

Additional file 2

Additional figures

Title: Associations of genetically predicted interleukin-6 and tumor necrosis factor signaling pathways with mortality among persons with colorectal cancer: A two-sample Mendelian Randomization

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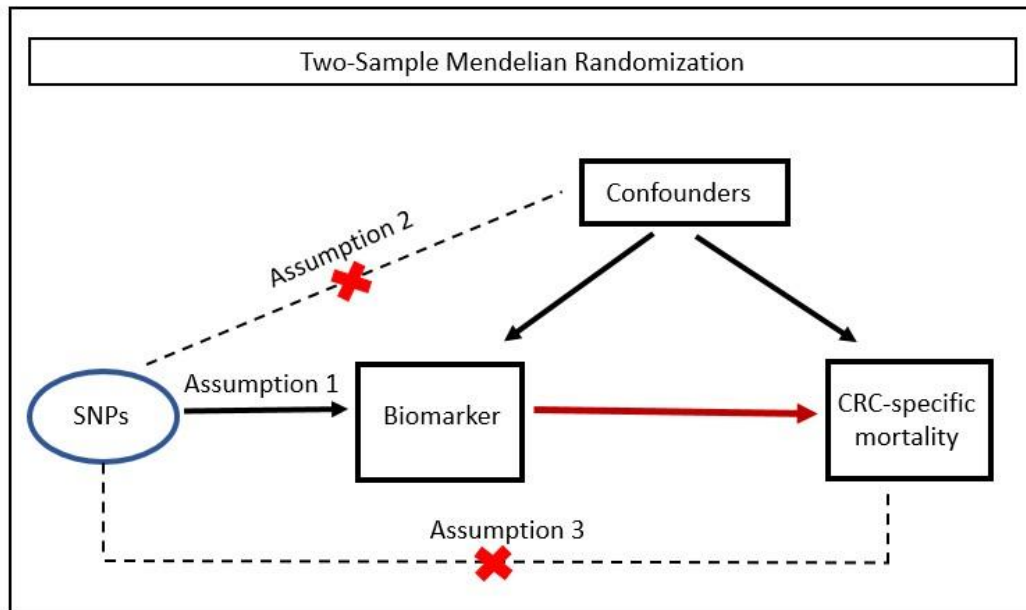
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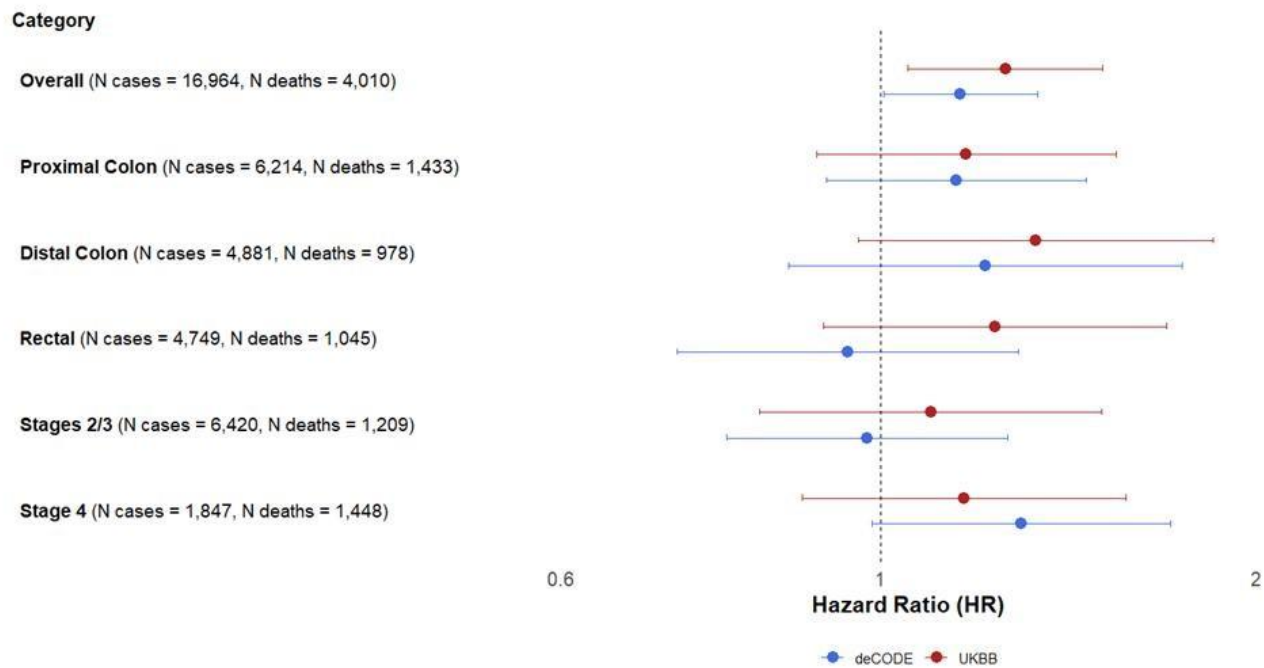
Additional Figure 17: Distribution of number of SNPs showing spurious association with BMI across 1,000 simulations.

Additional Figure 1: Two-sample Mendelian randomization (MR) analysis.



^aSNPs are used as instrumental variables to estimate the association between IL-6 or TNF- α and colorectal cancer-specific (CRC) mortality. The three core MR assumptions are illustrated: (1) the genetic variants (SNPs) are associated with the exposure (IL-6 or TNF- α); (2) the SNPs are not associated with confounders of the exposure-outcome relationship; and (3) the SNPs influence the outcome (CRC mortality) only through the exposure and not via alternative pathways. Red crosses indicate potential violations of assumptions 2 and 3.

