## Supplementary methods

## **Immunohistochemistry**

The tissue sections were dried in room temperature (RT) overnight and baked in 50°C for 12-24 hours. Prior to immunohistochemical staining the sections were deparaffinized in xylene, hydrated in graded alcohols and blocked for endogenous peroxidase in 0.3% hydrogen peroxide diluted in 95% ethanol. For antigen retrieval, a Decloaking chamber (Biocare Medical, Walnut Creek, CA) was used. Slides were immersed and boiled in Citrate buffer, pH6 (Lab Vision, Freemont, CA) for 4 min at 125°C and then allowed to cool to 90°C (the total program is approximately 40 minutes). Automated immunohistochemistry was performed essentially as previously described29,30 using an Autostainer 480 instrument (Thermo Fischer Scientific, Waltham, MA). The primary antibody towards AGPAT1 (HPA073355, Atlas Antibodies AB) was diluted in 1:600 UltraAb Diluent (Thermo Fisher Scientific) followed by incubation for 30 min at RT. The slides were further incubated with the secondary reagent anti-rabbit/mouse horseradish peroxidase conjugated UltraVision (Thermo Fischer Scientific) for 30 min at RT and developed for 10 min using Diaminobenzidine (DAB) Quanto (Thermo Fisher Scientific) as chromogen. All incubations were followed by rinse in wash buffer (Thermo Fisher Scientific) 2 X 5 min. Slides were counterstained in Mayers hematoxylin (Histolab, Gothenburg, Sweden) and cover slipped using Pertex (Histolab) as mounting medium. The stained slides were digitized with ScanScope AT2 (Leica Aperio, Vista, CA) using a 20x objective

## Supplementary table 1

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  |  | **P-value** | |
| **Protein** | **Mean diff.** | **95% CI** | **t-test** | **Mann-Whitney** |
| CD47 | 0.908 | 0.549 1.268 | 0.0002 | 0.0012 |
| TMEM192 | 0.206 | 0.121 0.290 | 0.0003 | 0.0007 |
| LSM7 | 0.954 | 0.531 1.377 | 0.0006 | 0.0033 |
| NDUFAF4 | 0.708 | 0.393 1.022 | 0.0006 | 0.0079 |
| AGPAT1 | -0.332 | -0.482 -0.182 | 0.0007 | 0.0079 |
| FANCI | 0.037 | 0.020 0.055 | 0.0010 | 0.0012 |
| ESD | 1.961 | 1.025 2.898 | 0.0011 | 0.0021 |
| TMEM33 | -1.064 | -1.575 -0.553 | 0.0011 | 0.0007 |
| UPF2 | -0.046 | -0.068 -0.024 | 0.0012 | 0.0021 |
| PARP9 | 0.185 | 0.095 0.275 | 0.0012 | 0.0012 |
| ECE1 | -0.130 | -0.193 -0.066 | 0.0013 | 0.0003 |
| LSS | -0.425 | -0.637 -0.212 | 0.0015 | 0.0021 |
| TLCD1 | -0.036 | -0.054 -0.018 | 0.0016 | 0.0051 |
| PELO | 0.064 | 0.032 0.096 | 0.0016 | 0.0007 |
| NCL | 4.903 | 2.428 7.378 | 0.0017 | 0.0012 |
| TRAPPC5 | -0.345 | -0.520 -0.170 | 0.0017 | 0.0012 |
| ABHD3 | -0.225 | -0.339 -0.110 | 0.0018 | 0.0012 |
| TXNRD1 | -0.426 | -0.643 -0.208 | 0.0018 | 0.0079 |
| NUDT5 | 0.363 | 0.176 0.549 | 0.0019 | 0.0033 |
| NTPCR | -0.914 | -1.389 -0.439 | 0.0021 | 0.0012 |
| COX15 | -0.244 | -0.371 -0.117 | 0.0021 | 0.0052 |
| EXOSC4 | -0.417 | -0.638 -0.196 | 0.0024 | 0.0052 |
| NDUFA13 | -4.506 | -6.912 -2.100 | 0.0025 | 0.0007 |
| DENR | 0.386 | 0.180 0.593 | 0.0025 | 0.0052 |
| BAG6 | -0.175 | -0.270 -0.080 | 0.0028 | 0.0079 |
| PGS1 | -0.046 | -0.071 -0.021 | 0.0030 | 0.0081 |
| NDUFS2 | -2.613 | -4.042 -1.184 | 0.0030 | 0.0033 |
| IVL | 0.648 | 0.293 1.004 | 0.0030 | 0.0042 |
| THEMIS2 | 0.062 | 0.028 0.096 | 0.0031 | 0.0033 |
| SLC25A13 | -1.308 | -2.030 -0.586 | 0.0032 | 0.0079 |
| SEC62 | 0.100 | 0.044 0.156 | 0.0034 | 0.0052 |
| SLC27A1 | -0.104 | -0.163 -0.046 | 0.0036 | 0.0079 |
| UTP20 | 0.334 | 0.145 0.523 | 0.0038 | 0.0052 |
| HNRNPH2 | -0.540 | -0.848 -0.232 | 0.0040 | 0.0079 |
| RNASEH2B | 0.084 | 0.036 0.132 | 0.0041 | 0.0195 |
| AKR7A2 | -1.534 | -2.416 -0.652 | 0.0042 | 0.0052 |
| B4GALT5 | -0.067 | -0.106 -0.029 | 0.0043 | 0.0079 |
| NHLRC2 | -0.152 | -0.241 -0.064 | 0.0046 | 0.0007 |
| UROD | -0.157 | -0.249 -0.065 | 0.0047 | 0.0033 |
| EIF2B5 | -0.085 | -0.134 -0.035 | 0.0048 | 0.0164 |
| GLTP | 0.348 | 0.141 0.555 | 0.0053 | 0.0052 |
| TMED7 | -2.530 | -4.037 -1.022 | 0.0054 | 0.0079 |
| HSP90AA1 | 13.903 | 5.599 22.207 | 0.0055 | 0.0052 |
| IBA57 | -0.538 | -0.860 -0.217 | 0.0055 | 0.0033 |
| MS4A6A | 0.062 | 0.025 0.099 | 0.0055 | 0.0108 |
| CMAS | -0.184 | -0.295 -0.074 | 0.0057 | 0.0080 |
| SNX6 | 0.554 | 0.220 0.887 | 0.0057 | 0.0079 |
| CPNE1 | -1.303 | -2.090 -0.516 | 0.0058 | 0.0052 |
| ZNF706 | 0.215 | 0.085 0.345 | 0.0060 | 0.0148 |
| LAGE3 | -0.144 | -0.231 -0.057 | 0.0060 | 0.0081 |

