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Comprehensive Experimental Workflow for Filovirus Infection Studies in iPSC-derived and Primary-derived Human Gut Organoids Part 2

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Abstract

Experimental Workflow:

This workflow outlines the step-by-step experimental pipeline for studying Ebola virus (EBOV) and Marburg virus (MARV) infection in human intestinal and colonic organoid systems. It integrates organoid differentiation from induced pluripotent stem cells (hiPSCs) and primary human colonic tissue, standardized infection procedures, functional assays, and downstream immunofluorescence and flow cytometry analyses. Each section provides detailed methods and reagents to ensure reproducibility in BSL-4-compatible systems and support comparative studies of filovirus pathogenesis in 3D human models.

Sections:

Protocol 3: Flow Cytometry (FACS) Analysis of Filovirus-Infected Human Gut Organoids

Downstream quantification and analysis of EBOV infection.

Protocol 4: Whole-Mount Immunofluorescence Staining of Human iPSC-Derived Gut Organoids for Filovirus Antigens

Microscopy-based detection and visualization of infection targets.

Protocol 5: Functional Assay for Barrier Integrity Using Forskolin-Induced Swelling

Assessment of epithelial barrier function.



Guidelines

- Adjust NIR stain concentration and incubation times depending on cell type and viability.
- Handle all filovirus-infected samples under appropriate biosafety conditions until verified inactivated.
- Include compensation and fluorescence-minus-one (FMO) controls for accurate gating.

Experimental Design and Replication

- Include control wells treated with DMSO and/or organoid culture medium in each experiment.
- Perform at least three replicate wells per treatment condition.
- Repeat imaging and analysis at each designated time point (24, 48, 72 hours).

Statistical Analysis

- Conduct statistical analyses using GraphPad Prism or equivalent software.
- Compare treatment groups using two-way ANOVA followed by Tukey's multiple comparisons test.
- Present data as mean $\pm$ SEM.
- Consider $p \leq 0.05$ statistically significant.

Materials

- Near-Infrared (NIR) Live/Dead Stain
- Dimethyl sulfoxide (DMSO)
- Phosphate-Buffered Saline (PBS), 1X
- Bovine Serum Albumin (BSA), 0.5% in PBS
- Ethylenediaminetetraacetic Acid (EDTA), 2 mM
- Trypsin, 0.5%
- Cell Recovery Solution (CRS)
- 3D Matrigel
- Paraformaldehyde (PFA), 4%
- Triton X-100
- Blocking buffer (5% goat serum in PBS-Tx)
- Rabbit anti-EBOV NP (primary antibody)
- Donkey anti-Rabbit Alexa Fluor 594 or 647 (secondary antibody)
- FACS buffer (0.5% BSA PBS + 2 mM EDTA)
- CellPro Flow Cytometry Tubes, 35 µm Strainer Cap
- Stratadigm Flow Cytometer
- Cell Recovery Solution (Corning)
- Paraformaldehyde (PFA), 4% solution (freshly prepared or commercial)
- Normal donkey serum (for blocking; Jackson ImmunoResearch or equivalent)
- Hoechst 33342 nuclear dye (ThermoFisher Scientific, 62249)
- Cavity slides (Millipore Sigma, BR475505)
- ProLong Diamond Antifade Mountant (ThermoFisher Scientific, P36961)
- 13 mm coverslips (ThermoFisher Scientific, 174950)
- Eppendorf tubes (1.5 mL)

Antibodies, Dyes, and Reagents for Immunostaining

- 4% Normal Goat Serum (NGS)

- Vendor: Jackson ImmunoResearch
- Catalog No.: 005-000-121
- Dilution/Notes: Blocking solution

- 13 mm Coverslips

- Vendor: ThermoFisher Scientific
- Catalog No.: 174950

- ProLong Diamond Antifade Mountant

- Vendor: ThermoFisher Scientific
- Catalog No.: P36961
- Dilution/Notes: Mounting medium