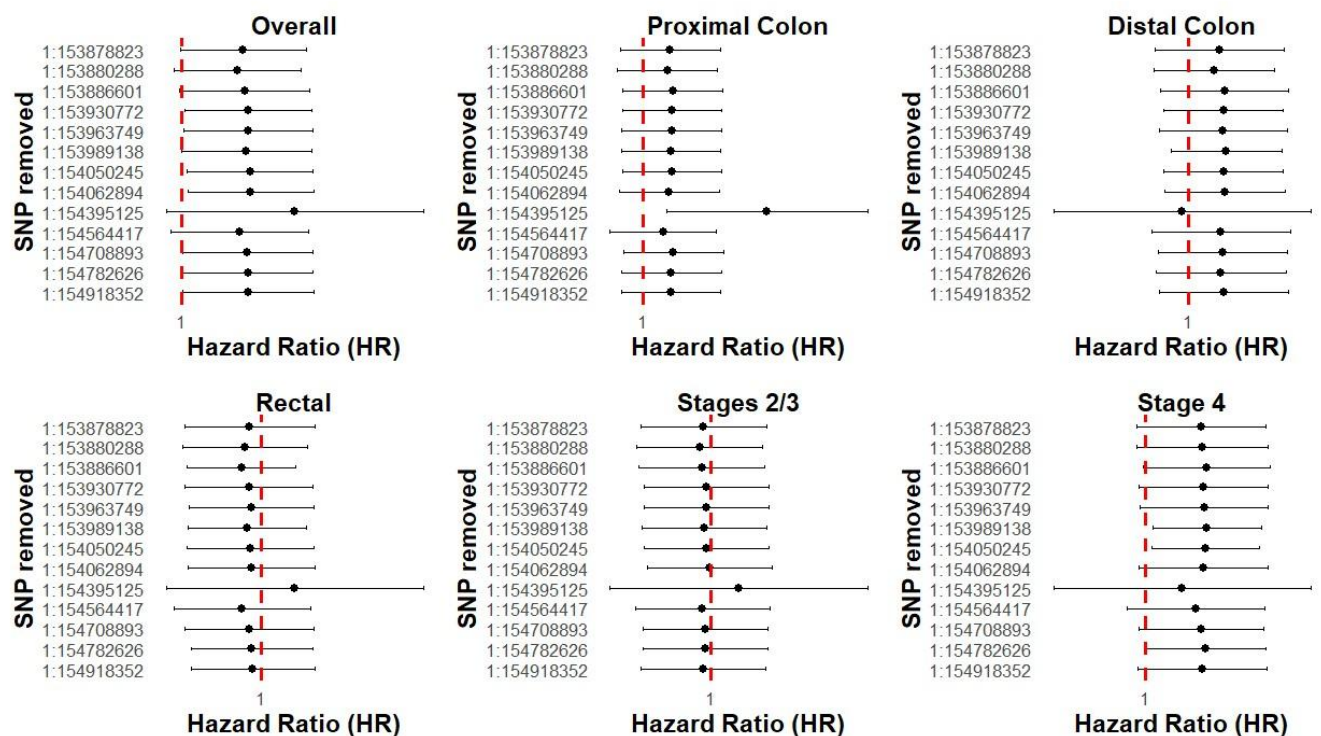
Additional Figure 2: MR estimates of genetically predicted sIL6R- α on CRC-specific mortality across subgroups in two GWAS.



^aHazard ratios (HRs) and 95% confidence intervals (CIs) are presented for the estimated effects of genetically predicted inflammatory biomarker on colorectal cancer-specific mortality, using Mendelian Randomization (MR) with the Inverse Variance Weighted (IVW) method.

^bGenetic cis instruments for biomarker levels were derived from two genome-wide association studies (GWAS): deCODE genetics (Ferkingstad et al., 2021, (1)) and UK Biobank (UKBB) (Sun et al., 2023 (2)). ^cHRs represent the effect per one standard deviation (SD) increase in biomarker levels.

Additional Figure 3: Leave-one-out MR analysis of genetically predicted sIL6R- α and CRC-specific mortality using deCODE GWAS.

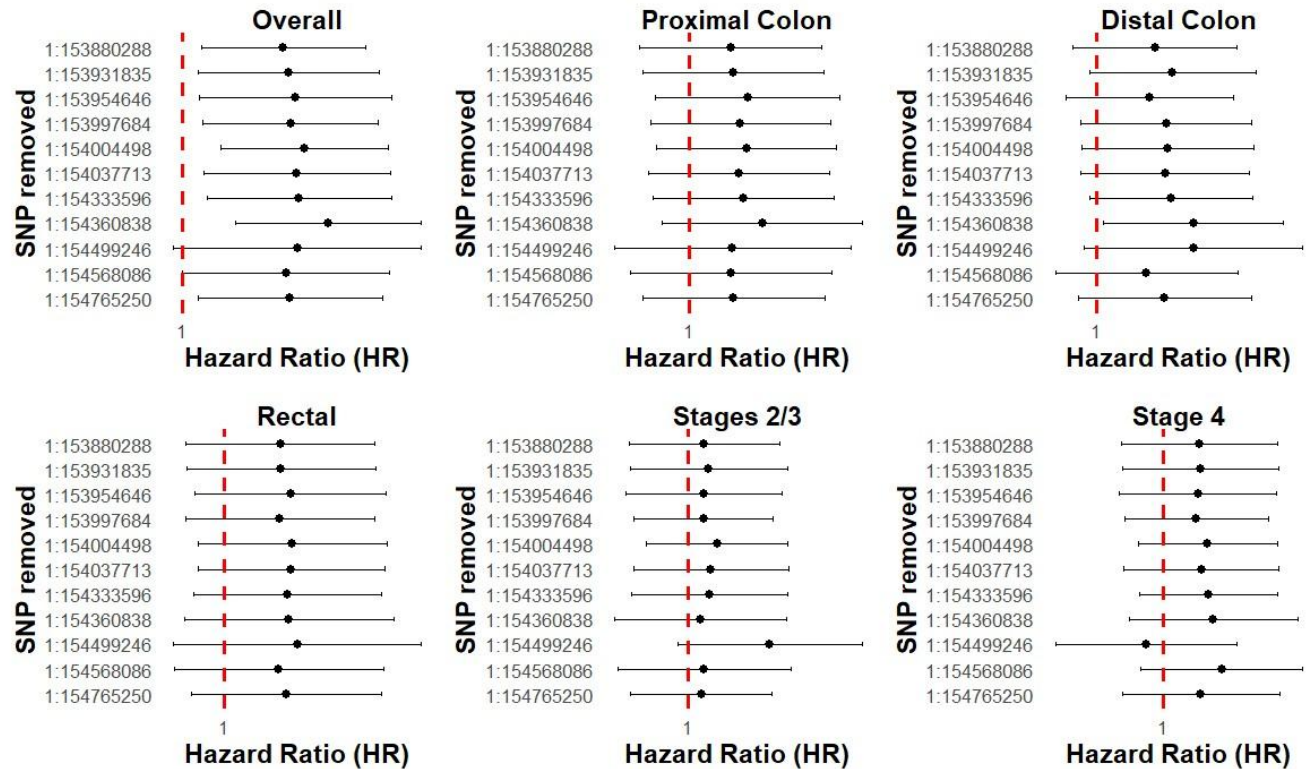


^aHazard ratios (HRs) and 95% confidence intervals (CIs) are presented for the estimated effects of genetically predicted inflammatory biomarker on colorectal cancer-specific mortality, using Mendelian Randomization (MR) with the Inverse Variance Weighted (IVW) method.

