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Supplementary Methods

Study population

The FOCUS consortium was initiated to investigate biomarkers related to Folate-dependent One-carbon metabolism in colorectal Cancer recurrence and Survival. The six prospective cohort studies included in this consortium have been described before. Briefly, the COLON [1] and EnCoRe [2] studies are ongoing prospective cohort studies that recruit newly diagnosed colorectal cancer patients since 2010 and 2012, respectively. Participants were recruited at time of diagnosis from eleven hospitals in the Netherlands for the COLON study and from three hospitals in the Netherlands for the EnCoRe study. CORSA is an ongoing study since 2003, recruiting colorectal cancer patients in cooperation with the province-wide screening project “Burgenland Prevention Trial of Colorectal Disease with Immunological Testing” (B-PREDICT) using fecal occult blood testing (FOBT). FOBT-positive tested individuals subsequently received a complete colonoscopy. Additional colorectal cancer patients were recruited at diagnosis at four hospitals in Vienna. ColoCare is an ongoing, international, prospective cohort study [3]. Patients were recruited since 2010 at the University Hospital of Heidelberg and the National Center for Tumor Diseases in Heidelberg, Germany (ColoCare HD). ColoCare patients were also recruited since 2015 at Huntsman Cancer Institute (Salt Lake City, U.S.)(ColoCare HCI) and since 2007 at Fred Hutchinson Cancer Research Center (Seattle, U.S.)(ColoCare FHCRC).

Data collection

Demographic and lifestyle characteristics including age at colorectal cancer diagnosis, sex, weight, height, smoking history, and dietary supplement use were collected through cohort-specific self-administered questionnaires (COLON, EnCoRe, CORSA and ColoCare) and home visits (EnCoRe). Total energy intake (kcal/day) and alcohol use (kcal/day) were assessed at diagnosis using standardized Food Frequency Questionnaires (FFQ) in COLON, EnCoRe and the ColoCare cohorts. Physical activity was assessed using the validated Short QUestionnaire to ASsess Health enhancing physical activity (SQUASH) in COLON and EnCoRe [1, 2]. For ColoCare, questions regarding
physical activity were included in cohort-specific questionnaires [3]. Participants included from CORSA did not have FFQ data, intake of dietary supplements, nor physical activity available as these variables are not routinely collected in this cohort.

Clinical data, including colorectal cancer stage, tumor site, resection status, and treatment regimen were obtained from medical records. Clinical outcome data, including vital status and disease recurrence, were abstracted from medical records and through linkage with national cancer registries.

Sample preparation

Blood samples were collected shortly after colorectal cancer diagnosis and mostly before neo-adjuvant therapy or surgery (87%). Samples were sent and measured in two batches; the first batch was measured between November 2016 and March 2017. The second batch was analyzed between November 2017 and March 2018, to allow for longer follow-up time of participants and therefore more samples to analyze. Samples were analyzed in 96-well plates; each plate consisted of 86 study samples supplemented by one blank sample (water), two different in-house quality control samples in duplicate and five calibrator samples.

In addition to BEVITAL in-house quality control samples, duplicates of COLON samples (n=97) were included in both batches. The mean value for duplicate samples was calculated for concentrations included in the current study. Regression coefficients for COLON duplicates were 0.94, 0.92, 0.90 and 0.71 for 5-mTHF, hmTHF, folic acid, pABG, and apABG, respectively.

Blood samples from the ColoCare cohorts were 1:2 diluted for analysis due to limited sample volume availability. The dilution factor was taken into account in concentrations by BEVITAL prior to providing biochemical analysis data.

Study end points

Recurrence was defined as locoregional (including local recurrence, in either the tumor bed or bowel wall, and affected regional lymph nodes) or distant (metastasis) recurrence after complete tumor resection. If tumor resection status was incomplete, newly diagnosed colorectal tumors were defined as a progression of the initial colorectal cancer diagnosis and were therefore not classified as a disease recurrence in the present study (n=10). Patients with a missing or unknown recurrence status (n=46)
were excluded from recurrence and disease-free survival analyses. Follow-up time for recurrence was calculated as the time between the date of blood collection and the date of recurrence. Date of colorectal cancer recurrence was defined as the date when recurrence status was updated or the last known date for follow-up (for example due to death or immigration), whichever came first. The date of recurrence was defined as the date of confirmatory imaging, or, in cases that needed tissue diagnosis confirmation, the date of biopsy.

The definition of overall survival was based on using death from any cause in the analysis. Follow-up time was calculated as the time between the date of blood collection to the date of death, the last date vital status was updated, or the date of end of follow-up (for example due to immigration), whichever came first.

Disease-free survival was defined by considering a recurrence or death from any cause as events in the analysis. Follow-up time was calculated as the time between the date of blood collection to the date of recurrence or death, whichever came first, or until the last date vital status was updated, the last date recurrence status was updated, or the date of end of follow-up (for example due to immigration), whichever came first.

For the cohorts COLON and EnCoRe, recurrence data was collected by the Netherlands Comprehensive Cancer Organization and survival data (date of death) were collected through linkage with the Municipal Personal Records Database. CORSA obtained recurrence data using the medical records in hospitals and survival data (date of death) using medical records and by checking with the Health Insurance Association. Lastly, the ColoCare sites collected data on recurrence using medical records and by sending questionnaires to the involved external physicians and oncologists. Date of death was obtained by the tumor registry of the National Center for Tumor Diseases (Heidelberg, ColoCare HD), by reviewing medical records, death registries, searching obituaries, and routine follow-up mailings (all ColoCare sites).

Statistical analysis

Clinical, demographic, and lifestyle characteristics were summarized for the total study population and by cohort using descriptive analysis. Hazard ratio (HR) estimates of Cox proportional

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hazard models for log2-transformed continuous variables may be interpreted as the risk associated with a 2-fold increase in concentration of circulating folate, pABG, apABG, or folic acid.

Subgroup analyses presented using forest plots were conducted to assess potential effect measure modification by cohort (COLON/EnCoRe/CORSA/ColoCare HD/ColoCare HCI/ColoCare FHCRC), disease stage (stage I/stage II/stage III, excluding unknowns (n=58)), tumor location (colon/rectal, excluding unknowns (n=4)), sex, neo- and/or adjuvant treatment (yes/no, excluding unknowns (n=16)), and dietary supplement use (any/containing folic acid/none, excluding unknowns (n=341)). Heterogeneity was explored with subgroup-specific risk estimates using a random-effects meta-analysis approach [4] and was evaluated using the $I^2$ index [5, 6]. In addition to the main analysis, dose-response plots for folate concentrations by cohort were conducted using study-specific tertiles of folate concentrations. This was done because folic acid fortification is present in the U.S. and using tertiles based on the total study population for dose-response curves per cohort would thus not give a representative presentation.