- **BRAND Cavity Slides**

- Vendor: Millipore Sigma
- Catalog No.: BR475505
- Dilution/Notes: For organoid mounting

- **Chicken anti-GFP IgY**

- Vendor: ThermoFisher Scientific
- Catalog No.: A10262
- Dilution/Notes: 1:200

- **Mouse anti-Villin**

- Vendor: Millipore
- Catalog No.: MAB1671
- Dilution/Notes: 1:200

- **Mouse anti-MUC2**

- Vendor: Santa Cruz Biotechnology
- Catalog No.: sc-515032
- Dilution/Notes: 1:200

- **Rabbit anti-EBOV NP**

- Vendor: IBT
- Catalog No.: 0301-012
- Dilution/Notes: 1:200

- **Rabbit anti-MARV NP**

- Vendor: IBT
- Catalog No.: 0303-012
- Dilution/Notes: 1:500

- **Rabbit anti-MARV NC**

- Vendor: Mühlberger Lab
- Dilution/Notes: 1:200

- **Donkey anti-Chicken Alexa Fluor 488**

- Vendor: Jackson ImmunoResearch Labs
- Catalog No.: 703-545-155
- Dilution/Notes: 1:500

- **Donkey anti-Rabbit Alexa Fluor 594 IgG (H+L)**

- Vendor: ThermoFisher Scientific
- Catalog No.: A-21207
- Dilution/Notes: 1:500

- Donkey anti-Mouse Alexa Fluor 488 IgG (H+L)

- Vendor: ThermoFisher Scientific
- Catalog No.: A-21202
- Dilution/Notes: 1:500

- Hoechst 33342

- Vendor: ThermoFisher Scientific
- Catalog No.: 62249
- Dilution/Notes: 1:2000

Equipment

- P1000 pipette with cut tips (to enlarge aperture)
- Rocking platform
- Centrifuge capable of 3c200 3d g
- Confocal microscope (e.g., Zeiss LSM 700) or Nikon widefield fluorescence microscope

Forskolin-Induced Functional Assay Materials**- Human iPSC-derived and primary-derived gut organoids**

- Distal colonic organoids (HCOs), proximal intestinal organoids (HIOs), primary-derived gut organoids
- Maintained in 3D Matrigel prior to dissociation

- 3D Matrigel

- Basement membrane matrix (Corning, pre-chilled)
- 3D scaffold for organoid replating

- Phosphate-buffered saline (PBS)

- Sterile
- For washing and rinsing organoids

- CKDCI or IMCK medium

- Defined organoid culture medium
- Used for plating and treatment recovery

- Forskolin

- Sigma, Cat# F3917
- Stimulating agent (5 μ M)

- DMSO

- Vehicle control for forskolin
- Same final concentration as in forskolin-treated wells



- **Tissue culture plates**

- 37°C pre-warmed
- For droplet plating of organoids

- **EVOS M5000 Imaging System**

- High-resolution imaging
- For acquisition of organoid morphology

- **OrganoSeg MATLAB plug-in**

- Open-source software
- Quantification of organoid cross-sectional area (CSA)

Safety warnings

- ! Handle paraformaldehyde, fluorescent dyes, and infected material according to institutional biosafety level protocols and dispose of hazardous waste properly.

Before start

- Prepare PBS-Tx (PBS + 0.5% Triton X-100).
- Prepare blocking buffer (PBS-Tx + 4% normal donkey serum).
- Use a cut pipette tip for all liquid handling steps to prevent mechanical damage to organoids.
- Keep organoids at room temperature (RT) during short incubations unless otherwise noted.

Protocol 3: Flow Cytometry (FACS) Protocol for Filovirus- Infected Human iPSC-Derived Gut Organoids

1 **Authors:** Elizabeth Yvonne Flores et al.

