

**Table S1. TaqMan™ probes used in real-time qPCR gene expression assay.**

| <b>Gene symbol</b> | <b>Description</b>                                     | <b>TaqMan™ probe</b> |
|--------------------|--------------------------------------------------------|----------------------|
| <i>IL10</i>        | Interleukin 10                                         | Hs00961622_m1        |
| <i>NOD2</i>        | Nucleotide Binding Oligomerization Domain Containing 2 | Hs01550753_m1        |
| <i>SUCNR1</i>      |                                                        | Hs00263701_m1        |
| <i>TLR4</i>        |                                                        | Hs00152939_m1        |

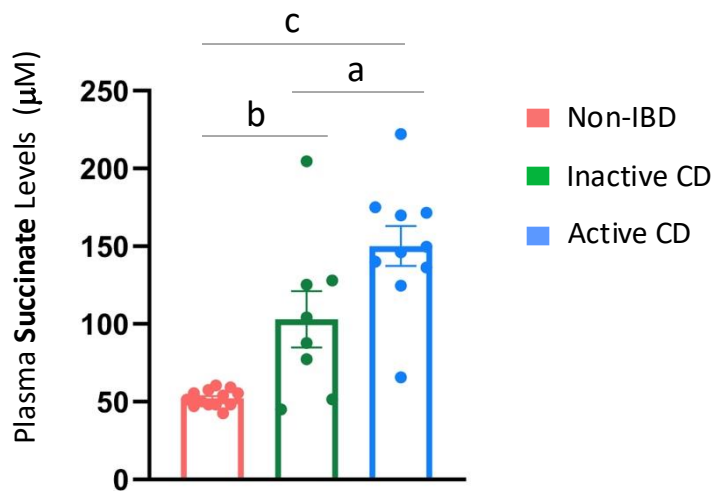
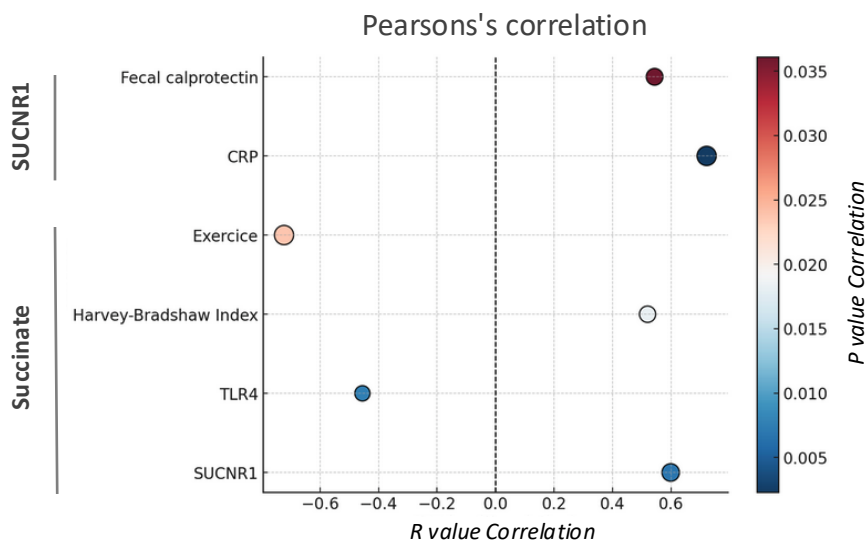
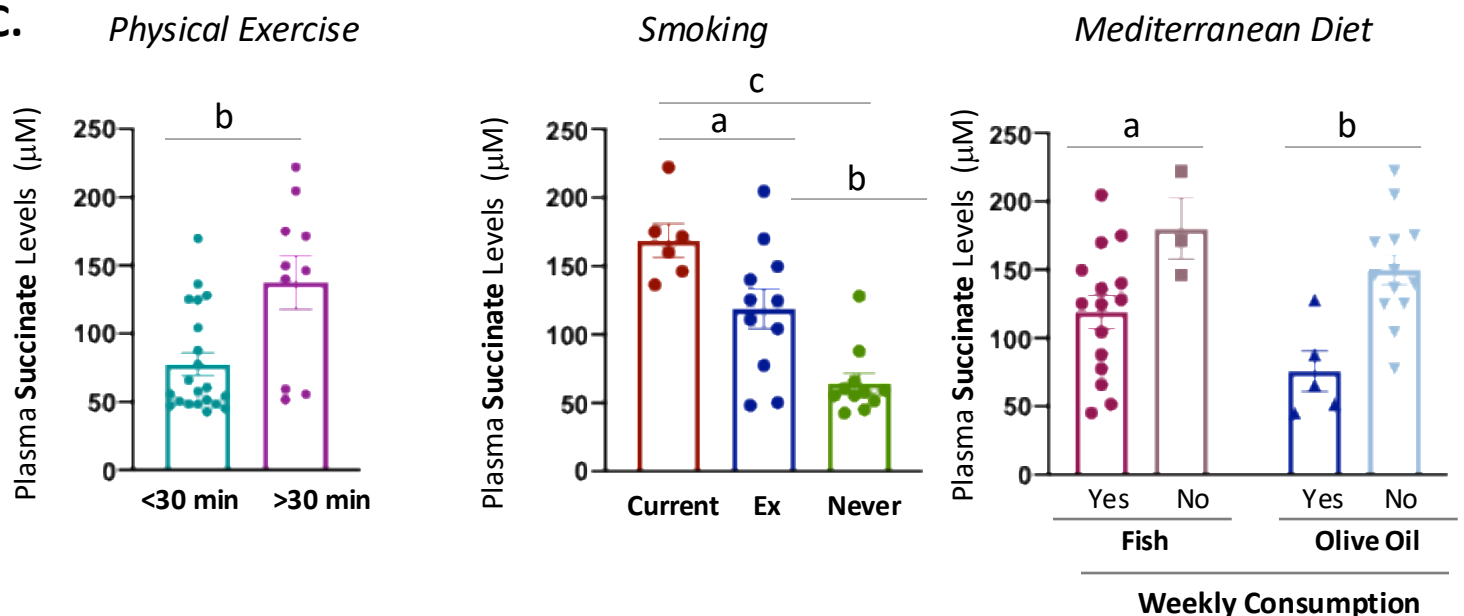
**Table S2. Demographic and epidemiological features of the validation cohort in the postoperative recurrence (POR) stratification.**