^bGenetic cis instruments for biomarker levels were derived from the genome-wide association study (GWAS) deCODE genetics (Ferkingstad et al., 2021).

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Additional Figure 4: Leave-one-out MR analysis of genetically predicted sIL6R- α and CRC-specific mortality using UKBB GWAS.

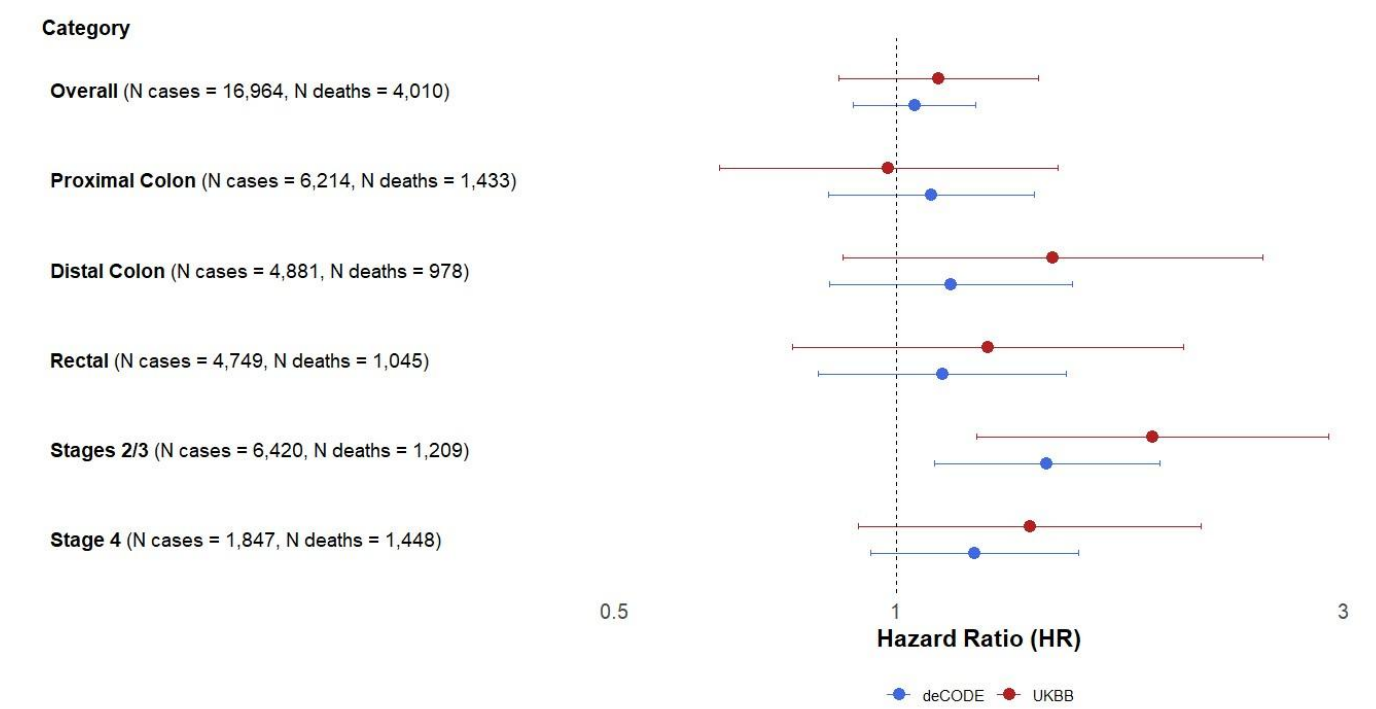


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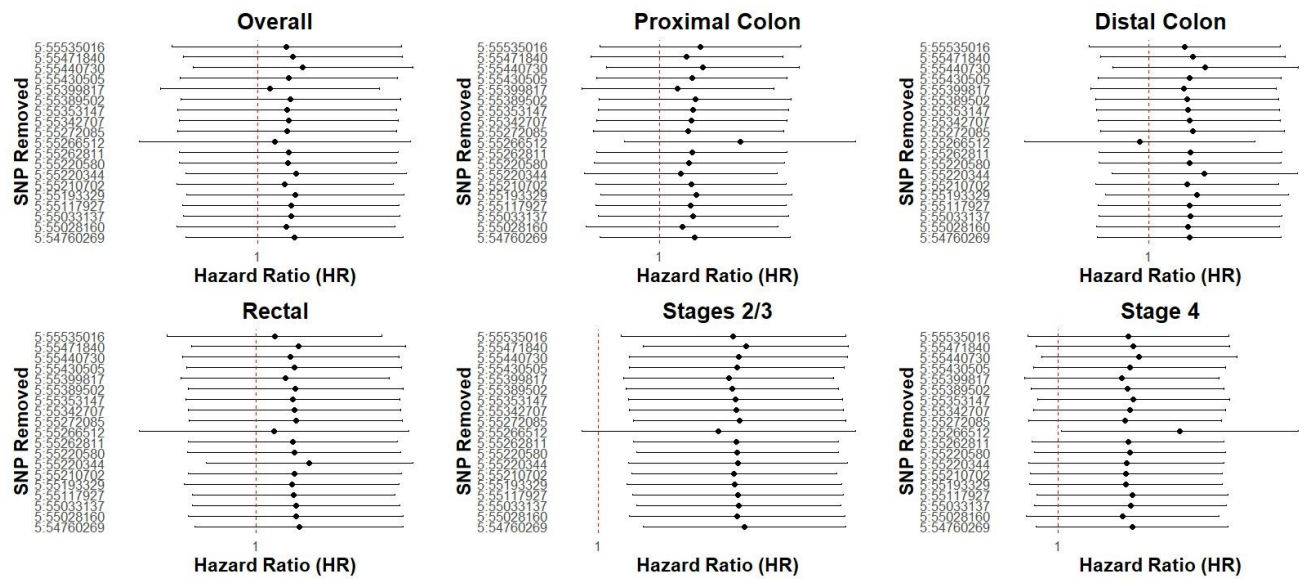
Additional Figure 5: MR estimates of genetically predicted IL6ST on CRC-specific mortality across subgroups in two GWAS.