Sensitivity analyses were performed by 1) excluding participants from whom blood was collected during or after any type of treatment, i.e. (neo-) adjuvant radio- or chemotherapy and/or surgery (n=275), by 2) excluding participants who died or experienced a recurrence within the first 100 days after diagnosis (n=33), by 3) excluding patients who did not receive surgery (n=57) or with unknown surgery status (n=4), and by 4) limiting analysis to recurrence events within the first two years after diagnosis (n=188 recurrence events).
References

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2. van Roekel EH, Bours MJ, de Brouwer CP, et al. The applicability of the International classification of functioning, disability and health to study lifestyle and quality of life of colorectal cancer survivors. Cancer Epidem Biomar 2014:cebp. 1144.2013.

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4. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7(3):177-188.

5. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21(11):1539-1558.

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### Supplementary Tables

#### Supplementary Table 1. Baseline characteristics stratified by tertiles of folate

| Characteristics                        | Tertile 1 (n=675) | Tertile 2 (n=675) | Tertile 3 (n=674) |
|----------------------------------------|-------------------|-------------------|-------------------|
| Men, n (%)                             | 466 (69.0)        | 443 (65.6)        | 395 (58.6)        |
| Age at diagnosis (years), median (IQR) | 66.8 (61.2-73.0)  | 66.2 (61.0-72.9)  | 65.2 (58.0-72.1)  |
| Body mass index (kg/m²), median (IQR)  | 26.6 (24.2-29.4)  | 26.2 (24.0-29.0)  | 26.6 (24.1-30.0)  |
| Underweight, <18.5, n (%)              | 0 (0.5)           | 1 (1.1)           | 1 (1.1)           |
| Normal weight, 18.5-24.9, n (%)        | 224 (33.6)        | 241 (36.1)        | 219 (33.0)        |
| Overweight, 25-29.9, n (%)             | 283 (42.5)        | 306 (45.8)        | 274 (41.3)        |
| Obese, ≥30, n (%)                      | 156 (23.4)        | 114 (17.1)        | 163 (24.6)        |
| Smoking, n (%)                         |                   |                   |                   |
| Current                                | 94 (14.5)         | 87 (13.4)         | 70 (11.2)         |
| Former                                 | 354 (54.7)        | 365 (56.1)        | 309 (49.3)        |
| Never                                  | 199 (30.8)        | 199 (30.6)        | 248 (39.6)        |
| Unknown/missing                        | 28                | 24                | 47                |
| Stage of disease, n (%)                |                   |                   |                   |
| I                                      | 178 (26.4)        | 177 (26.2)        | 188 (27.9)        |
| II                                     | 190 (28.2)        | 190 (28.2)        | 219 (32.5)        |
| III                                    | 286 (42.4)        | 290 (43.0)        | 248 (36.8)        |
| Unspecified/unknown, n                 | 21 (3.1)          | 18 (2.7)          | 19 (2.8)          |
| Tumor location*, n (%)                 |                   |                   |                   |
| Colon                                  | 436 (77.3)        | 411 (60.9)        | 405 (60.3)        |
| Rectal                                 | 237 (35.2)        | 264 (39.1)        | 267 (39.7)        |
| Unknown/missing                        | 2                 | 0                 | 2                 |
| Neo-adjuvant treatment, n (%)          |                   |                   |                   |
| Yes                                    | 153 (22.7)        | 163 (24.2)        | 142 (21.1)        |
| No                                     | 521 (77.3)        | 512 (75.9)        | 531 (78.9)        |
| Unknown/missing                        | 1                 | 0                 | 1                 |
| Surgery, n (%)                         |                   |                   |                   |
| Yes                                    | 650 (96.4)        | 658 (97.8)        | 655 (97.3)        |
| No                                     | 24 (3.6)          | 15 (2.2)          | 18 (2.7)          |
| Unknown/missing                        | 1                 | 2                 | 1                 |
| Adjuvant treatment, n (%)              |                   |                   |                   |
| Yes                                    | 175 (26.2)        | 207 (31.0)        | 214 (32.1)        |
| No                                     | 493 (73.8)        | 461 (69.0)        | 452 (67.9)        |
| Unknown/missing                        | 7                 | 7                 | 8                 |
| Cohort, n (%)                          |                   |                   |                   |
| COLON                                  | 487 (72.2)        | 384 (56.9)        | 223 (33.1)        |
| EnCoRe                                 | 111 (15.4)        | 119 (17.6)        | 67 (9.9)          |
| CORSA                                  | 51 (7.6)          | 76 (11.3)         | 82 (12.2)         |
| ColoCare HD                            | 25 (3.7)          | 85 (12.6)         | 150 (22.3)        |
| ColoCare HCI                           | 0 (0.0)           | 1 (0.2)           | 45 (6.7)          |
| ColoCare FHCRC                         | 1 (0.2)           | 10 (1.5)          | 107 (15.9)        |

Adherence to physical activity guidelines†,
| Description                                      | Yes          | No           | Unknown/missing, n |
|-------------------------------------------------|--------------|--------------|--------------------|
| Dietary supplement use‡, n (%)                  | 179 (29.7)   | 212 (37.2)   | 291 (57.1)         |
| Contains folic acid                             | 65 (10.8)    | 105 (18.4)   | 172 (33.7)         |
| Unknown supplement use                          | 33 (4.9)     | 69 (12.0)    | 199 (39.5)         |
| Total folate concentration (nmol/l), median (IQR)| 8.5          | 15.0         | 30.9               |
| Participants with detectable folic acid concentrations§, n (%) | 33 (4.9)     | 69 (12.0)    | 199 (39.5)         |
| Detectable folic acid concentrations (nmol/l), median (IQR) | 0.7 (0.6-1.0) | 0.8 (0.6-1.2) | 1.2 (0.8-2.3)      |
| Participants with detectable pABG concentrations¶, n (%) | 653 (96.7)   | 639 (94.7)   | 654 (97.0)         |
| Detectable pABG concentrations (nmol/l), median (IQR) | 3.3 (1.5-6.0) | 2.3 (0.9-5.5) | 1.8 (0.8-4.9)      |
| Participants with detectable apABG concentrations#, n (%) | 581 (86.1)   | 599 (88.7)   | 621 (92.1)         |
| Detectable apABG concentrations (nmol/l), median (IQR) | 0.6 (0.4-0.8)| 0.7 (0.5-0.9)| 0.9 (0.7-1.3)      |
| Total energy intake (kcal/day), median (IQR)    | 1867 (1562-2279)| 1944 (1612-2355)| 1807 (1462-2199)|
| Unknown/missing, n                              | 94           | 169          | 282                |
| Alcohol intake (g/day), median (IQR)            | 7.6          | 9.0          | 8.0                |
| Unknown/missing, n                              | 79           | 113          | 201                |
| Follow-up time**, median (range)                | 3.8y         | 3.6y         | 3.6y               |
| Deceased††, n (%)                               | 98 (14.5)    | 100 (14.8)   | 90 (13.4)          |
| Location of the recurrence, n (%)               | 25 (3.7)     | 18 (2.7)     | 23 (3.6)           |
| Locoregional                                    | 75 (11.2)    | 68 (10.3)    | 71 (11.2)          |
| Unknown location                                | 1 (0.2)      | 3 (0.5)      | 3 (0.5)            |
| Disease-free survival§§, n (%)                  | 138 (20.5)   | 150 (22.8)   | 140 (21.8)         |