|                                                  | NR          | MR          | NR vs MR<br>(P-Value) | SR         | NR vs SR<br>(P-Value) | MR vs SR<br>(P-Value) |
|--------------------------------------------------|-------------|-------------|-----------------------|------------|-----------------------|-----------------------|
| <b>n</b>                                         | 22          | 30          |                       | 14         |                       |                       |
| <b>Age, yr</b>                                   | 35 (29-44)  | 37 (25-50)  | ns                    | 36 (28-42) | ns                    | ns                    |
| <b>Female sex; n (%)</b>                         | 14 (64)     | 12 (40)     | ns                    | 5 (36)     | ns                    | ns                    |
| <b>BMI</b>                                       | 22 (19-23)  | 23 (19-25)  | ns                    | 22 (19-24) | ns                    | ns                    |
| <b>Active smoking; n (%)</b>                     |             |             |                       |            |                       |                       |
| Never                                            | 7 (32)      | 12 (40)     |                       | 5 (36)     |                       |                       |
| Ex-smoker                                        | 2 (9)       | 5 (17)      | ns                    | 0          | ns                    | ns                    |
| Active                                           | 13 (59)     | 13 (43)     |                       | 9 (64)     |                       |                       |
| <b>CD location; n (%)</b>                        |             |             |                       |            |                       |                       |
| Ileal                                            | 15 (68)     | 19 (63)     |                       | 9 (64)     |                       |                       |
| Ileocolic                                        | 7 (32)      | 11 (37)     | ns                    | 5 (36)     | ns                    | ns                    |
| <b>Perianal disease; n (%)</b>                   | 6 (27)      | 10 (33)     | ns                    | 5 (36)     | ns                    | ns                    |
| <b>Family background; n (%)</b>                  | 3 (14)      | 3 (10)      | ns                    | 2 (14)     | ns                    | ns                    |
| <b>Time from CD diagnosis to surgery, months</b> | 69 (27-141) | 67 (32-103) | ns                    | 39 (10-91) | ns                    | ns                    |
| <b>Treatment before surgery; n (%)</b>           |             |             |                       |            |                       |                       |
| Nothing or aminosalicylates                      | 3 (14)      | 3 (13)      |                       | 5 (36)     |                       |                       |
| IMM                                              | 11 (50)     | 10 (42)     |                       | 3 (21)     |                       |                       |
| Anti-TNF                                         | 2 (9)       | 0           | ns                    | 3 (21)     | ns                    | ≤0.05                 |
| Anti-TNF+IMM                                     | 5 (23)      | 8 (33)      |                       | 3 (21)     |                       |                       |
| Undetermined                                     | 1 (5)       | 3 (13)      |                       | 0          |                       |                       |
| <b>Surgical indication; n (%)</b>                |             |             |                       |            |                       |                       |
| Stricture disease                                | 11 (50)     | 14 (47)     |                       | 4 (29)     |                       |                       |
| Penetrating disease                              | 11 (50)     | 14 (47)     | ns                    | 10 (71)    | ns                    | ns                    |
| Inflammatory phenotype                           | 0           | 2 (6)       |                       | 0          |                       |                       |