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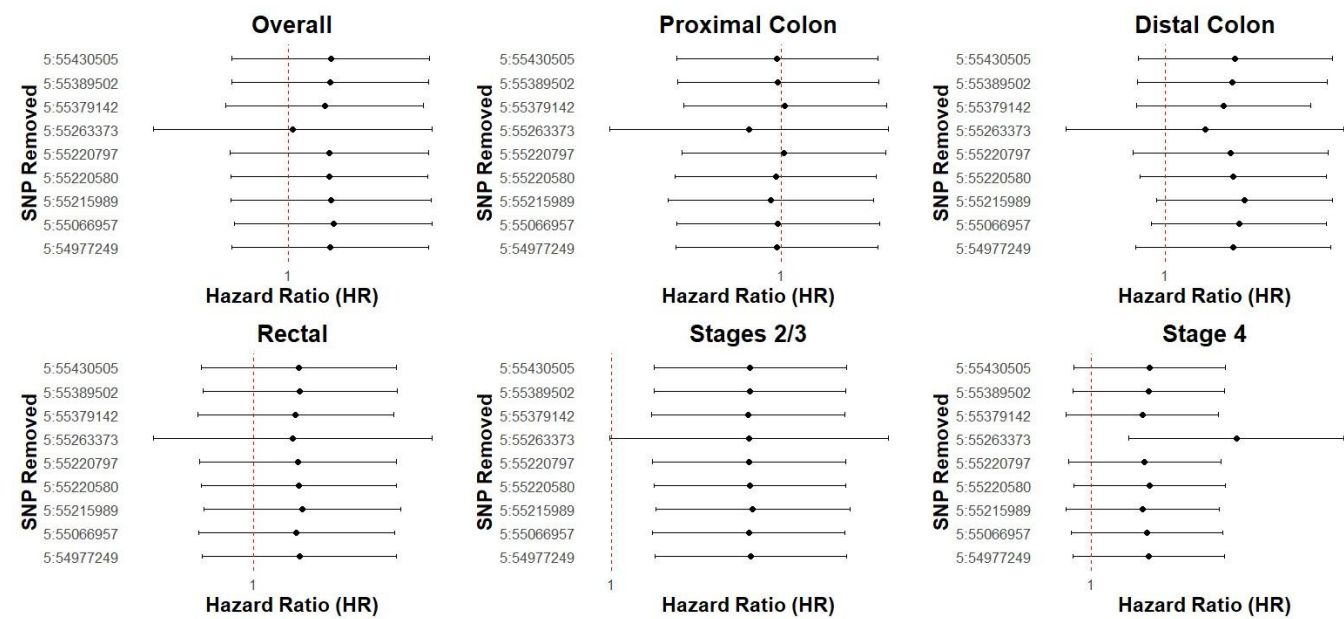
Additional Figure 6: Leave-one-out MR analysis of genetically predicted IL6ST and CRC-specific mortality using deCODE GWAS.



^aHazard ratios (HRs) and 95% confidence intervals (CIs) are presented for the estimated effects of genetically predicted inflammatory biomarker on colorectal cancer-specific mortality, using Mendelian Randomization (MR) with the Inverse Variance Weighted (IVW) method.

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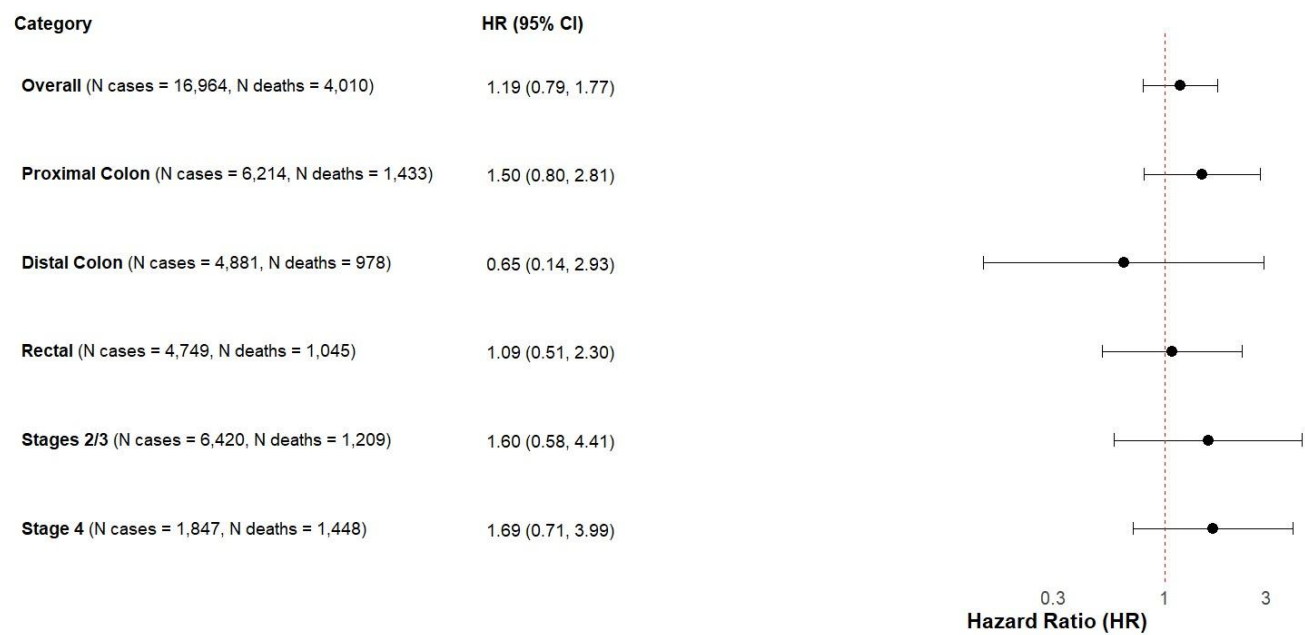
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Additional Figure 8: MR estimates of genetically predicted TNF- α on CRC-specific mortality across subgroups using UKBB GWAS.

