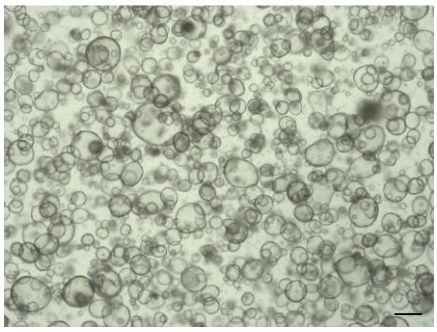
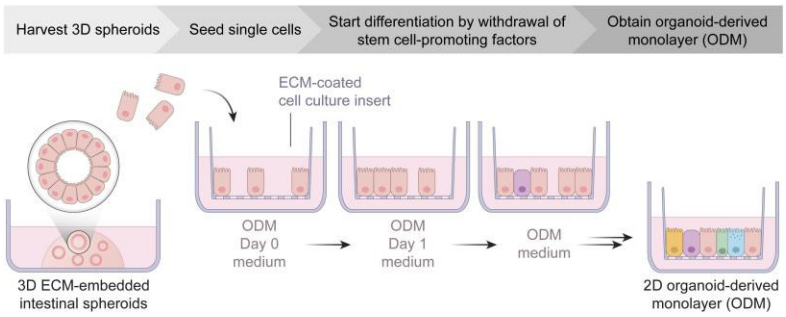
SUPPLEMENTARY INFORMATION