Abbreviations: CD, Crohn's disease; BMI, body mass index; Anti-TNF, tumour necrosis factor-alpha inhibitor; IMM, immunomodulators; NR, non-recurrent; MR, mild-recurrent; SR, severe recurrent; ns, non-significant. Statistics: Quantitative variables were analyzed using the Student's *t* test and qualitative variables were compared using the  $\chi^2$  test.

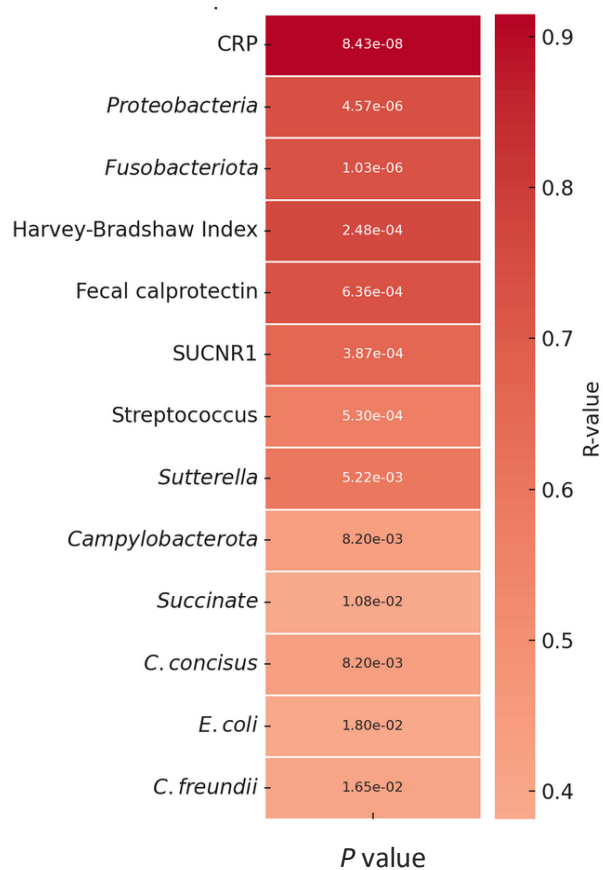
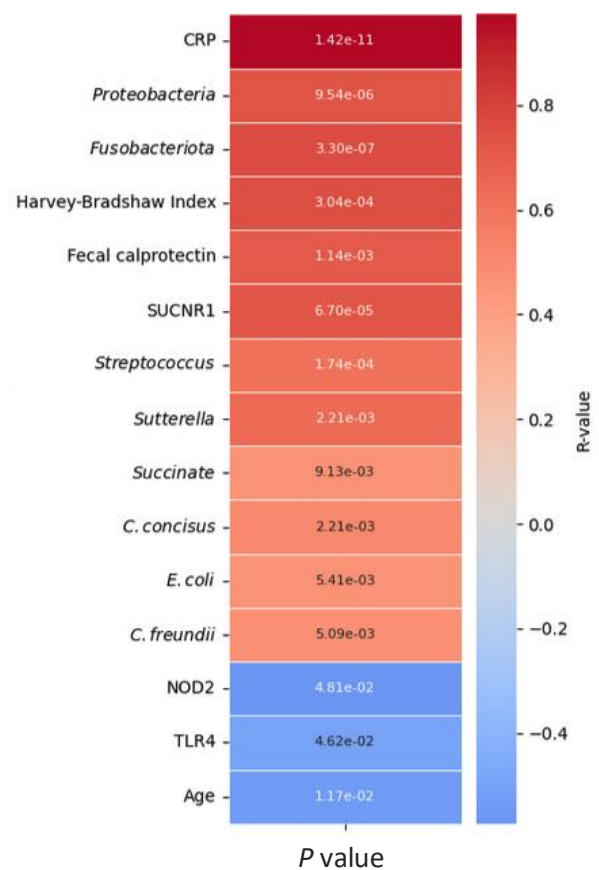
**Table S3.** Circulating succinate levels and PBMC gene expression in the HJ23 cohort.