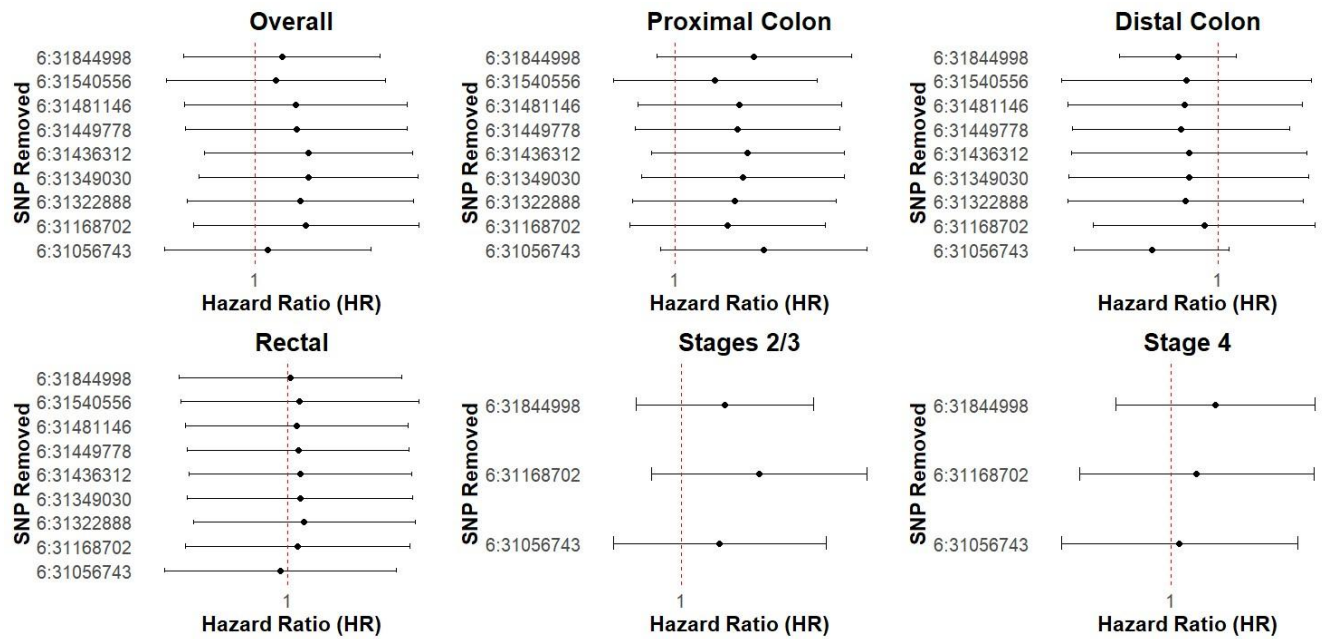


^aHazard ratios (HRs) and 95% confidence intervals (CIs) are presented for the estimated effects of genetically predicted inflammatory biomarker on colorectal cancer-specific mortality, using Mendelian Randomization (MR) with the Inverse Variance Weighted (IVW) method.

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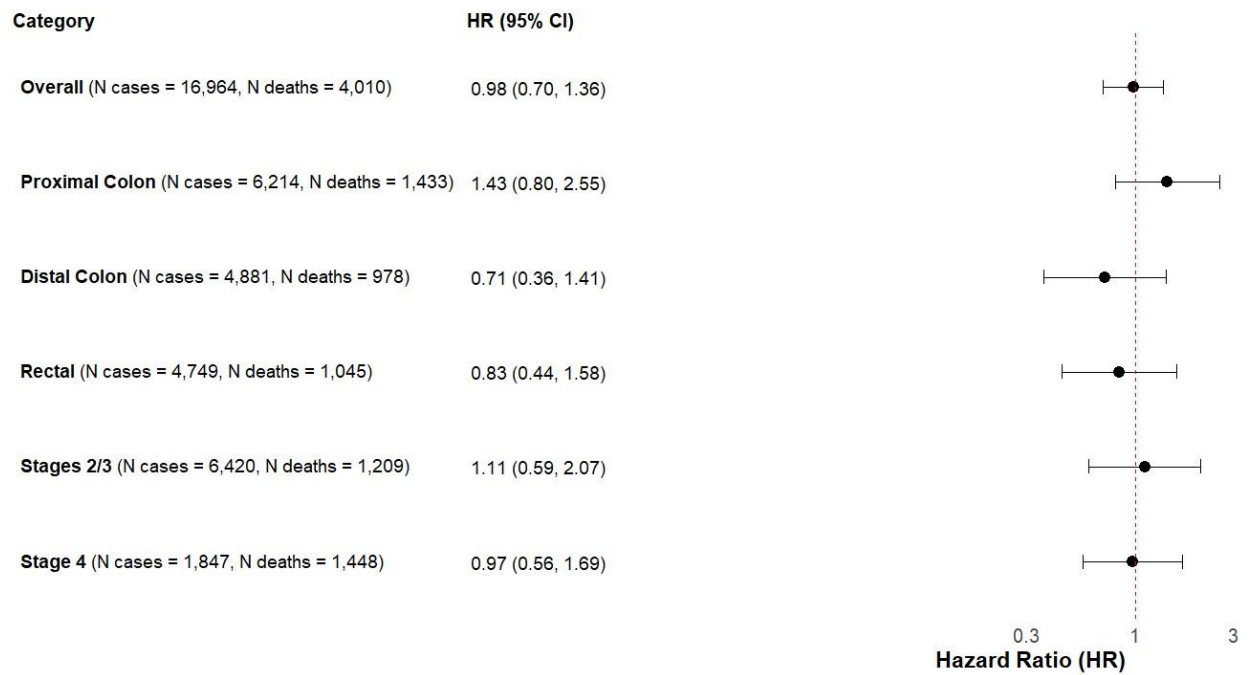
Additional Figure 9: Leave-one-out MR analysis of genetically predicted TNF- α and CRC-specific mortality using UKBB GWAS.



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Additional Figure 10: MR estimates of genetically predicted sTNF-R1 on CRC-specific mortality across subgroups using deCODE GWAS.

