA2* |
| *BRIP1* | *BRIP1* |
| *CDH1* | *CDH1* |
| *CDC73* | *CDK4* |
| *CDK4* | *CDKN2A* |
| *CDKN2A* | *CHEK2* |
| *CEBPA* | *DICER1* |
| *CHEK2* | *EGFR* |
| *DICER1* | *EPCAM* |
| *EGFR* | *FAM175A* (Abraxas) |
| *EPCAM* | *FH* |
| *ERBB2* | *FLCN* |
| *ERCC3* | *GATA2* |
| *ETV6* | *GREM1* |
| *FAM175A* (Abraxas) | *HRAS* |
| *FANCA* | *JAK2* |
| *FANCC* | *KIT* |
| *FH* | *KRAS* |
| *FLCN* | *MAX* |
| *GATA2* | *MEN1* |
| *HOXB13* | *MET* |
| *HRAS* | *MITF* |
| *KIT* | *MLH1* |
| *KRAS* | *MRE11A* |
| *MAX* | *MSH2* |
| *MEN1* | *MSH6* |
| *MET* | *MUTYH* |
| *MITF* | *NBN* |
| *MLH1* | *NF1* |
| *MRE11A* | *NF2* |
| *MSH2* | *NRAS* |

| | |
|---|---|
| *MSH3* | *PALB2* |
| *MSH6* | *PAX5* |
| *MUTYH* | *PDGFRA* |
| *NBN* | *PHOX2B* |
| *NF1* | *PMS2* |
| *NF2* | *POLE* |
| *NRAS* | *PTCH1* |
| *NTHL1* | *PTEN* |
| *PALB2* | *RAD50* |
| *PAX5* | *RAD51* |
| *PDGFRA* | *RAD51B* |
| *PHOX2B* | *RAD51C* |
| *PMS2* | *RAD51D* |
| *POLD1* | *RB1* |
| *POLE* | *RECQL4* |
| *PTCH1* | *RET* |
| *PTEN* | *RUNX1* |
| *RAD50* | *SDHA* |
| *RAD51* | *SDHAF2* |
| *RAD51B* | *SDHB* |
| *RAD51C* | *SDHC* |
| *RAD51D* | *SDHD* |
| *RB1* | *SMAD3* |
| *RECQL* | *SMAD4* |
| *RECQL4* | *SMARCA4* |
| *RET* | *SMARCB1* |
| *RTEL1* | *STK11* |
| *RUNX1* | *SUFU* |
| *SDHA* | *TERT* |
| *SDHAF2* | *TGFBR1* |
| *SDHB* | *TGFBR2* |
| *SDHC* | *TMEM127* |
| *SDHD* | *TP53* |
| *SMAD3* | *TSC1* |
| *SMAD4* | *TSC2* |
| *SMARCA4* | *VHL* |
| *SMARCB1* | *WT1* |
| *STK11* | |
| *SUFU* | |
| *TERT* | |
| *TGFBR1* | |
| *TGFBR2* | |
| *TMEM127* | |
| *TP53* | |
| *TSC1* | |
| *TSC2* | |
| *VHL* | |
| *WT1* | |
| *YAP1* | |

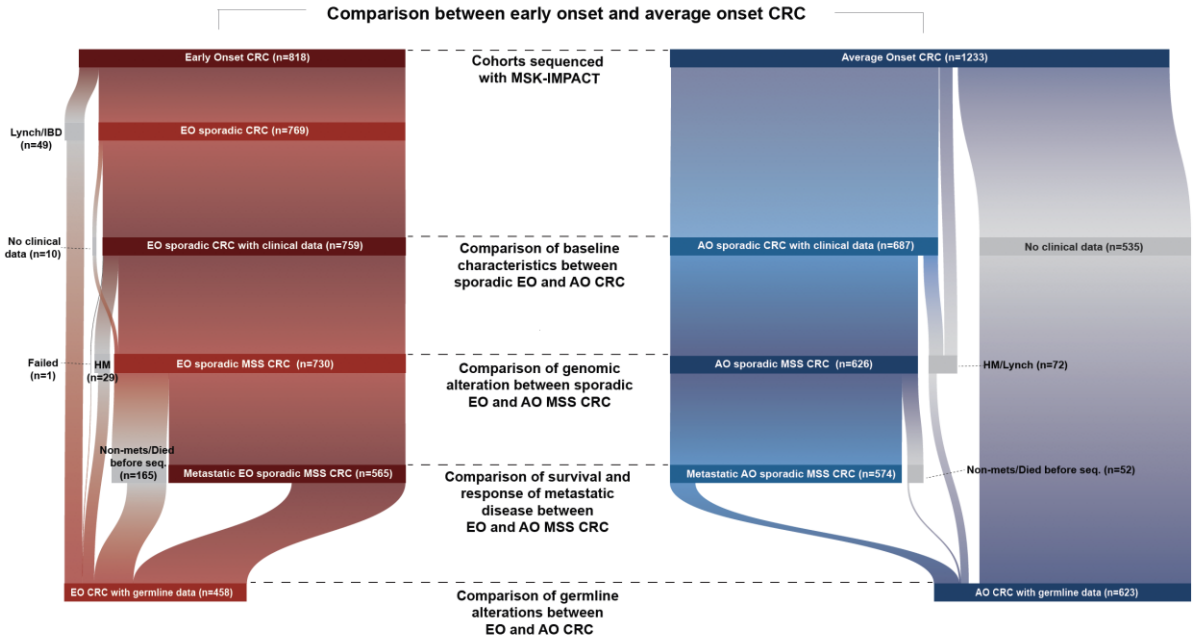
**Supplementary Table 3.** Patient- and sample-level clinical and genomic characteristics of EO-CRC genomically profiled with MSK-IMPACT (N = 818) (*available for separate download*).