**Supplemental table 1. Five candidate biomarkers from the discovery step were chosen for LC-MS/MS replication.** List of top-50 colonic proteins (represented with gene name) in the discovery step, sorted by p-values according to a linear regression model. PSC-UC is chosen as reference group. Only proteins with at least 5 non-zero values were included in the analysis. Proteins (n=5) selected for the replication step are marked with blue color.

## Supplementary table 2

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Protein | UniProt ID | Mean diff | 95% CI | P-value | | |
| IBD type | Thiopurine | Age |
| CD47 | [Q08722](http://www.uniprot.org/entry/Q08722) | 0.723 | (0.311, 1.134) | 0.0049 | 0.1959 | 0.3701 |
| TMEM192 | [Q8IY95](http://www.uniprot.org/entry/Q8IY95) | 0.172 | (0.069, 0.276) | 0.0068 | 0.5531 | 0.3980 |
| LSM7 | [Q9UK45](http://www.uniprot.org/entry/Q9UK45) | 0.747 | (0.251, 1.243) | 0.0121 | 0.2859 | 0.3616 |
| NDUFAF4 | [Q9P032](http://www.uniprot.org/entry/Q9P032) | 0.652 | (0.255, 1.049) | 0.0073 | 0.4768 | 0.9063 |
| AGPAT1 | [Q99943](http://www.uniprot.org/entry/Q99943) | -0.256 | (-0.432, -0.079) | 0.0150 | 0.443 | 0.258 |

Linear regression model adjusted for IBD type, age and thipopurines.

Abbreviations: Uniprot: Universal Protein Resourse; ND: not detected; CD47: Leukocyte surface antigen CD47; TMEM192: Transmembrane protein 192, LSM7: U6 SnRNA-associated Sm-like protein LSm7; NDUFAF4 NADH dehydrogenase ubiquinone 1 alpha subcomplex assembly factor 4; AGPAT1: 1-acetylglycerol-3-phosphate O-acyltransferas 1 .