*Participants with at least one event

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1. Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, reverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.
2. At least 150 minutes per week of moderate-to-vigorous physical activity.
3. Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for COLON and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.
4. Pearson correlation coefficient between log2 transformed folate concentrations and log2 transformed folic acid concentrations is 0.45 (n=301).
5. Detectable folic acid concentrations > 0.52nmol/l.
6. Detectable pABG concentrations > 0.08nmol/l.
7. Detectable apABG concentrations > 0.13nmol/l.
8. Follow-up time calculated using overall survival.
9. Overall survival events were investigated by using death from any cause in the analysis.
10. Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection.
11. Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis.
### Supplementary Table 2. Baseline characteristics of participants with and without detectable folic acid concentrations and stratified by tertiles of folic acid

| Characteristics                              | Participants without detectable folic acid concentrations (n=1723) | Participants with detectable folic acid concentrations (n=301) | Tertile 1 (n=100) | Tertile 2 (n=101) | Tertile 3 (n=100) |
|----------------------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|-------------------|-------------------|-------------------|
| Men, n (%)                                   | 1139 (66.1)                                                  | 165 (54.8)                                                   | 57 (57.0)         | 55 (54.5)         | 53 (53.0)         |
| Age at diagnosis (years), median (IQR)       | 66.0 (60.0-72.5)                                             | 67.0 (60.1-73.4)                                             | 67.3              | 66.8              | 66.4              |
| Body mass index (kg/m²), median (IQR)        | 26.5 (24.2-29.4)                                             | 26.5 (23.9-29.9)                                             | 26.3              | 25.8              | 27.7              |
| Underweight, <18.5, n (%)                    | 15 (0.9)                                                     | 2 (0.7)                                                      | 0 (0.0)           | 2 (2.0)           | 0 (0.0)           |
| Normal weight, 18.5-24.9, n (%)              | 576 (33.8)                                                   | 108 (36.6)                                                   | 39 (40.6)         | 38 (38.0)         | 31 (31.3)         |
| Overweight, 25-29.9, n (%)                   | 751 (44.1)                                                   | 112 (38.0)                                                   | 35 (36.5)         | 43 (43.0)         | 34 (34.3)         |
| Obese, ≥30, n (%)                            | 360 (21.2)                                                   | 73 (24.8)                                                    | 22 (22.9)         | 17 (17.0)         | 34 (34.3)         |
| Smoking, n (%)                               | 215 (13.1)                                                   | 36 (12.6)                                                    | 11 (11.6)         | 15 (15.8)         | 10 (10.5)         |
| Stage of disease, n (%)                      | 451 (26.2)                                                   | 92 (30.6)                                                    | 36 (36.0)         | 28 (27.7)         | 28 (28.0)         |
| Tumor location*, n (%)                       | 1069 (62.2)                                                  | 183 (61.0)                                                   | 62 (62.0)         | 67 (66.3)         | 54 (54.6)         |
| Neo-adjuvant treatment, n (%)                | 395 (23.0)                                                   | 63 (20.9)                                                    | 20 (20.0)         | 19 (18.8)         | 24 (24.0)         |
| Surgery, n (%)                               | 2 (0.0)                                                      | 0 (0.0)                                                      | 0 (0.0)           | 0 (0.0)           | 0 (0.0)           |
| Category                                      | Yes                  | No                  | Unknown/missing, n |
|-----------------------------------------------|----------------------|---------------------|--------------------|
| Adjuvant treatment, n (%)                     | 1672 (97.2)          | 48 (2.8)            | 3                  |
| Adherence to physical activity guidelines†, n (%) | 964 (66.4)           | 487 (33.6)          | 272                |
| Dietary supplement use‡, n (%)                | 520 (36.0)           | 277                 | 277                |
| Total folate concentration§ (nmol/l), median (IQR) | 13.9 (9.4-21.1)      | 19.9 (16.8-46.9)    | 0.0 (0.0-0.0) |
| Detectable folic acid concentrations§, n (%)  | 0.0 (0.0-0.0)        | 1.0 (0.7-1.9)       | 0.6 (0.6-0.7) |
| Detectable pABG concentrations§, n (%)        | 2.3 (0.9-4.9)        | 4.1 (1.6-10.4)      | 4.6 (1.6-8.2) |
| Participants with detectable apABG concentrations§, n (%) | 1528 (88.7)          | 273 (90.7)          | 85 (85.0)          |
| Cohort, n (%)                                 | COLON 940 (54.6)     | 250 (14.5)          | 4 (0.0)            |
| EnCoRe                                       | 35 (11.6)            | 77 (27.8)           | 0 (0.0)            |
| CORSA                                        | 146 (14.3)           | 47 (15.6)           | 0 (0.0)            |
| ColoCare HD                                  | 34 (2.0)             | 12 (4.0)            | 0 (0.0)            |
| ColoCare HCI                                 | 79 (4.6)             | 39 (13.0)           | 0 (0.0)            |
| ColoCare FHCRC                               | 964 (66.4)           | 487 (33.6)          | 272                |
| Detectable folic acid concentrations§, n (%)  | 0.0 (0.0-0.0)        | 1.0 (0.7-1.9)       | 0.6 (0.6-0.7) |
| Detectable pABG concentrations§, n (%)        | 2.3 (0.9-4.9)        | 4.1 (1.6-10.4)      | 4.6 (1.6-8.2) |
| Participants with detectable apABG concentrations§, n (%) | 1528 (88.7)          | 273 (90.7)          | 85 (85.0)          |
| Detectable apABG concentrations \( (\text{nmol/l}) \), median (IQR) | 0.7 (0.5-1.0) | 0.9 (0.6-1.4) | 0.8 (0.6-0.9) | 0.9 (0.6-1.2) | 1.3 (0.8-2.0) |
|---------------------------------------------------------------|----------------|----------------|----------------|----------------|----------------|
| Total energy intake (kcal/day), median (IQR)                 | 1884           | 1860           | 1865           | 1888           | 1846           |
| Unknown/missing, n                                           | 457            | 88             | 28             | 28             | 40             |
| Alcohol intake (g/day), median (IQR)                         | 8.5            | 6.4            | 7.0            | 8.1            | 3.3            |
| Unknown/missing, n                                           | 314            | 79             | 20             | 24             | 35             |
| Follow-up time\*, median (range)                            | 3.7y           | 4.0y           | 3.8y           | 4.1y           | 4.1y           |
| Deceased\††, n (%)                                          | 237 (13.8)     | 51 (16.9)      | 10 (10.0)      | 18 (17.8)      | 23 (23.0)      |
| Yes                                                          | 216 (12.9)     | 42 (14.2)      | 8 (8.0)        | 14 (14.1)      | 20 (20.2)      |
| Unknown/missing, n                                           | 42             | 4              | 0              | 2              | 4              |
| Location of the recurrence, n (%)                            | 48 (2.9)       | 18 (6.1)       | 3 (3.1)        | 8 (8.1)        | 7 (7.1)        |
| Locoregional                                                 |                |                |                |                |                |
| Distant                                                      | 182 (10.9)     | 32 (10.8)      | 6 (6.1)        | 11 (11.1)      | 15 (15.2)      |
| Unknown location                                             | 6 (0.4)        | 1 (0.3)        | 0 (0.0)        | 1 (1.0)        | 1 (1.0)        |
| Disease-free survival\‡‡, n (%)                              | 354 (21.1)     | 74 (25.0)      | 15 (15.3)      | 27 (27.3)      | 32 (32.3)      |
| Participants with at least one event                         |                |                |                |                |                |

\*Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

\†At least 150 minutes per week of moderate-to-vigorous physical activity.