SUPPLEMENTARY FIGURES

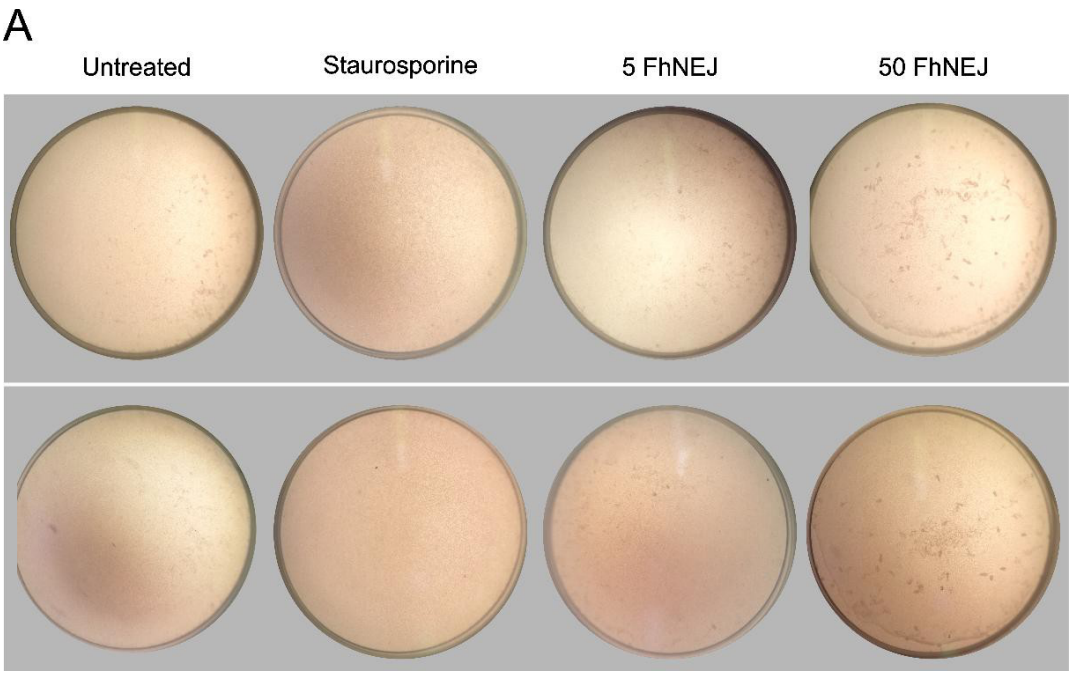
A



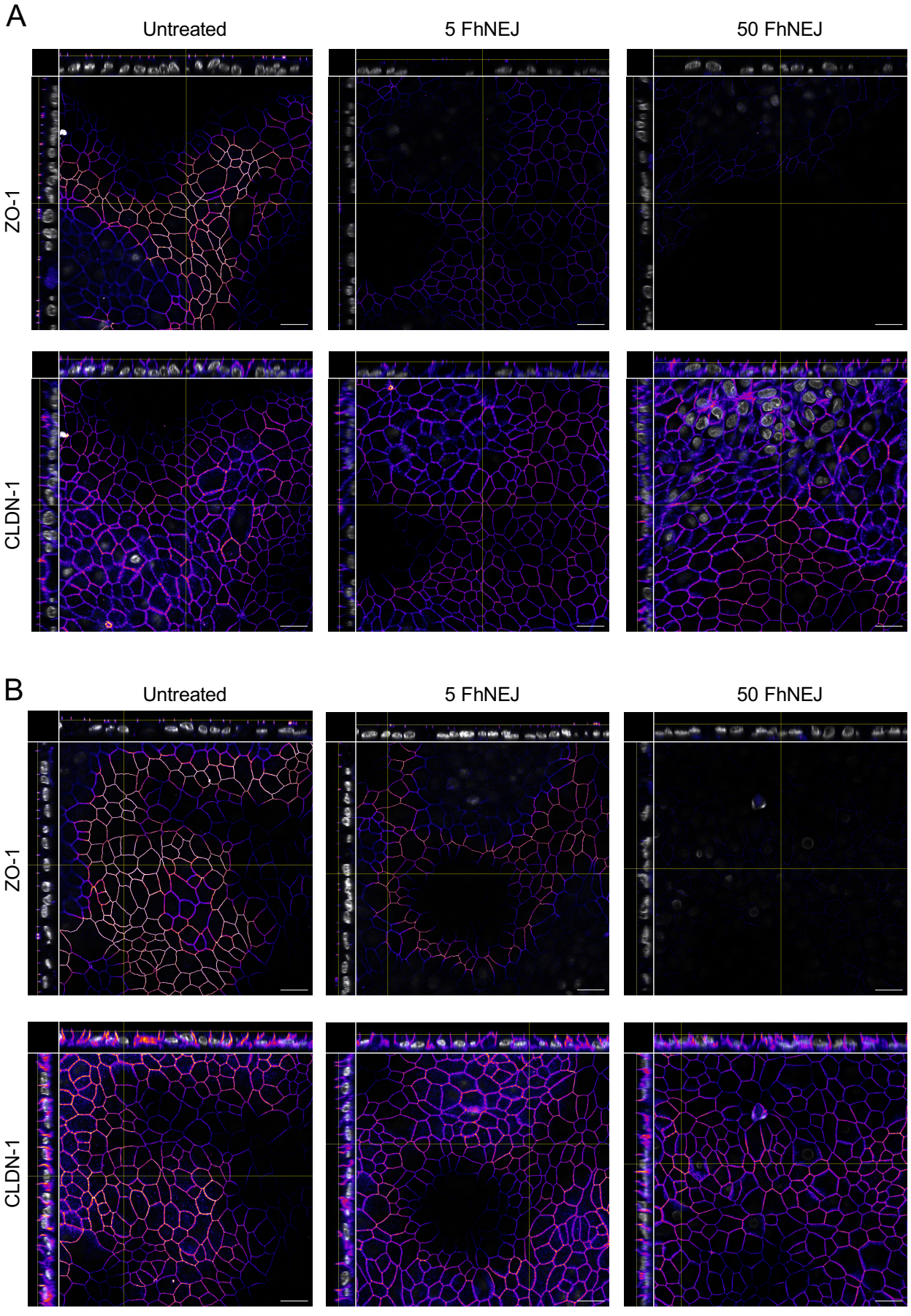
B



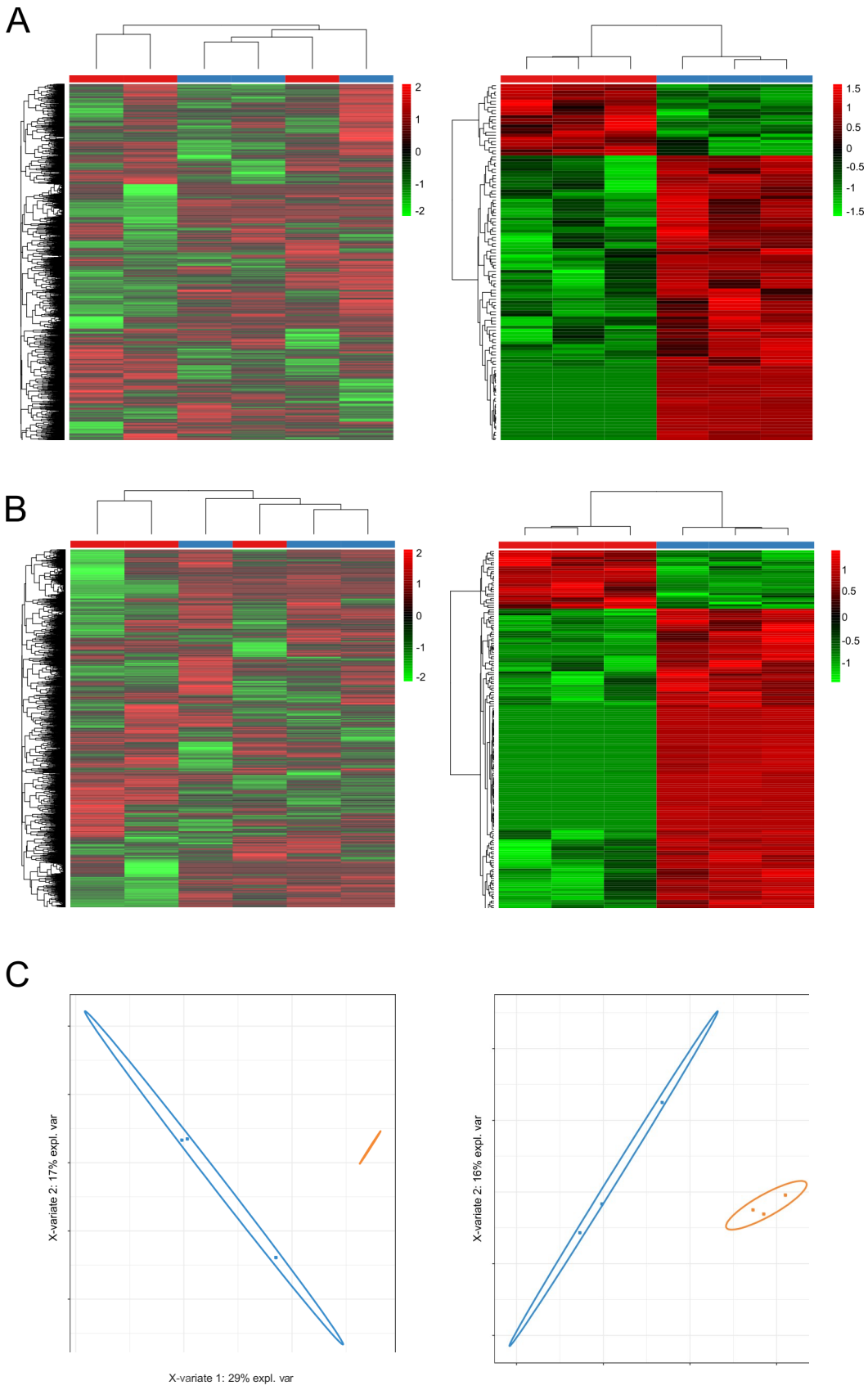
Supplementary Figure 1. Generation of ODMs from 3D organoids derived from human duodenum samples. A) Phase contrast image of 3D, stem-cell-enriched organoid culture at passage 23 and 10 days after seeding, derived from human duodenum biopsy samples and used for ODM generation. Scale bar, 200 µm. **B)** Schematic representation of the experimental procedures employed for ODM generation. From Warschkau et al. (2022)[64].