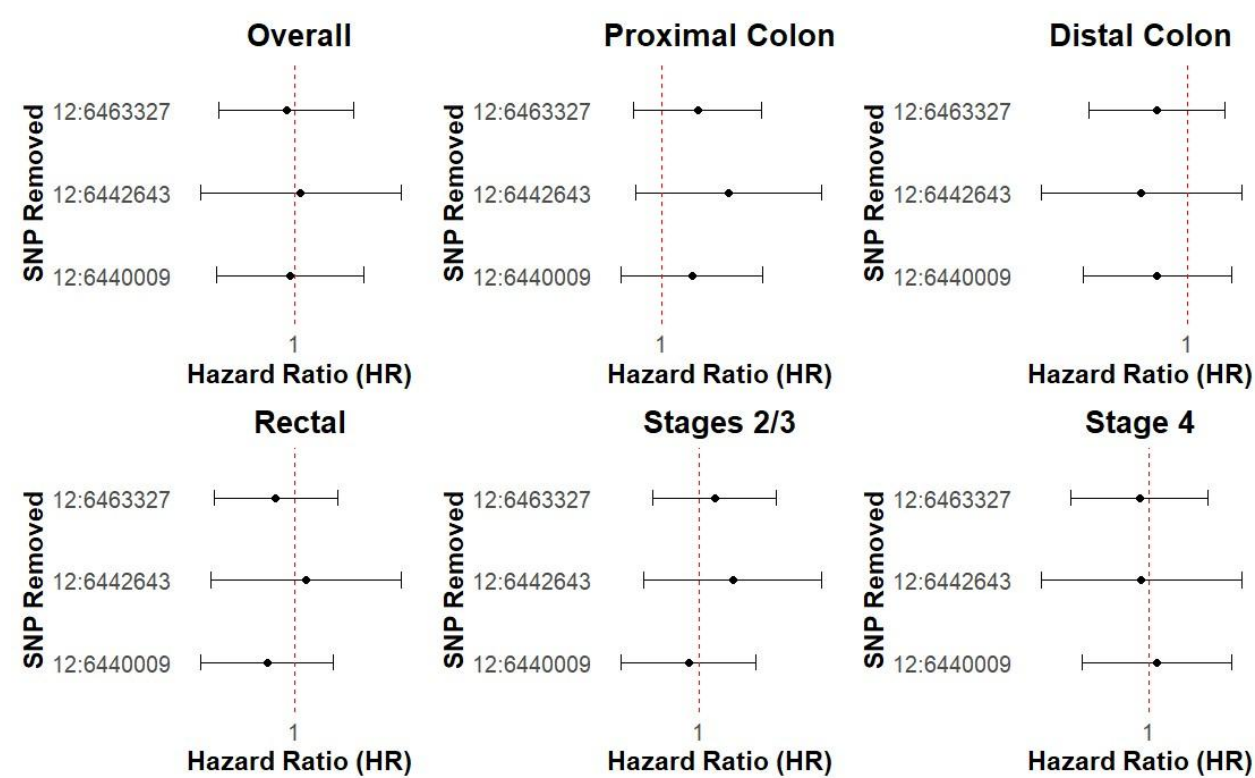


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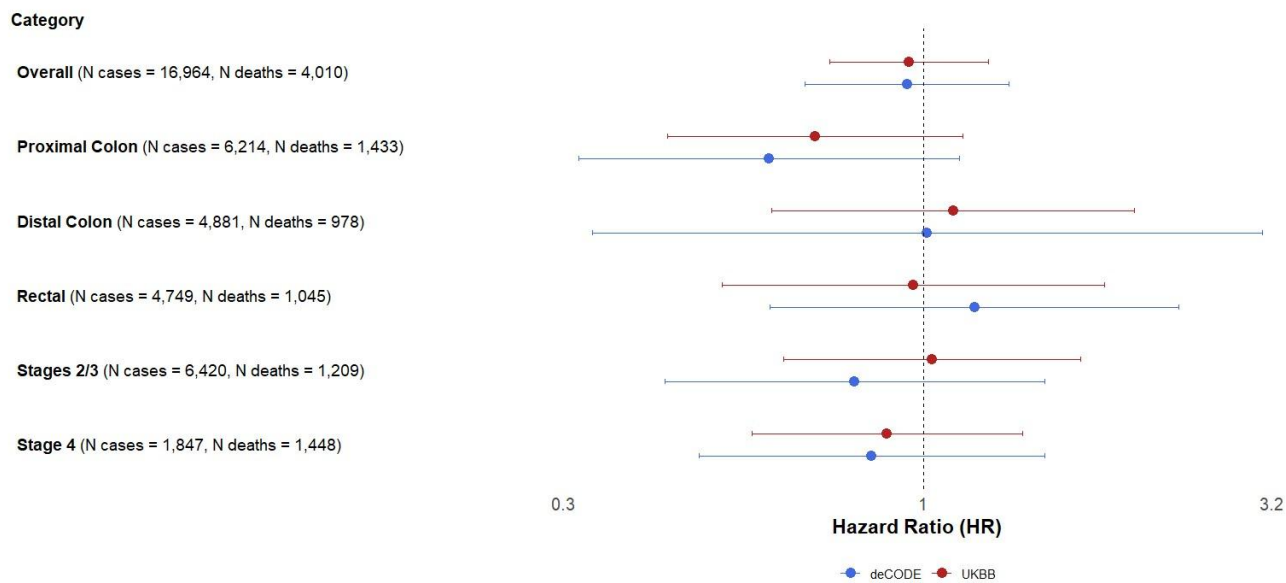