## Supplementary table 3

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Protein** | **Step 1** | | |  | **Step 2** | | |  | **Meta-analyses** | | |
|  | *Coeff* | *SD* | *P-value* |  | *Coeff* | *SD* | *P-value* |  | *Coeff* | *SD* | *P-value* |
| AGPAT1 | -0.332 | 0.077 | 0.0007 |  | -0.214 | 0.078 | 0.0094 |  | -0.274 | 0.059 | 3.6e-06 |
| ESD | 1.961 | 0.478 | 0.0011 |  | 1.366 | 0.854 | 0.1187 |  | 1.819 | 0.417 | 1.3e-05 |
| C1orf122 | 0.224 | 0.072 | 0.0076 |  | 0.172 | 0.059 | 0.0065 |  | 0.193 | 0.046 | 2.5e-05 |
| PELO | 0.064 | 0.016 | 0.0016 |  | 0.047 | 0.032 | 0.1516 |  | 0.061 | 0.015 | 3.6e-05 |
| TXNRD1 | -0.426 | 0.111 | 0.0018 |  | -0.322 | 0.259 | 0.2225 |  | -0.410 | 0.102 | 6.0e-05 |
| NHLRC2 | -0.152 | 0.045 | 0.0046 |  | -0.100 | 0.044 | 0.0276 |  | -0.125 | 0.031 | 6.6e-05 |
| NUDT5 | 0.363 | 0.095 | 0.0019 |  | 0.221 | 0.206 | 0.2904 |  | 0.338 | 0.087 | 9.6e-05 |
| DENR | 0.386 | 0.105 | 0.0025 |  | 0.340 | 0.278 | 0.2294 |  | 0.380 | 0.099 | 0.0001 |
| HNRNPH2 | -0.540 | 0.157 | 0.0040 |  | -0.612 | 0.390 | 0.1257 |  | -0.550 | 0.146 | 0.0002 |
| ABHD6 | -0.221 | 0.215 | 0.3221 |  | -0.243 | 0.068 | 0.0010 |  | -0.241 | 0.065 | 0.0002 |
| SLC35F6 | -0.037 | 0.199 | 0.8543 |  | 0.107 | 0.028 | 0.0006 |  | 0.104 | 0.028 | 0.0002 |
| PTGES | -0.102 | 0.059 | 0.1046 |  | -0.149 | 0.046 | 0.0024 |  | -0.131 | 0.036 | 0.0003 |
| GATA6 | 0.077 | 0.032 | 0.0284 |  | 0.042 | 0.012 | 0.0016 |  | 0.048 | 0.013 | 0.0004 |
| BPI | -0.128 | 0.178 | 0.4840 |  | -0.263 | 0.075 | 0.0012 |  | -0.243 | 0.069 | 0.0004 |
| TUBGCP4 | 0.012 | 0.015 | 0.4236 |  | 0.022 | 0.007 | 0.0015 |  | 0.021 | 0.006 | 0.0005 |
| SNX5 | 0.375 | 0.155 | 0.0298 |  | 0.663 | 0.202 | 0.0024 |  | 0.489 | 0.141 | 0.0005 |
| OLFM4 | -2.033 | 1.522 | 0.2027 |  | -1.720 | 0.536 | 0.0029 |  | -1.755 | 0.506 | 0.0005 |
| PUM1 | 0.117 | 0.042 | 0.0142 |  | 0.083 | 0.039 | 0.0416 |  | 0.099 | 0.029 | 0.0005 |
| PDLIM4 | 0.873 | 2.777 | 0.7578 |  | -1.804 | 0.506 | 0.0011 |  | -1.718 | 0.498 | 0.0006 |
| GLTP | 0.348 | 0.106 | 0.0053 |  | 0.251 | 0.239 | 0.3008 |  | 0.333 | 0.097 | 0.0006 |
| DAD1 | -3.783 | 1.652 | 0.0380 |  | -6.787 | 1.951 | 0.0014 |  | -5.105 | 1.492 | 0.0006 |
| MFSD1 | 0.036 | 0.047 | 0.4493 |  | 0.053 | 0.016 | 0.0022 |  | 0.051 | 0.015 | 0.0007 |
| MYADM | -0.651 | 0.373 | 0.1031 |  | -0.712 | 0.246 | 0.0065 |  | -0.694 | 0.205 | 0.0007 |
| ADAM15 | -0.031 | 0.014 | 0.0401 |  | -0.016 | 0.006 | 0.0111 |  | -0.018 | 0.005 | 0.0008 |
| SECISBP2 | 0.013 | 0.005 | 0.0143 |  | 0.015 | 0.008 | 0.0676 |  | 0.013 | 0.004 | 0.0008 |
| AMDHD2 | -0.061 | 0.024 | 0.0218 |  | -0.066 | 0.031 | 0.0382 |  | -0.063 | 0.019 | 0.0008 |
| VPS33A | -0.206 | 0.204 | 0.3310 |  | -0.140 | 0.044 | 0.0029 |  | -0.143 | 0.043 | 0.0008 |
| ATP6V1C1 | 0.302 | 0.122 | 0.0270 |  | 0.306 | 0.136 | 0.0311 |  | 0.304 | 0.091 | 0.0008 |
| RPS18 | 14.989 | 7.637 | 0.0699 |  | 14.635 | 5.488 | 0.0115 |  | 14.756 | 4.457 | 0.0009 |
| ZNF706 | 0.215 | 0.066 | 0.0060 |  | 0.143 | 0.182 | 0.4370 |  | 0.206 | 0.062 | 0.0009 |
| AAAS | -0.114 | 0.053 | 0.0476 |  | -0.084 | 0.033 | 0.0158 |  | -0.093 | 0.028 | 0.0010 |
