#### Supporting Information

#### N-terminomics/TAILS profiling of proteases and their substrates in ulcerative colitis

All peptides and proteins identified in the LC-MS/MS proteomics and N-terminomics study (Supplementary Tables 1-6).

**Supplementary Table 1** | N-terminomics TAILS peptides from colon biopsies identified without constraint by enzyme specificity rules during spectrum-to-sequence matching.

**Supplementary Table 2** | N-terminomics TAILS peptides significantly elevated from the healthy colon biopsies.

**Supplementary Table 3** | N-terminomics TAILS peptides significantly elevated from the ulcerative colitis colon biopsies.

**Supplementary Table 4** | Shotgun proteomics peptides from colon biopsies identified without constraint by enzyme specificity rules during spectrum-to-sequence matching.

**Supplementary Table 5** | Reactome Pathway of Shotgun proteomics data.

**Supplementary Table 6** | Identification of differential proteolytic processing of proteins from N-terminomics.

Supplementary Figures 1-8 (PDF)



**Supplementary Figure 1**: (A) Determination of dimethylation ratio cutoffs for identifying high-confidence N-termini. Log-distribution of dimethylated peptides from N-terminal/TAILS experiment. Significance was determined using Box-Plot analysis.



**Supplementary Figure 2**: Heat map representing (**A**) shotgun and (**B**) N-terminomics/TAILS data of differentially expressed peptides of healthy and ulcerative colitis biopsies.



Supplementary Figure 3: Radar plots integrated analysis of healthy (A) and ulcerative colitis (B) protein-protein interactions reactome pathways.

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Protease web of Caspase-1 in Healthy patients (PathFINDer)



Protease web of Caspase-1 in Ulcerative Colitis patients (PathFINDer)



**Supplementary Figure 4**: Protease Web Analysis Using PathFINDer. All N-termini identified in healthy (A) or ulcerative colitis (B) colon biopsies in Figure 1A and Supplementary Table 2-3 were analyzed using PathFINDer analysis tool. Caspase-1, CASP1 (blue), was the query protease. The numbers are the known cleavage site from the topFIND database, inhibitors are shown in red, substrates in black, and known cleavage sites are shown in grey.

Protease web of Caspase-7 in Healthy patients (PathFINDer)



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Protease web of Caspase-7 in Ulcerative Colitis patients (PathFINDer)



**Supplementary Figure 5**: Protease Web Analysis Using PathFINDer. All N-termini identified in healthy (A) or ulcerative colitis (B) colon biopsies in Figure 1A and Supplementary Table 2-3 were analyzed using PathFINDer analysis tool. Caspase-7, CASP7 (blue), was the query protease. The numbers are the known cleavage site from the topFIND database, inhibitors are shown in red, substrates in black, and known cleavage sites are shown in grey.

Protease web of Granzyme B in Healthy patients (PathFINDer)

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Protease web of Granzyme B in Ulcerative Colitis patients (PathFINDer)



**Supplementary Figure 6**: Protease Web Analysis Using PathFINDer. All N-termini identified in healthy (A) or ulcerative colitis (B) colon biopsies in Figure 1A and Supplementary Table 2-3 were analyzed using PathFINDer analysis tool. Granzyme B, GZMB (blue), was the query protease. The numbers are the known cleavage site from the topFIND database, inhibitors are shown in red, substrates in black, and known cleavage sites are shown in grey.

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Protease web of Tryptase alpha/beta-1 in Healthy patients (PathFINDer)



Protease web of Tryptase alpha/beta-1in Ulcerative Colitis patients (PathFINDer)



**Supplementary Figure 7**: Protease Web Analysis Using PathFINDer. All N-termini identified in healthy (A) or ulcerative colitis (B) colon biopsies in Figure 1A and Supplementary Table 2-3 were analyzed using PathFINDer analysis tool. Tryptase alpha/beta-1, TPSAB1 (blue), was the query protease. The numbers are the known cleavage site from the topFIND database, inhibitors are shown in red, substrates in black, and known cleavage sites are shown in grey.

Colon Biopsies

Calpain-1 Triple points biologics Domain-I large subunit



Tryptase FL 275 Santa Cruz Biotechnology







Calpain-2 Triple points biologics Domain-IV large subunit



STAT1 #9172S lot 25 Cell signaling Technologies



Caspase-3 #9622ss Cell Signaling Technologies



Supplementary Figure 8: Full Western blots from Figure 6.