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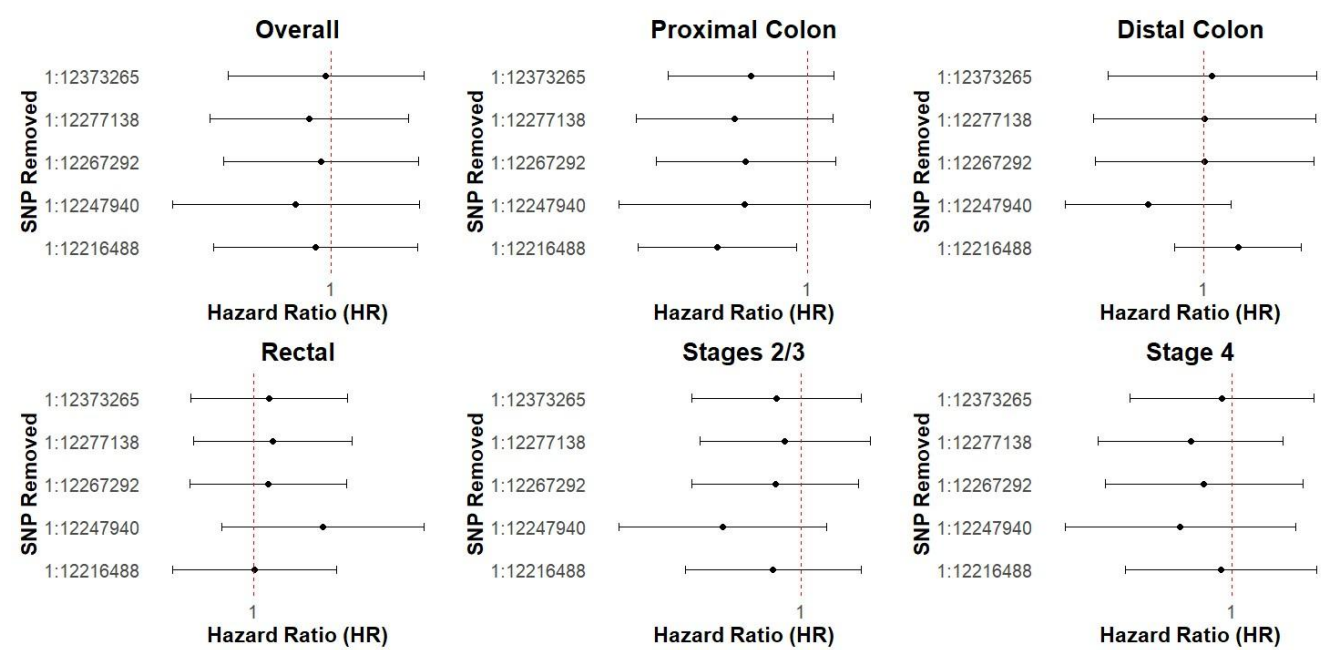
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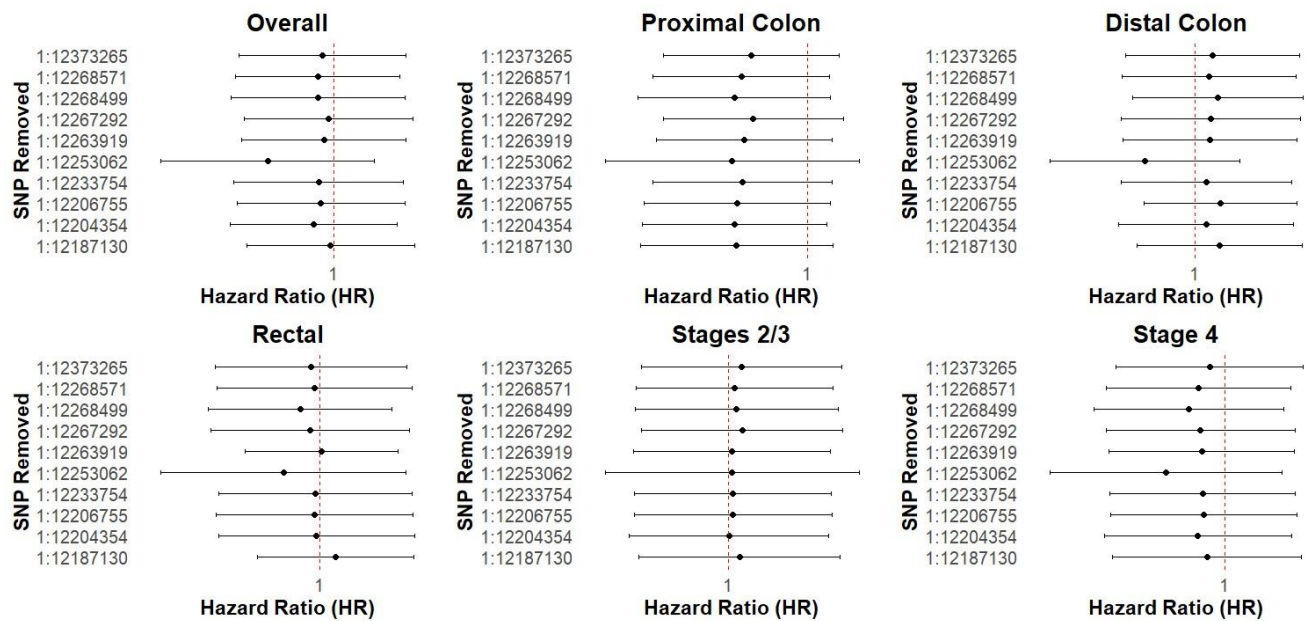
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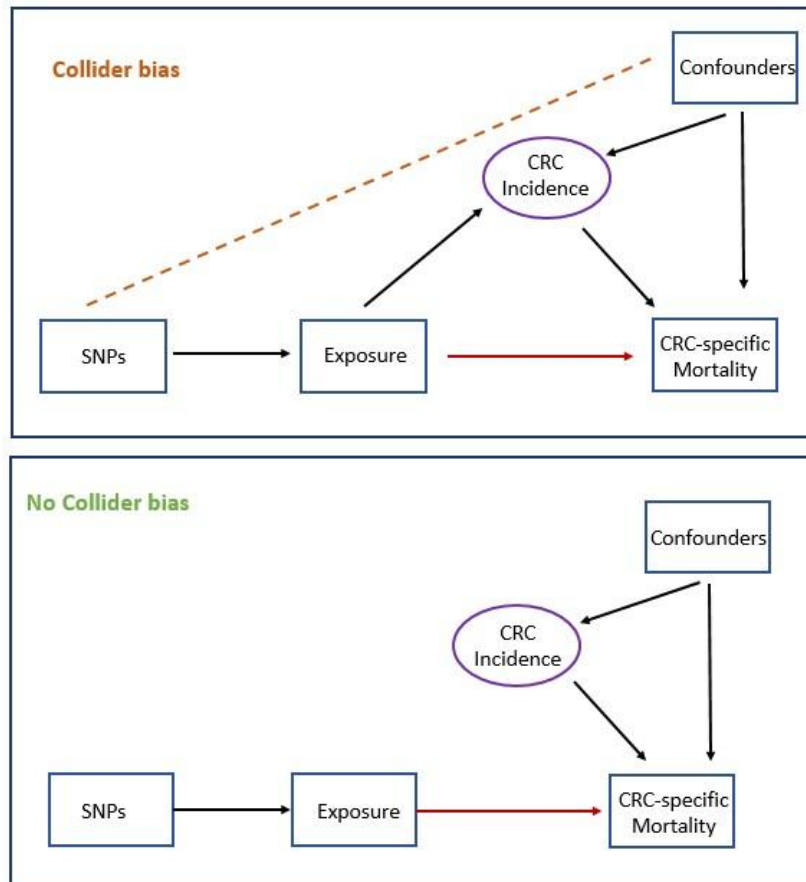
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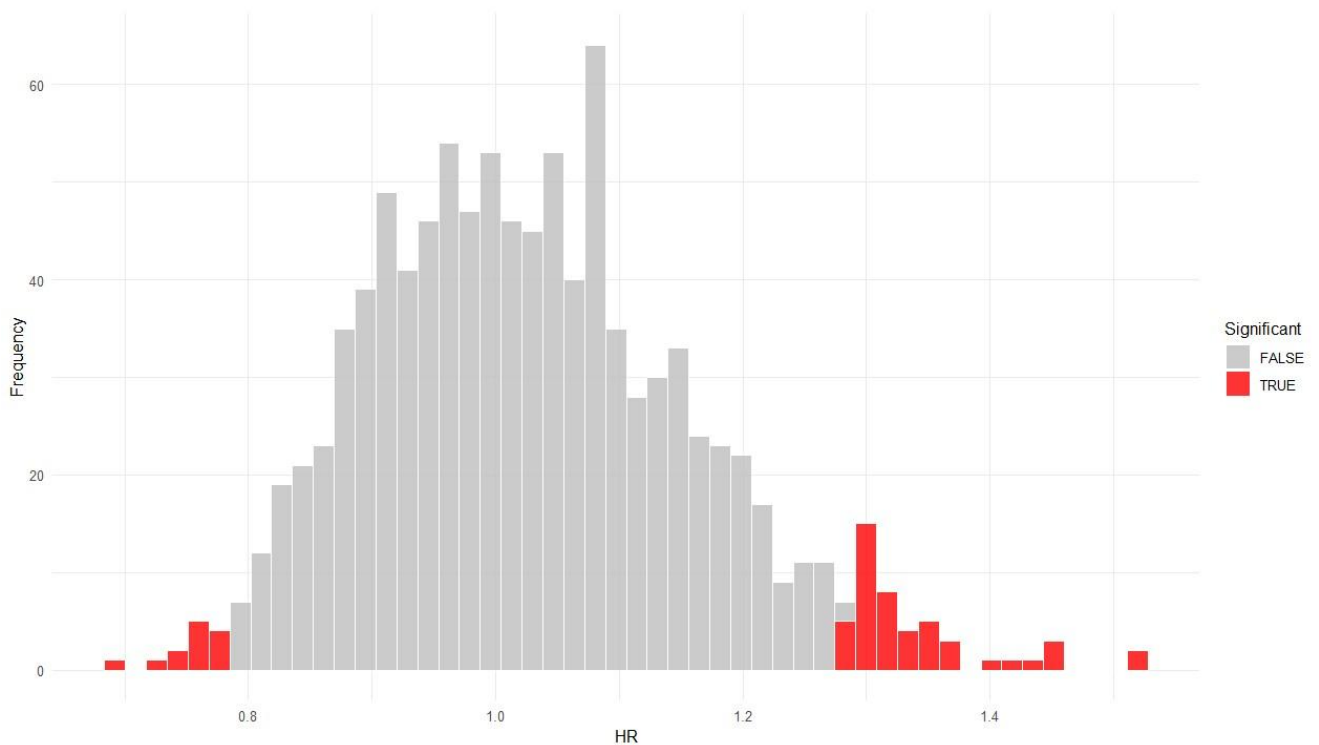
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Additional Figure 16: Distribution of Simulated MR Hazard Ratio Estimates of TNF- α on CRC-Specific Mortality.