\‡Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for COLON and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.

\§Pearson correlation coefficient between log2 transformed folate concentrations and log2 transformed folic acid concentrations is 0.45 (n=301).

\∥Detectable folic acid concentrations > 0.52nmol/l.

\¶Detectable pABG concentrations > 0.08nmol/l.

\#Detectable apABG concentrations > 0.13nmol/l.

\**Follow-up time calculated using overall survival.

\††Overall survival events were investigated by using death from any cause in the analysis.

\‡‡Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection.

\§§Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis.

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| Characteristics                        | pABG tertiles | apABG tertiles |
|---------------------------------------|---------------|----------------|
|                                       | Tertile 1 (n=647) | Tertile 2 (n=649) | Tertile 3 (n=650) | Tertile 1 (n=600) | Tertile 2 (n=600) | Tertile 3 (n=601) |
| Men, n (%)                            | 442 (68.3)    | 420 (74.7)    | 388 (59.7)    | 388 (64.7)    | 387 (64.5)    | 398 (66.2)    |
| Age at diagnosis (years), median (IQR)| 66.0          | 66.0          | 66.3          | 66.0          | 65.8          | 67.0          |
| Body mass index (kg/m²), median (IQR) | 27.0          | 26.6          | 25.8          | 26.1          | 26.6          | 26.8          |
| Underweight, <18.5, n (%)             | 6 (1.0)       | 4 (0.6)       | 6 (0.9)       | 5 (0.9)       | 5 (0.8)       | 6 (1.0)       |
| Normal weight, 18.5-24.9, n (%)       | 195 (30.8)    | 210 (32.7)    | 256 (39.7)    | 211 (35.8)    | 206 (34.7)    | 180 (30.5)    |
| Overweight, 25-29.9, n (%)            | 277 (43.7)    | 276 (43.0)    | 274 (42.5)    | 260 (44.1)    | 256 (43.1)    | 258 (43.7)    |
| Obese, ≥30, n (%)                     | 156 (24.6)    | 152 (23.7)    | 109 (16.9)    | 113 (19.2)    | 127 (21.4)    | 147 (24.9)    |
| Unknown/missing, n                    | 13            | 7             | 11            | 6             | 10            |
| Smoking, n (%)                        |               |               |               |               |               |               |
| Current                               | 82 (13.6)     | 82 (13.1)     | 70 (11.2)     | 85 (14.5)     | 71 (12.5)     | 60 (10.7)     |
| Never                                 | 299 (49.7)    | 358 (57.2)    | 346 (55.5)    | 309 (52.8)    | 312 (55.0)    | 297 (53.1)    |
| Never                                 | 221 (36.7)    | 186 (29.7)    | 208 (33.3)    | 191 (32.7)    | 184 (32.5)    | 202 (36.1)    |
| Unknown/missing, n                    | 45            | 23            | 26            | 15            | 33            | 42            |
| Stage of disease, n (%)               |               |               |               |               |               |               |
| I                                     | 188 (29.1)    | 163 (25.1)    | 171 (26.2)    | 168 (28.0)    | 165 (27.5)    | 154 (25.6)    |
| II                                    | 191 (29.5)    | 192 (29.6)    | 190 (29.2)    | 155 (25.8)    | 176 (29.3)    | 182 (30.3)    |
| III                                   | 249 (38.5)    | 273 (42.1)    | 273 (42.0)    | 261 (43.6)    | 238 (39.7)    | 249 (41.4)    |
| Unspecified/unknown                   | 19 (2.9)      | 21 (3.2)      | 16 (2.5)      | 16 (2.7)      | 21 (3.5)      | 16 (2.7)      |
| Tumor location*, n (%)                |               |               |               |               |               |               |
| Colon                                 | 389 (60.2)    | 410 (63.5)    | 416 (64.0)    | 391 (65.4)    | 365 (60.8)    | 355 (59.3)    |
| Rectal                                | 257 (39.8)    | 236 (36.5)    | 234 (36.0)    | 207 (34.6)    | 235 (39.2)    | 244 (40.7)    |
| Unknown/missing, n                    | 1             | 3             | 0             | 2             | 2             |
| Neo-adjuvant treatment, n (%)         |               |               |               |               |               |               |
| Yes                                   | 130 (20.1)    | 150 (23.2)    | 155 (23.9)    | 131 (21.8)    | 130 (21.7)    | 144 (24.0)    |
| No                                    | 516 (79.9)    | 498 (76.9)    | 495 (76.2)    | 469 (78.2)    | 469 (78.3)    | 457 (76.0)    |
| Unknown/missing, n                    | 1             | 1             | 0             | 1             | 0             |
| Surgery, n (%)                        |               |               |               |               |               |               |
| Yes                                   | 621 (33.2)    | 624 (96.3)    | 642 (98.9)    | 571 (95.3)    | 584 (97.5)    | 589 (98.2)    |
| No | 24 (3.7) | 24 (3.7) | 7 (1.1) | 28 (4.7) | 15 (2.5) | 11 (1.8) |
| Unknown/missing, n | 2 | 1 | 1 | 1 | 1 | 1 |
| Adjuvant treatment, n (%) | | | | | | |
| Yes | 212 (33.2) | 182 (28.4) | 169 (26.2) | 177 (29.9) | 184 (31.1) | 177 (29.7) |
| No | 426 (66.8) | 458 (71.6) | 477 (73.8) | 416 (70.2) | 407 (68.9) | 419 (70.3) |
| Unknown/missing, n | 9 | 9 | 4 | 7 | 9 | 5 |
| Cohort, n (%) | | | | | | |
| COLON | 171 (26.4) | 371 (57.2) | 545 (83.9) | 391 (65.2) | 295 (49.2) | 264 (43.9) |
| EnCoRe | 82 (12.7) | 151 (23.3) | 64 (9.9) | 93 (15.5) | 103 (17.2) | 75 (12.5) |
| CORSA | 139 (21.5) | 38 (5.9) | 17 (2.6) | 95 (15.8) | 62 (10.3) | 38 (6.3) |
| ColoCare HD | 150 (23.2) | 50 (7.7) | 13 (2.0) | 17 (2.8) | 90 (15.0) | 122 (20.3) |
| ColoCare HCl | 27 (4.2) | 11 (1.7) | 3 (0.5) | 2 (0.3) | 8 (1.3) | 35 (5.8) |
| ColoCare FHCRC | 78 (12.1) | 28 (4.3) | 8 (1.2) | 2 (0.3) | 42 (7.0) | 67 (11.2) |
| Adherence to physical activity guidelines†, n (%) | | | | | | |
| Yes | 255 (56.7) | 411 (70.6) | 434 (71.4) | 356 (72.2) | 324 (65.2) | 293 (57.2) |
| No | 195 (43.3) | 171 (29.4) | 174 (28.6) | 137 (27.8) | 173 (34.8) | 219 (42.8) |
| Unknown/missing, n | 197 | 67 | 42 | 107 | 103 | 89 |
| Dietary supplement use‡, n (%) | | | | | | |
| Any | 148 (33.6) | 213 (36.9) | 311 (50.9) | 173 (35.2) | 203 (41.4) | 234 (46.3) |
| Containing folic acid | 39 (8.8) | 101 (17.5) | 202 (33.1) | 95 (19.4) | 93 (19.0) | 111 (21.9) |
| Unknown supplement use, n | 206 | 72 | 39 | 109 | 110 | 95 |
| Total folate concentration (nmol/l), median (IQR) | | | | | | |
| Participants with detectable folic acid concentrations§, n (%) | 64 (9.9) | 87 (13.4) | 150 (23.1) | 57 (9.5) | 71 (11.8) | 145 (24.1) |
| Detectable folic acid concentrations (nmol/l), median (IQR) | 1.1 (0.7-1.7) | 1.2 (0.7-2.2) | 0.9 (0.7-1.9) | 0.9 (0.6-1.2) | 0.8 (0.6-1.1) | 1.3 (0.9-2.3) |
| Participants with detectable pABG concentrations§, n (%) | 647 (100.0) | 649 (100.0) | 650 (100.0) | 584 (97.3) | 571 (95.2) | 582 (96.8) |
| Detectable pABG concentrations (nmol/l), median (IQR) | 0.7 (0.5-1.0) | 2.5 (1.9-3.2) | 7.7 (5.4-11.7) | 2.7 (1.0-5.8) | 2.4 (1.0-5.0) | 2.1 (1.0-5.0) |
| Participants with detectable apABG concentrations§, n (%) | 588 (90.9) | 582 (89.7) | 567 (87.2) | 600 (100.0) | 600 (100.0) | 601 (100.0) |
| Detectable apABG concentrations (nmol/l), median (IQR) | 0.7 (0.5-1.1) | 0.7 (0.5-1.0) | 0.7 (0.5-1.0) | 0.4 (0.4-0.5) | 0.7 (0.7-0.8) | 1.2 (1.0-1.5) |