Abstract

2 This protocol describes the preparation, staining, and analysis of human iPSC-derived gut organoids infected with filovirus (EBOV) for flow cytometric quantification of infected cells. The method includes dissociation of 3D organoids, viability staining with a near- infrared (NIR) live/dead dye, fixation/inactivation, intracellular immunostaining for viral nucleoproteins, and acquisition using a Stratedigm flow cytometer.

Materials and Reagent

3

Reagent / Material	Vendor	Catalog No . / Details	Notes
Near-Infrared (NIR) Live/Dead Stain	—	—	For live/dead discrimination
Dimethyl sulfoxide (DMSO)	—	—	Solvent for NIRdye
Phosphate-Buffered Saline (PBS), 1X	—	—	For washing
Bovine Serum Albumin (BSA), 0.5% in PBS	—	—	For FACS buffer
Ethylenediaminetetraacetic Acid (EDTA), 2 mM	—	—	Prevents cell clumping
Trypsin, 0.5%	—	—	For single-cell dissociation
Cell Recovery Solution (CRS)	Corning	—	To recover organoids from Matrigel

	3D Matrigel	Corning	—	Organoid culture matrix
	Paraformaldehyde (PFA), 4%	—	—	For fixation and viral inactivation
	Triton X-100	—	—	For cell permeabilization
	Blocking buffer (5% goat serum in PBS-Tx)	—	—	For antibody blocking
	Rabbit anti-EBOV NP (primary antibody)	IBT	0301-012	Dilution range: 1:250–1:1000
	Donkey anti-Rabbit Alexa Fluor 594 or 647 (secondary antibody)	Thermo Fisher	A-21207 or equivalent	1:1000 dilution
	FACS buffer (0.5% BSA PBS + 2 mM EDTA)	—	—	Final resuspension buffer
	CellPro Flow Cytometry Tubes, 35 µm Strainer Cap	—	—	For filtration
	Stratedigm Flow Cytometer	CRoM Core	—	For sample acquisition

Methods

4 Step 1 — NIR Dye Preparation

1. Reconstitute the NIR Live/Dead dye in 100 µL DMSO.
2. Prepare 1:800 dilution aliquots (10 µL each) for single use.
3. Protect aliquots from light by wrapping in foil and store at –20°C.B. FACS Buffer Preparation
4. Prepare FACS buffer by combining 0.5% BSA in 1X PBS with 2 mM EDTA.
5. Mix thoroughly and keep at 4°C until use

5 Step 2 — In the BSL-4 Laboratory

1. Organoid Recovery from Matrigel: Add 1 mL Cell Recovery Solution (CRS) to each Matrigel dome and incubate on ice for 30 minutes.
2. Collect the organoid suspension and centrifuge at 300 × g for 5 minutes.

3. Organoid Dissociation: Resuspend the pellet in 0.5% trypsin and incubate for 3–5 minutes at 37°C.
4. Pipette gently to dissociate into single cells, then centrifuge at 300× g for 5 minutes.
5. Wash and Filter: Wash the pellet once with 1X PBS and filter through 35 µm strainer-capped flow tubes.
6. Live/Dead Staining: Stain cells with NIR dye (1:800 dilution in 250 µL PBS).
7. Incubate for 15–30 minutes at room temperature or 4°C, protected from light. Mix gently and wash once with PBS.
8. Fixation and Inactivation: Resuspend cells in 4% PFA and incubate for 6 hours at RT.
9. Following inactivation, transfer samples to BSL-2 and wash with 1X PBS.

6 Step 3 — In the BSL-2 Laboratory

1. Washing: Wash cells twice with 1X PBS to remove residual PFA.
2. Permeabilization: Incubate in PBS containing 0.5% Triton X-100 for 10 minutes at RT.
3. Blocking: Wash once with FACS buffer and block in 5% goat serum for 20 minutes at RT.
4. Primary Antibody Incubation: Wash with PBS, add rabbit anti-EBOV NP antibody (IBT 0301-012) diluted 1:250–1:1000 in blocking buffer, and incubate for 1 hour at RT.
5. Secondary Antibody Incubation: Wash twice with PBS, add donkey anti-rabbit Alexa Fluor 594 or 647 (1:1000 dilution), and incubate for 1 hour in the dark.
6. Final Washing and Resuspension: Wash twice with PBS and resuspend in 350 µL FACS buffer.