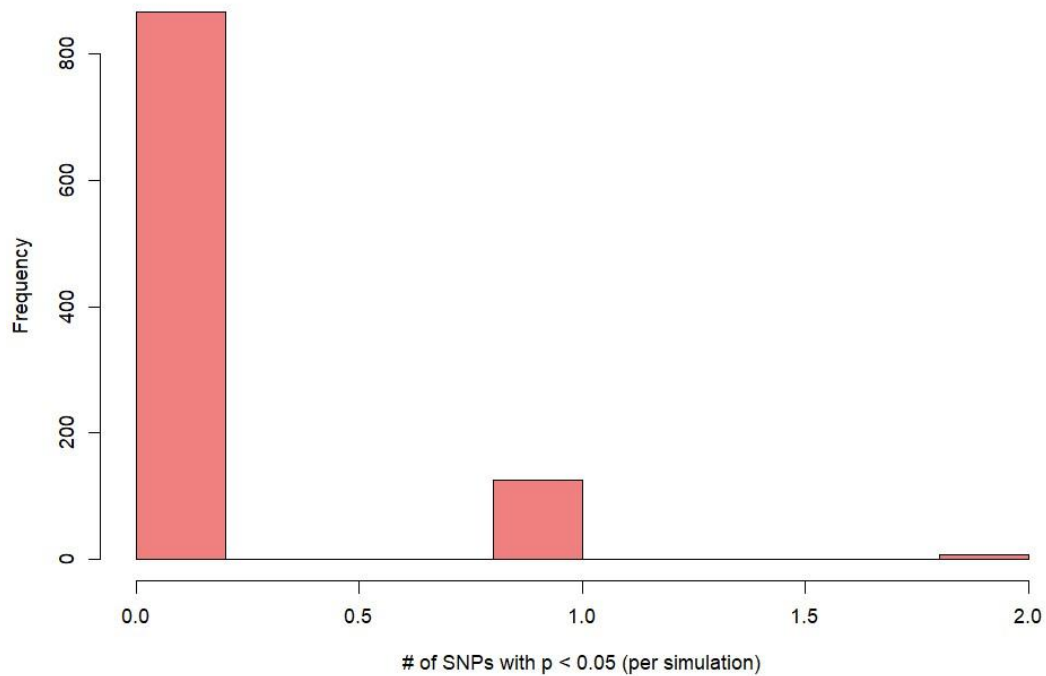


^aResults are based on 1,000 simulated Mendelian randomization analyses under the null hypothesis of no causal effect of genetically predicted TNF- α on CRC-specific mortality. ^bBars represent the frequency distribution of Hazard Ratio (HR) estimates across simulations.

^cRed bars indicate simulations where the MR estimate reached nominal statistical significance ($p < 0.05$); grey bars indicate non-significant estimates.

^dThe distribution illustrates the small upward bias in HR estimates and the modest inflation in type I error due to collider bias when conditioning on CRC incidence.

Additional Figure 17: Distribution of number of SNPs showing spurious association with BMI across 1,000 simulations.



^aEach bar represents the frequency of simulations in which 0, 1, or 2 SNPs showed a nominal association with BMI after conditioning on CRC incidence.

^bIn ~87% of simulations, no SNPs were spuriously associated with BMI; in ~13% at least one SNP reached nominal significance.

^cThese findings illustrate that collider bias can occasionally induce spurious SNP–confounder associations, though the overall frequency was low.