Supplementary Materials
| Total energy intake (kcal/day), median | 1886 | 1923 | 1836 | 1848 | 1907 | 1881 |
|--------------------------------------|------|------|------|------|------|------|
| (IQR)                                | (1528-2301) | (1597-2359) | (1506-2184) | (1550-2245) | (1594-2304) | (1487-2292) |
| Unknown/missing, n                   | 312  | 113  | 62   | 125  | 174  | 194  |
| Alcohol intake (g/day), median (IQR) | 7.7  | 7.9  | 8.5  | 7.7  | 8.9  | 8.5  |
| Unknown/missing, n                   | (0.8-21.5) | (1.1-20.5) | (1.0-20.8) | (0.9-19.5) | (1.4-21.9) | (0.5-22.0) |
| Follow-up time, median (range)       | 3.5y | 3.5y | 4.3y | 4.1y | 3.5y | 3.3y |
| Deceased**, n (%)                    | 104 (16.1) | 86 (13.3) | 85 (13.1) | 79 (13.2) | 89 (14.8) | 93 (15.5) |
| Recurrence††, n (%)                  | Yes  | 86 (13.6) | 74 (11.7) | 89 (14.0) | 73 (12.4) | 86 (14.7) | 73 (12.5) |
| Location of the recurrence, n (%)    | 35   | 12   | 3    | 4    | 17   | 17   |
| Locoregional                         | 22 (3.5) | 12 (1.9) | 31 (4.9) | 22 (3.8) | 20 (3.4) | 19 (3.2) |
| Distant                              | 66 (10.4) | 66 (10.4) | 74 (11.7) | 62 (10.6) | 74 (12.7) | 58 (9.9) |
| Unknown location                     | 6 (1.0) | 0 (0.0) | 1 (0.2) | 3 (0.5) | 2 (0.3) | 3 (0.5) |
| Disease-free survival‡‡, n (%)       | Participants with at least one event | 159 (25.1) | 122 (19.2) | 129 (20.3) | 120 (20.4) | 135 (23.1) | 132 (22.5) |

*Tumor location is defined as colon (cecum, appendix and ascending colon, hepatic flexure, transverse colon, splenic flexure, descending colon and sigmoid colon) and rectal (rectosigmoid junction and rectum) cancer.

†At least 150 minutes per week of moderate-to-vigorous physical activity.

‡Dietary supplement use is defined as the use of single micronutrients and/or the use of multivitamins in the last year for COLON and EnCoRe and in the last month for the ColoCare sites. Dietary supplement use was not collected in the CORSA study.

§Detectable folic acid concentrations > 0.52 nmol/l.

||Detectable pABG concentrations > 0.08 nmol/l.

¶Detectable apABG concentrations > 0.13 nmol/l.

#Follow-up time calculated using overall survival.

**Overall survival events were investigated by using death from any cause in the analysis.

††Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection.

‡‡Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis.
### Supplementary Table 4. Associations between cohort-specific tertiles of circulating concentrations of folate, folic acid, pABG, and apABG and recurrence and survival