| Identifier | Succinate<br>levels ( $\mu\text{M}$ ) | Clinical<br>status | Relative gene expression |            |            |            |
|------------|---------------------------------------|--------------------|--------------------------|------------|------------|------------|
|            |                                       |                    | SUCNR1                   | NOD2       | TLR4       | IL10       |
| G1         | 87,8                                  | iCD                | 0,67791079               | 0,53658644 | 0,41472547 | 0,25411801 |
| G2         | 104,3                                 | iCD                | 0,74171994               | 0,67634665 | 1,02224173 | 1,07600099 |
| G3         | 125,4                                 | iCD                | 0,81732504               | 0,50277491 | 0,70750917 | 0,83165753 |
| G4         | 128                                   | iCD                | 2,50608521               | 1,38970935 | 1,64559033 | 3,95053178 |
| G5         | 222,2                                 | aCD                | 2,56205199               | 0,33048961 | 0,07748937 | 0,59016938 |
| G6         | 169,9                                 | aCD                | 1,60286728               | 0,65414055 | 0,64660122 | 0,72193818 |
| G7         | 149,6                                 | aCD                | 0,26026364               | 0,33181348 | 0,0613357  | 0,26368319 |
| G8         | 146,1                                 | aCD                | 1,48867842               | 0,76714871 | 0,27919613 | 0,68518352 |
| G9         | 204,7                                 | iCD                | ND                       | ND         | ND         | ND         |
| G10        | 136,4                                 | aCD                | 1,97875106               | 0,75196758 | 0,42025672 | 0,72072518 |
| G11        | 140                                   | aCD                | 0,45739359               | 0,87045278 | 0,80534797 | 0,76756422 |
| G12        | 171,6                                 | aCD                | 5,63760395               | 0,07913315 | 0,04352801 | 0,22756366 |
| G14        | 124,6                                 | aCD                | 0,6305394                | 0,8613713  | 0,97629612 | 1,69417718 |
| G15        | 175,1                                 | aCD                | ND                       | ND         | ND         | ND         |
| G16        | 65,82                                 | aCD                | 0,10859828               | 1,19099201 | 0,55473129 | 1,10463767 |
| G17        | 45,12                                 | iCD                | 1,03511383               | 1,11363677 | 1,20707768 | 2,20143721 |
| G18        | 77,37                                 | iCD                | 8,05240848               | 0,36023469 | 0,89914696 | 8,82614289 |
| G19        | 51,49                                 | iCD                | ND                       | ND         | ND         | ND         |
| C01        | 55,5                                  | Non-IBD            | 1,12023038               | 2,71624844 | 2,29935853 | 1,94684346 |
| C02        | 59,19                                 | Non-IBD            | 0,46339047               | 1,33137535 | 1,12344434 | 1,24187382 |
| C03        | 48,16                                 | Non-IBD            | 1,18034983               | 1,36665266 | 1,23054802 | 0,8783754  |
| C04        | 51,34                                 | Non-IBD            | ND                       | ND         | ND         | ND         |
| C05        | 42,56                                 | Non-IBD            | 0,6184172                | 1,50860949 | 1,8398659  | 1,62911814 |
| C06        | 47,17                                 | Non-IBD            | 1,04183782               | 1,10973234 | 1,13869436 | 0,45623754 |
| C07        | 50,37                                 | Non-IBD            | 1,61777004               | 0,33974742 | 0,29378054 | 0,45221452 |
| C08        | 48,23                                 | Non-IBD            | 0,87575628               | 0,58279028 | 0,67787183 | 1,29249644 |
| C09        | 54,14                                 | Non-IBD            | 1,78794058               | 0,61039126 | 0,75401369 | 1,08391164 |
| C10        | 48,15                                 | Non-IBD            | 6,52201947               | 0,14754963 | 0,07855248 | 7,90994396 |
| C11        | 55,58                                 | Non-IBD            | 2,04673025               | 0,81150934 | 1,54708212 | 2,76911538 |
| C12        | 57,62                                 | Non-IBD            | 7,7970849                | 0,45080425 | 0,14654846 | 7,2909405  |
| C13        | 60,52                                 | Non-IBD            | 0,48858417               | 1,23227171 | 0,6463781  | 0,36112616 |

**A.****B.****C.**

**Figure S1. Elevated circulating succinate levels correlate with clinical and biological parameters.**

**A:** Circulating succinate levels across the cohort, including non-inflammatory bowel diseases (IBD) individuals, active CD patients, and inactive Crohn's disease (CD) patients. Statistical comparisons were performed using one-way ANOVA followed by post hoc tests for multiple comparisons. **B:** Pearson's correlation analysis between circulating succinate levels, SUCNR1 expression, and key clinical and biological parameters. **C:** Plasma succinate levels stratified by physical activity, smoking status, and adherence to a Mediterranean diet. Differences among the three groups were assessed using one-way ANOVA, while pairwise comparisons were conducted using the Mann-Whitney U test. <sup>a</sup> $P < 0.05$ ; <sup>b</sup> $P < 0.01$ ; <sup>c</sup> $P < 0.001$ .

**A.**Pearson's correlations with *Prevotella* genus**B.**Pearson's correlations with *Prevotella buccae*

**Figure S2. *Prevotella* and *P. buccae* are strongly associated with inflammatory and microbial alterations in Crohn's disease (CD).** **A:** Pearson correlation analysis of the genus *Prevotella* with clinical, microbial, and biological variables. **B:** Pearson correlation analysis of *P. buccae* with the same set of parameters. The colour scale represents the correlation coefficient (*R* value), with positive correlations shown in red and negative correlations in blue. The corresponding *p*-values are displayed within each cell of the heatmap. Statistically significant correlations ( $P < 0.05$ ) are explicitly indicated.