Supplementary Figure 2. Stereoscope images of ODMs after incubation with FhNEJ. Images from ODMs exposed to 5 FhNEJ, 50 FhNEJ, or left untreated at 48 hours after addition of the parasites are shown for two additional independent experiments.


A
Untreated
5 FhNEJ
50 FhNEJ
ZO-1
CLDN-1
B
Untreated
5 FhNEJ
50 FhNEJ
ZO-1
CLDN-1

Supplementary Figure 3. Orthogonal stacks of representative IFA images of ZO-1 and CLDN-1 48 hours after addition of FhNEJ. A,B) Specimens from two additional experiments were visualised in a Leica Stellaris 8 confocal microscope using the 63× objective. ZO-1 and CLDN-1 are displayed with colour-coded intensities; DAPI is shown in grey. Scale bars, 20 µm. Every panel displays one independent experiments.


A
2
1
0
-1
-2
1.5
1
0.5
0
-0.5
-1
-1.5
B
2
1
0
-1
-2
1
0.5
0
-0.5
-1
C
X-variate 2: 17% expl. var
X-variate 1: 29% expl. var
X-variate 2: 16% expl. var

Supplementary Figure 4. Feature selection by elastic-net and PLS-DA. A,B) An elastic-net penalised regression model was performed to select for proteins that were specifically expressed in each experimental condition. The figures show heatmap representations of Z-score normalised protein expression levels of all the proteins identified (left panels) or proteins that were selected as specific to each experimental condition through elastic-net regularised regression (right panels), for the untreated vs. 5 FhNEJ **(A)** and untreated vs. 50 FhNEJ **(B)** comparisons. Red, untreated; blue, ODMs treated with FhNEJ. **C)** PLS-DA was performed to identify the proteins most relevant for distinguishing between experimental conditions. Only those proteins that contributed substantially to the newly projected bidimensional space (as determined by vip>1.5) were considered specific to each experimental condition. Plots are shown for untreated vs. 5 FhNEJ (left panel) and untreated vs. 50 FhNEJ conditions (right panel). Blue, untreated; orange, ODMs treated with FhNEJ.

DESCRIPTION OF ADDITIONAL SUPPLEMENTARY FILES

File name: Supplementary Movie 1.

Live imaging of ODMs stimulated with FhNEJ. Time-lapse imaging of ODMs from 0 to 16 hours after addition of FhNEJ showed that the parasites were alive and actively moved throughout the epithelial monolayer. One image every five minutes was acquired in a ZEISS AxioObserver.Z1 imaging system. SpyDNA555 (orange) labelled dead host cells.

Download link: https://saco.csic.es/s/rnnNmSYax68JmQN

File name: Supplementary Movie 2.

Video reconstruction of all planes acquired along the entire Z stack of a fixed transwell incubated with FhNEJ, from the apical to the basal side, using a step size of 0.30 µm. The video shows one example of FhNEJ that was recovered under the epithelial monolayer. The specimens were visualised in a Leica Stellaris 8 confocal microscope using the 40× objective. Scale bar, 50 µm.

Download link: https://saco.csic.es/s/zm8FpeEZF6zyQLw

File name: Supplementary Table 1.

Differentially abundant proteins identified in ODMs stimulated with FhNEJ when compared to their untreated controls, including information about their functions as annotated in the Uniprot database.

Download link: https://saco.csic.es/s/bdLxaDiF97t6ggo