| Folate species | Median (IQR) | No. of events/at risk | Crude HR (95% CI) | Adj. HR (95% CI)§ | No. of events/at risk | Crude HR (95% CI) | Adj. HR (95% CI)§ | No. of events/at risk | Crude HR (95% CI) | Adj. HR (95% CI)§ |
|---------------|-------------|----------------------|-------------------|-------------------|----------------------|-------------------|-------------------|----------------------|-------------------|-------------------|
| Folate (nmol/l) |             |                      |                   |                   |                      |                   |                   |                      |                   |                   |
| T1‖           | 8.5 (6.3 to 10.3) | 85/662               | 1.00 (referent)   | 1.00 (referent)   | 103/678              | 1.00 (referent)   | 1.00 (referent)   | 146/662              | 1.00 (referent)   | 1.00 (referent)   |
| T2‖           | 14.1 (12.0 to 18.2) | 91/665               | 1.09 (0.81 to 1.47) | 1.10 (0.82 to 1.48) | 104/676              | 1.01 (0.77 to 1.32) | 0.99 (0.75 to 1.30) | 154/665              | 1.07 (0.85 to 1.34) | 1.05 (0.84 to 1.32) |
| T3‖           | 26.8 (20.0 to 38.6) | 82/646               | 1.00 (0.74 to 1.35) | 1.08 (0.80 to 1.47) | 81/670              | 0.73 (0.54 to 0.97) | 0.80 (0.60 to 1.07) | 128/646              | 0.86 (0.68 to 1.09) | 0.92 (0.73 to 1.17) |
| Pₚend         |             | 0.98                 | 0.59              |                   |                      |                   |                   |                      |                   |                   |
| Folic acid (nmol/l) |         |                      |                   |                   |                      |                   |                   |                      |                   |                   |
| T1‖           | 0.6 (0.6 to 0.7) | 10/100               | 1.00 (referent)   | 1.00 (referent)   | 12/102              | 1.00 (referent)   | 1.00 (referent)   | 19/100               | 1.00 (referent)   | 1.00 (referent)   |
| T2‖           | 0.9 (0.8 to 1.4) | 15/101               | 1.60 (0.72 to 3.57) | 1.67 (0.74 to 3.75) | 22/103             | 1.79 (0.89 to 3.63) | 2.05 (0.99 to 4.22) | 29/101               | 1.55 (0.87 to 2.76) | 1.66 (0.92 to 3.00) |
| T3‖           | 2.6 (1.5 to 5.6) | 17/95                | 1.87 (0.86 to 4.09) | 2.45 (1.09 to 5.49) | 17/96              | 1.41 (0.67 to 2.95) | 1.58 (0.74 to 3.38) | 26/95                | 1.47 (0.81 to 2.66) | 1.61 (0.88 to 2.97) |
| Pₚend         |             | 0.12                 | 0.03              |                   |                      |                   |                   |                      |                   |                   |
| pABG (nmol/l)  |             |                      |                   |                   |                      |                   |                   |                      |                   |                   |
| T1‖           | 0.9 (0.5 to 1.6) | 82/637               | 1.00 (referent)   | 1.00 (referent)   | 94/650              | 1.00 (referent)   | 1.00 (referent)   | 145/637              | 1.00 (referent)   | 1.00 (referent)   |
| T2‖           | 3.0 (1.1 to 4.4) | 78/635               | 0.92 (0.67 to 1.25) | 0.94 (0.69 to 1.28) | 84/653             | 0.81 (0.60 to 1.09) | 0.87 (0.64 to 1.16) | 128/635              | 0.81 (0.64 to 1.03) | 0.84 (0.66 to 1.07) |
| T3‖           | 7.7 (3.9 to 11.8) | 89/632               | 1.03 (0.77 to 1.40) | 1.06 (0.78 to 1.44) | 97/643             | 0.92 (0.70 to 1.23) | 1.02 (0.76 to 1.36) | 137/632              | 0.86 (0.68 to 1.08) | 0.91 (0.72 to 1.15) |
| Pₚend         |             | 0.82                 | 0.70              |                   |                      |                   |                   |                      |                   |                   |
| apABG (nmol/l) |             |                      |                   |                   |                      |                   |                   |                      |                   |                   |
| T1‖           | 0.4 (0.4 to 0.5) | 76/587               | 1.00 (referent)   | 1.00 (referent)   | 70/603              | 1.00 (referent)   | 1.00 (referent)   | 116/587              | 1.00 (referent)   | 1.00 (referent)   |
| T2‖           | 0.7 (0.6 to 0.8) | 84/590               | 1.14 (0.83 to 1.55) | 1.09 (0.80 to 1.49) | 92/605             | 1.35 (0.99 to 1.85) | 1.24 (0.91 to 1.70) | 140/590              | 1.24 (0.97 to 1.59) | 1.17 (0.91 to 1.50) |
| T3‖           | 1.2 (1.0 to 1.6) | 72/581               | 1.04 (0.75 to 1.44) | 1.04 (0.75 to 1.44) | 99/593             | 1.67 (1.23 to 2.27) | 1.42 (1.04 to 1.93) | 131/581              | 1.30 (1.02 to 1.67) | 1.19 (0.92 to 1.53) |
| Pₚend         |             | 0.79                 | 0.82              |                   |                      |                   |                   |                      |                   |                   |

*Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection. A HR > 1.00 should be interpreted as an increased risk of recurrence.

†Overall survival events were investigated by using death from any cause in the analysis. A HR > 1.00 should be interpreted as a reduced overall survival (more deaths).

‡Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis. A HR > 1.00 should be interpreted as a reduced disease-free survival (more deaths and/or recurrences).

§Adjusted for age, sex, chemotherapy status, and cohort for folate and folic acid. pABG and apABG were additionally adjusted for log2 transformed creatinine concentrations.

**Supplementary Materials**
Stage was tested as potential confounder in all models, but it did not influence the effect estimates.

\[ \text{Supplementary Materials} \]

Tertile cut-off values for folate were 9.6 nmol/l and 16.0 nmol/l for COLON, 13.3 nmol/l and 22.4 nmol/l for EnCoRe, 10.6 nmol/l and 16.5 nmol/l for CORSA, 18.1 nmol/l and 26.7 nmol/l for ColoCare HD, 51.0 nmol/l and 80.8 nmol/l for ColoCare HCI, 35.2 nmol/l and 61.0 nmol/l for ColoCare FHCRC; analysis performed using non-log2 transformed concentrations.

Analysis only performed for participants with detectable folic acid, pABG, or apABG; analysis performed using non-log2 transformed concentrations.

Tertile cut-off values for folic acid were 0.7 nmol/l and 1.0 nmol/l for COLON, 0.7 nmol/l and 0.9 nmol/l for EnCoRe, 0.7 nmol/l and 2.4 nmol/l for CORSA, 1.4 nmol/l and 1.7 nmol/l for ColoCare HD, 1.6 nmol/l and 3.6 nmol/l for ColoCare HCI, 1.5 nmol/l and 3.0 nmol/l for ColoCare FHCRC.

Tertile cut-off values for pABG were 2.7 nmol/l and 6.5 nmol/l for COLON, 1.6 nmol/l and 3.2 nmol/l for EnCoRe, 0.5 nmol/l and 1.2 nmol/l for CORSA, 0.7 nmol/l and 1.4 nmol/l for ColoCare HD, 0.6 nmol/l and 1.4 nmol/l for ColoCare HCI, 0.7 nmol/l and 1.3 nmol/l for ColoCare FHCRC.