7 Step 4 — Flow Cytometry Analysis

1. Acquire samples on a Stratified flow cytometer using standard fluorescence channels (594 nm or 647 nm for secondary antibody, NIR for viability, GFP for CDX2 expression).
2. Avoid green fluorophores in secondary staining to prevent overlap with CDX2-GFP signal.

Experimental Conditions

8

Condition	EBOV	Mock
Unstained control	✓	✓
NIR only	✓	✓
Secondary antibody only	✓	✓
Primary antibody dilutions (1:250, 1:500, 1:750, 1:1000) +secondary	✓	✓



Apply all conditions across experimental time points (e.g., 1, 3 dpi).

Quality Control and Reproducibility

- 9
 1. Adjust NIR stain concentration and incubation times depending on cell type and viability.
 2. Handle all filovirus-infected samples under appropriate biosafety conditions until verified inactivated.
 3. Include compensation and fluorescence-minus-one (FMO) controls for accurate gating.

Protocol 4: Whole-Mount Immunofluorescence Staining of Human iPSC-Derived Gut Organoids for Detection of FilovirusUntitled section

- 10 **Authors:** Adapted from Aleks Syzmaniak and Katie McCauley; revised by Elizabeth Yvonne Flores, September 2022

Abstract

- 11 This protocol describes whole-mount immunofluorescence staining of human iPSC-derived gut organoids expressing CDX2-GFP, as well as the intestinal markers Villin and MUC2, for the detection of filovirus (EBOV and MARV) nucleoproteins localized to the perinuclear region. The method preserves organoid architecture, minimizes structural damage during processing, and includes optimized steps for gentle handling, permeabilization, and high-resolution imaging by confocal microscopy.

Materials and Reagents

- 12
 - Targets: CDX2-GFP, Villin, MUC2, EBOV NP, MARV NP, MARV NC
 - Species: human iPSC-derived gut organoids
 - Method type: Whole-mount immunofluorescence staining
 - Estimated duration: ~2 days
- 13
 - Cell Recovery Solution (Corning)
 - Phosphate-buffered saline (PBS)
 - Paraformaldehyde (PFA), 4% solution (freshly prepared or commercial)
 - Triton X-100
 - Normal donkey serum (for blocking; Jackson ImmunoResearch or equivalent)
 - Hoechst 33342 nuclear dye (ThermoFisher Scientific, 62249)
 - Cavity slides (Millipore Sigma, BR475505)
 - ProLong Diamond Antifade Mountant (ThermoFisher Scientific, P36961)

- 13 mm coverslips (ThermoFisher Scientific, 174950)
- Eppendorf tubes (1.5 mL)

14 Antibodies, Dyes, and Reagents for Immunostaining

Category	Target /Reagent	Vendor	Catalog N o.	Dilution / Notes
IFA Reagents	4% Normal Goat Serum (NGS)	Jackson ImmunoResearch	005-000-121	Blocking solution
IFA Reagents	13 mm Coverslips	ThermoFisher Scientific	174950	
IFA Reagents	ProLong Diamond Antifade Mountant	ThermoFisher Scientific	P36961	Mounting medium
IFA Reagents	BRAND CavitySlides	Millipore Sigma	BR475505	For organoid mounting
Primary Antibodies	Rabbit anti-EBOV NP	IBT	0301-012	1:200
Primary Antibodies	Rabbit anti-MARV NP	IBT	0303-012	1:500
Primary Antibodies	Rabbit anti-MARV NC	Mühlberger Lab	—	1:200
Secondary Antibodies	Donkey anti-Chicken Alexa Fluor 488	Jackson ImmunoResearch Labs	703-545-155	1:500
Secondary Antibodies	Donkey anti-Rabbit Alexa Fluor 594 IgG(H+L)	ThermoFisher Scientific	A-21207	1:500
Secondary Antibodies	Donkey anti-Mouse Alexa Fluor 488 IgG (H+L)	ThermoFisher Scientific	A-21202	1:500