**Supplementary Table 4.** Patient- and sample-level clinical and genomic characteristics of AO-CRC genomically profiled with MSK-IMPACT (N = 698) (*available for separate download*).

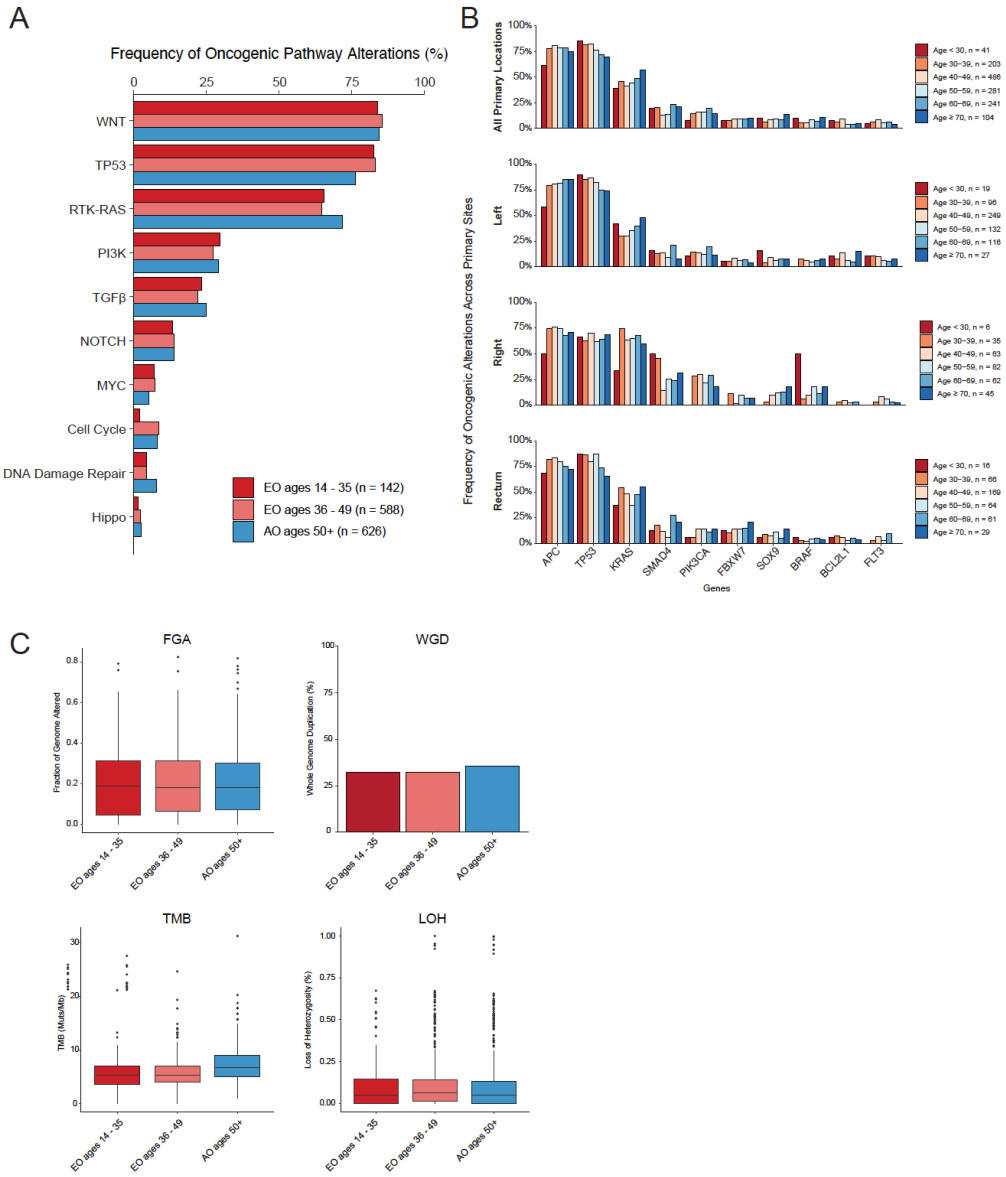
**Supplementary Table 5.** Frequency of cancer-related presenting symptoms across primary tumor locations (*available for separate download*).

## Supplementary Figures

**Supplementary Figure 1.** A detailed breakdown of early-onset and average-onset colorectal cancer cohort comparisons with sample sizes. EO = early-onset; AO = average-onset; CRC = colorectal cancer; MSS = microsatellite stable; IBD = inflammatory bowel disease; HM = hypermutated tumors.



**Supplementary Figure 2. Comparison of Oncogenic Pathway Alterations and Molecular Features** Comparison of (A) frequency of oncogenic pathway alterations by age group (EO 14-35, EO 36-49, and AO 50+). The frequency of oncogenic driver mutations did not differ between age ≤35, 36-49, and AO-CRC groups (B) frequency of oncogenic alterations adjusted for tumor location (left vs. right vs. rectum) by decades. The differences in the prevalence of TP53 and RTK-RAS pathway alterations were no longer significant at the gene or pathway level once adjusted for sidedness. (C) Tumor mutational burden (TMB), fraction of genome altered (FGA), whole genome duplication (WGD), and loss of heterozygosity (LOH) in early-onset (n=730) and average-onset colorectal cancer (n=626) cohorts divided by age. No significant differences in tumor mutational burden (TMB), fraction of genome altered (FGA), whole genome duplication (WGD), or loss of heterozygosity (LOH) were observed.



**Supplementary Figure 3. Germline Mutations** Pathogenic/likely pathogenic germline mutations with biallelic inactivation by age group, EO 14-35, EO 36-49, and AO 50+, and by gene penetrance level. EO = early-onset; AO = average-onset