| CTSS | 1.386 | 0.554 | 0.0254 |  | 1.893 | 0.866 | 0.0356 |  | 1.534 | 0.467 | 0.0010 |
| CMAS | -0.184 | 0.057 | 0.0057 |  | -0.104 | 0.419 | 0.8051 |  | -0.183 | 0.056 | 0.0011 |
| EIF2S2 | 0.789 | 0.251 | 0.0071 |  | 0.440 | 0.384 | 0.2602 |  | 0.685 | 0.210 | 0.0011 |
| DYSF | -0.049 | 0.025 | 0.0680 |  | -0.056 | 0.022 | 0.0135 |  | -0.053 | 0.016 | 0.0011 |
| HRNR | -0.979 | 1.915 | 0.6173 |  | 0.227 | 0.070 | 0.0024 |  | 0.226 | 0.070 | 0.0012 |
| ESYT2 | -0.191 | 0.164 | 0.2632 |  | -0.290 | 0.094 | 0.0042 |  | -0.265 | 0.082 | 0.0012 |
| COX6B1 | 1.751 | 1.254 | 0.1843 |  | 3.301 | 1.084 | 0.0044 |  | 2.638 | 0.820 | 0.0013 |
| RSU1 | -1.022 | 0.608 | 0.1148 |  | -1.966 | 0.572 | 0.0015 |  | -1.516 | 0.472 | 0.0013 |
| DYM | 0.106 | 0.173 | 0.5501 |  | 0.070 | 0.022 | 0.0033 |  | 0.071 | 0.022 | 0.0014 |
| PPM1G | 0.290 | 0.107 | 0.0172 |  | 0.211 | 0.119 | 0.0844 |  | 0.254 | 0.080 | 0.0014 |
| STBD1 | -0.152 | 0.057 | 0.0177 |  | -0.200 | 0.113 | 0.0858 |  | -0.162 | 0.051 | 0.0014 |
| STXBP5 | -0.064 | 0.026 | 0.0294 |  | -0.045 | 0.021 | 0.0412 |  | -0.052 | 0.017 | 0.0015 |
| CLPTM1 | -0.257 | 0.088 | 0.0113 |  | -0.185 | 0.146 | 0.2133 |  | -0.238 | 0.075 | 0.0016 |
| EVL | 0.015 | 0.349 | 0.9666 |  | 0.344 | 0.105 | 0.0024 |  | 0.317 | 0.101 | 0.0017 |
| DDX58 | 0.049 | 0.016 | 0.0097 |  | 0.028 | 0.025 | 0.2530 |  | 0.043 | 0.014 | 0.0017 |
| TROVE2 | 0.169 | 0.085 | 0.0661 |  | 0.194 | 0.080 | 0.0208 |  | 0.183 | 0.058 | 0.0018 |
| TGM2 | -2.313 | 1.940 | 0.2529 |  | -3.719 | 1.269 | 0.0059 |  | -3.298 | 1.062 | 0.0019 |
| CXXC1 | 0.026 | 0.010 | 0.0168 |  | 0.020 | 0.013 | 0.1280 |  | 0.024 | 0.008 | 0.0019 |
| PTMA | 4.892 | 1.650 | 0.0102 |  | 6.469 | 6.907 | 0.3554 |  | 4.978 | 1.605 | 0.0019 |

**Supplemental Table 3. Meta-analysis of the two proteomic datasets.** List of the top-50 proteins with the smallest p-value according to meta-analysis of linear regression results from both LC-MS/MS steps. Only proteins that had more than 5 non-zero values in each step were accepted, resulting in 6964 proteins included in the analysis.

Supplementary figure 1

**Assessment of immunostaining for AGPAT1.** For assessment of AGPAT1 IHC intensity, immune-stained colon sections were analysed using the free image software QuPath, version 0.2.3. Positively stained areas intensity was categorized using three different treshhold: weak, moderate, strong. For each biopsy, the proportions (%) of each DAB-staining (AGPAT1 positive) category were calculated using an artificial intelligence-based subtraction model, illustrated by representative photos: a) whole biopsy demarcated, b) areas with at least weak staining intensity, and c) at least moderately stained areas.

## Supplementary figure 2

**En bild som visar skärmbild, karta

Automatiskt genererad beskrivning**

**Random forest analysis supports colonic candidate biomarker selection.** Variable importance plot from random forest. The 5 biomarker candidates (highlighted in red) are all found among the 50 proteins being of highest discriminative importance (between PSC-UC and UC).