Tertile cut-off values for apABG were 0.5 nmol/l and 0.8 nmol/l for COLON, 0.6 nmol/l and 0.8 nmol/l for EnCoRe, 0.5 nmol/l and 0.7 nmol/l for CORSA, 0.8 nmol/l and 1.1 nmol/l for ColoCare HD, 1.0 nmol/l and 1.6 nmol/l for ColoCare HCI, 0.8 nmol/l and 1.4 nmol/l for ColoCare FHCRC.
### Supplementary Table 5. Associations between circulating concentrations of folate, folic acid, pABG, and apABG and recurrence and survival excluding participants of whom blood was collected during or after any type of treatment

| Folate species | Median (IQR) | No. of events/at risk | Crude HR (95% CI) | Adj. HR (95% CI)§ | No. of deaths/at risk | Crude HR (95% CI) | Adj. HR (95% CI)§ | No. of events/at risk | Crude HR (95% CI) | Adj. HR (95% CI)§ |
|----------------|--------------|-----------------------|-------------------|-------------------|----------------------|-------------------|-------------------|----------------------|-------------------|-------------------|
| **Folate (nmol/l)** | | | | | | | | | | |
| Continuous† | 14.8 (9.6 to 23.3) | 212/1711 | 1.04 (0.91 to 1.19) | 1.03 (0.88 to 1.21) | 245/1749 | 0.98 (0.87 to 1.12) | 0.93 (0.80 to 1.08) | 359/1711 | 1.01 (0.91 to 1.12) | 0.98 (0.86 to 1.11) |
| T1‡ | 8.4 (6.2 to 9.6) | 73/570 | 1.00 (referent) | 1.00 (referent) | 84/583 | 1.00 (referent) | 1.00 (referent) | 119/570 | 1.00 (referent) | 1.00 (referent) |
| T2‡ | 14.9 (13.0 to 17.3) | 62/571 | 0.86 (0.61 to 1.20) | 0.87 (0.62 to 1.23) | 85/583 | 1.01 (0.74 to 1.36) | 0.93 (0.68 to 1.26) | 120/571 | 0.99 (0.77 to 1.28) | 0.95 (0.73 to 1.23) |
| T3‡ | 29.5 (23.7 to 41.0) | 77/570 | 1.06 (0.77 to 1.46) | 1.09 (0.76 to 1.56) | 76/583 | 0.90 (0.66 to 1.23) | 0.80 (0.57 to 1.13) | 120/571 | 0.97 (0.75 to 1.25) | 0.90 (0.68 to 1.20) |

| Folic acid (nmol/l) | | | | | | | | | | |
| **Undetectable** | †† | | | | | | | | | |
| Detectable‡ | 0.9 (0.7 to 1.8) | 35/259 | 1.12 (0.78 to 1.61) | 1.20 (0.83 to 1.74) | 43/264 | 1.18 (0.85 to 1.63) | 1.24 (0.89 to 1.73) | 62/259 | 1.17 (0.89 to 1.53) | 1.23 (0.93 to 1.62) |

| **Continuous** | ‡‡ | | | | | | | | | |
| T1†† | 0.6 (0.6 to 0.7) | 5/86 | 1.00 (referent) | 1.00 (referent) | 8/88 | 1.00 (referent) | 1.00 (referent) | 11/86 | 1.00 (referent) | 1.00 (referent) |
| T2†† | 0.9 (0.8 to 1.1) | 14/87 | 3.02 (1.09 to 8.40) | 3.76 (1.29 to 10.91) | 16/89 | 1.99 (0.85 to 4.66) | 1.61 (0.67 to 3.83) | 24/87 | 2.22 (1.09 to 4.55) | 2.41 (1.15 to 5.07) |
| T3†† | 2.6 (1.8 to 5.5) | 16/86 | 3.28 (1.20 to 8.96) | 4.48 (1.40 to 14.34) | 19/87 | 2.46 (1.08 to 5.62) | 1.48 (0.60 to 3.66) | 27/86 | 2.56 (1.27 to 5.16) | 2.54 (1.15 to 5.63) |

| pABG (nmol/l) | | | | | | | | | | |
| **Undetectable** | †† | | | | | | | | | |
| Detectable‡ | 2.6 (1.1 to 5.5) | 206/1666 | 0.95 (0.42 to 2.13) | 1.00 (0.43 to 2.33) | 236/1697 | 0.72 (0.37 to 1.40) | 0.95 (0.47 to 1.89) | 347/1666 | 0.78 (0.44 to 1.39) | 0.96 (0.53 to 1.74) |

| **Continuous** | ‡‡ | | | | | | | | | |
| T1†† | 0.7 (0.5 to 1.1) | 69/556 | 1.00 (referent) | 1.00 (referent) | 86/564 | 1.00 (referent) | 1.00 (referent) | 134/556 | 1.00 (referent) | 1.00 (referent) |
| T2†† | 2.6 (1.9 to 3.3) | 62/555 | 0.93 (0.66 to 1.32) | 0.94 (0.65 to 1.38) | 76/568 | 0.98 (0.72 to 1.34) | 1.29 (0.91 to 1.82) | 104/555 | 0.88 (0.68 to 1.14) | 0.98 (0.73 to 1.30) |
| T3†† | 7.9 (5.5 to 12.3) | 75/555 | 1.06 (0.76 to 1.47) | 1.05 (0.71 to 1.55) | 74/565 | 0.78 (0.57 to 1.07) | 1.17 (0.79 to 1.72) | 109/555 | 0.81 (0.63 to 1.05) | 0.94 (0.69 to 1.27) |

| apABG (nmol/l) | | | | | | | | | | |
| **Undetectable** | †† | | | | | | | | | |
| Detectable‡ | 0.7 (0.5 to 1.0) | 190/1520 | 1.17 (0.75 to 1.82) | 1.22 (0.78 to 1.92) | 222/1551 | 1.40 (0.91 to 2.15) | 1.24 (0.80 to 1.92) | 325/1520 | 1.32 (0.93 to 1.88) | 1.25 (0.87 to 1.80) |

**Supplementary Materials**
Recurrence is defined as colorectal cancer recurrence (event) after complete tumor resection. A HR > 1.00 should be interpreted as an increased risk of recurrence.

Overall survival events were investigated by using death from any cause in the analysis. A HR > 1.00 should be interpreted as a reduced overall survival (more deaths).

Disease-free survival was investigated by using a recurrence or death from any cause as events in the analysis. A HR > 1.00 should be interpreted as a reduced disease-free survival (more deaths and/or recurrences).

Adjusted for age, sex, chemotherapy status, and cohort for folate and folic acid. pABG and apABG were additionally adjusted for log2 transformed creatinine concentrations.

Stage was tested as potential confounder in all models, but it did not influence the effect estimates.

Analysis performed using log2 transformed concentrations. Thus, HRs represent a doubling in folate concentrations.

Analysis only performed for participants with detectable folic acid, pABG, or apABG. Tertile cut-off values were 0.71 nmol/l and 1.32 nmol/l for folic acid, 1.45 nmol/l and 4.26 nmol/l for pABG, and 0.57 nmol/l and 0.86 nmol/l for apABG; analysis performed using non-log2 transformed concentrations.

LOD: level of detection.

| Continuous** | 0.7 (0.5 to 1.0) | 0.95 (0.79 to 1.15) | 0.92 (0.75 to 1.14) | 222/1551 | 1.21 (1.03 to 1.43) | 1.08 (0.89 to 1.30) | 325/1520 | 1.10 (0.96 to 1.27) | 1.00 (0.85 to 1.17) |
| T1†† | 0.4 (0.4 to 0.5) | 0.95 (0.79 to 1.15) | 0.92 (0.75 to 1.14) | 222/1551 | 1.21 (1.03 to 1.43) | 1.08 (0.89 to 1.30) | 325/1520 | 1.10 (0.96 to 1.27) | 1.00 (0.85 to 1.17) |
| T2†† | 0.7 (0.6 to 0.8) | 1.23 (0.88 to 1.73) | 1.21 (0.85 to 1.71) | 1.55 (1.12 to 2.15) | 1.28 (0.89 to 1.83) | 1.05 (1.00 to 1.72) | 1.15 (0.85 to 1.54) |
| T3†† | 1.2 (1.0 to 1.5) | 0.97 (0.67 to 1.40) | 0.95 (0.64 to 1.41) | 78/517 | 1.55 (1.12 to 2.15) | 1.28 (0.89 to 1.83) | 108/507 | 1.31 (1.00 to 1.72) | 1.15 (0.85 to 1.54) |
| P_trend | 0.75 | 0.66 | 0.01 | 0.23 | 0.06 | 0.47 |
Supplementary Figure 1. Dose-response relationship of folate concentration (nmol/l) by cohort for recurrence, overall and disease to free survival. Hazard Ratios (HR) and corresponding 95% confidence intervals are shown for cohort to specific tertiles with the lowest tertile as the reference. Arrows represent an upper limit above a HR of 3.00 or a lower limit close to a HR of 0.00.
### Recurrence

| Subgroup          | Median IQR | Events At risk | Hazard Ratio [95% CI] |
|-------------------|------------|----------------|-----------------------|
| **Cohort**        |            |                |                       |
| COLOX             | 4.2 (2.0-6.3) | 144 1082       | 0.99 [0.89, 1.10]     |
| EnCoRe            | 2.3 (1.3-3.9) | 31 297         | 1.35 [0.97, 1.88]     |
| CORSRC            | 0.7 (0.4-1.5) | 23 194         | 0.83 [0.61, 1.13]     |
| CokCoCase HD      | 0.9 (0.6-1.6) | 27 186         | 0.86 [0.58, 1.30]     |
| CokCase HCl       | 0.9 (0.6-1.6) | 4 37           | 2.06 [1.32, 3.21]     |
| CokCoCase HGREC   | 1 (0.6-1.5)  | 20 108         | 1.11 [0.86, 1.42]     |
| **RE Model for Subgroup** | (Q = 15.09, df = 4, p = 0.00, I² = 73.50%) | | |

### Death from any cause (overall survival)

| No. of deaths At risk | Hazard Ratio [95% CI] |
|-----------------------|-----------------------|
| 137 1087              | 0.97 [0.86, 1.09]     |
| 28 297                | 1.69 [1.09, 1.49]     |
| 61 194                | 1.01 [0.81, 1.21]     |
| 23 213                | 1.13 [0.78, 1.64]     |
| 2 41                  | 4.16 [0.14, 123.60]   |
| 24 114                | 1.78 [1.20, 2.64]     |
| **RE Model for Subgroup** | (Q = 9.41, df = 5, p = 0.09; I² = 46.9%) | | |

### Recurrence or death from any cause (disease-free survival)

| Events At risk | Hazard Ratio [95% CI] |
|----------------|-----------------------|
| 214 1082       | 0.96 [0.77, 1.26]     |
| 49 297         | 1.19 [0.93, 1.52]     |
| 70 194         | 0.99 [0.80, 1.31]     |
| 42 186         | 0.93 [0.71, 1.22]     |
| 5 37           | 1.51 [1.06, 2.15]     |

### Disease stage

| Stage | Median IQR | Events At risk | Hazard Ratio [95% CI] |
|-------|------------|----------------|-----------------------|
| I     | 2.3 (0.9-5.3) | 17 510        | 0.96 [0.59, 1.52]     |
| II    | 2.5 (1.0-5.4) | 59 561        | 1.17 [0.50, 2.74]     |
| III   | 2.5 (1.0-5.5) | 167 777       | 1.03 [0.59, 1.82]     |
| **RE Model for Subgroup** | (Q = 1.82, df = 2, p = 0.40; I² = 0.99%) | | |

### Tumor location

| Location | Median IQR | Events At risk | Hazard Ratio [95% CI] |
|----------|------------|----------------|-----------------------|
| Colon    | 2.6 (1.0-5.5) | 135 1189      | 1.06 [0.92, 1.23]     |
| Rectal   | 2.3 (1.0-5.4) | 114 712       | 1.00 [0.86, 1.19]     |
| **RE Model for Subgroup** | (Q = 0.23, df = 1, p = 0.03; I² = 14.5%) | | |

### Sex

| Sex | Median IQR | Events At risk | Hazard Ratio [95% CI] |
|-----|------------|----------------|-----------------------|
| Men | 2.3 (1.0-5.0) | 170 1222      | 1.01 [0.90, 1.13]     |
| Women | 2.7 (1.6-6.6) | 79 682        | 1.07 [0.92, 1.24]     |
| **RE Model for Subgroup** | (Q = 0.35, df = 1, p = 0.55; I² = 0.00%) | | |

### Neo- and/or adjuvant treatment

| Treatment | Median IQR | Events At risk | Hazard Ratio [95% CI] |
|-----------|------------|----------------|-----------------------|
| Neo-adjuvant | 2.5 (1.2-5.6) | 74 430        | 0.91 [0.76, 1.09]     |
| Adjuvant   | 2.2 (0.9-4.8) | 103 550       | 1.05 [0.51, 2.12]     |
| No         | 2.6 (1.5-5.6) | 146 1354      | 1.00 [0.89, 1.12]     |
| **RE Model for Subgroup** | (Q = 1.49, df = 2, p = 0.47; I² = 0.00%) | | |

### Dietary supplement use

| Supplement | Median IQR | Events At risk | Hazard Ratio [95% CI] |
|-----------|------------|----------------|-----------------------|
| Any       | 3.7 (1.5-8.1) | 77 661        | 1.13 [0.96, 1.33]     |
| Containing folate | 5.4 (2.1-12.1) | 39 341   | 1.17 [0.92, 1.52]     |
| None      | 2.6 (1.4-9.9) | 126 938       | 0.95 [0.63, 1.45]     |
| **RE Model for Subgroup** | (Q = 3.64, df = 2, p = 0.16; I² = 45.1%) | | |

**Supplementary Figure 2.** Forest plots of subgroup analyses reporting hazard ratios and corresponding 95% CI for a doubling in pABG concentrations and recurrence, disease-free and overall survival. Weights of the effect estimates are from random effects meta-analysis; square dots represent the hazard ratio of each subgroup while the diamonds represent the hazard ratio of all subgroups combined. Heterogeneity among subgroups was evaluated using the I² index.

**Supplementary Materials**
Supplementary Figure 3. Forest plots of subgroup analyses reporting hazard ratios and corresponding 95% CI for a doubling in apABG concentrations and recurrence, disease-free and overall survival. Weights of the effect estimates are from random effects meta-analysis; square dots represent the hazard ratio of each subgroup while the diamonds represent the hazard ratio of all subgroups combined. Heterogeneity among subgroups was evaluated using the $I^2$ index.