	Dyes	Hoechst33342	ThermoFisher Scientific	62249	1:2000

Equipment

- 15
- P1000 pipette with cut tips (to enlarge aperture)
 - Rocking platform
 - Centrifuge capable of $<200 \times g$
 - Confocal microscope (e.g., Zeiss LSM 700) or Nikon widefield fluorescence microscope

Before You Begin

- 16
- Prepare PBS-Tx (PBS + 0.5% Triton X-100).
 - Prepare blocking buffer (PBS-Tx + 4% normal donkey serum).
 - Use a cut pipette tip for all liquid handling steps to prevent mechanical damage to organoids. Keep organoids at room temperature (RT) during short incubations unless otherwise noted.

Safety Information

- 17
- Handle paraformaldehyde, fluorescent dyes, and infected material according to institutional biosafety level protocols and dispose of hazardous waste properly.

Methods

- 18 **Step 1 — Organoid Recovery**
1. Recover organoids from Matrigel using Cell Recovery Solution (Corning) according to the manufacturer's instructions on ice (~30–60 min).
 2. Wash organoids with PBS to remove residual matrix.
 3. For large organoids: allow to settle by gravity for ~10 min.
 4. For small organoids: centrifuge at $<200 \times g$ for 2 min at 4°C to pellet.
- 19 **Step 2 — Fixation**
1. Fix organoids with 4% PFA for 30 min at RT.
 2. Wash organoids with PBS to remove residual fixative.
- 20 **Step 3 — Transfer and Handling**
1. Transfer organoids into 1.5 mL Eppendorf tubes (1 tube per staining condition).
 2. Between each subsequent step, centrifuge briefly (2 min, $<200 \times g$, RT) and pipette gently to avoid mechanical disruption.

21 Step 4 — Permeabilization

1. Incubate organoids in PBS-Tx (PBS + 0.5% Triton X-100) for 15 min at RT with gentle rocking.

22 Step 5 — Blocking

1. Incubate organoids in 500 μ L blocking buffer (PBS-Tx + 4% normal donkey serum) for 30 min at RT with rocking.

23 Step 6 — Primary Antibody Incubation

1. Prepare primary antibodies in blocking buffer at the indicated dilutions (see antibody table).
2. Incubate organoids with 250 μ L antibody solution overnight at 4°C with gentle rocking.
3. Tip: Overnight incubation enhances antibody specificity and penetration in 3D tissue.

24 Step 7 — Wash

1. Wash organoids at least 3 times in PBS-Tx, 15–30 min each with gentle rocking.

25 Step 8 — Secondary Antibody Incubation

1. Dilute appropriate donkey secondary antibodies (see table) 1:500 in blocking buffer.
2. Incubate organoids in 250 μ L secondary antibody solution for 1 hr at RT with gentle rocking.

26 Step 9 — Wash

1. Wash organoids ≥ 3 times in PBS-Tx, 15–30 min each.

27 Step 10 — Nuclear Staining

1. Stain nuclei with Hoechst 33342 (1:2000 dilution) for 30 min at RT.
2. Note: Hoechst is preferred over DAPI to avoid edge fluorescence artifacts common in 3D

28 Step 11 — Mounting and Imaging

1. Wash organoids with PBS and resuspend in up to 45 μ L PBS.
2. Mount the suspension in the center of a cavity slide and place a coverslip.
3. Invert the slide (coverslip facing down) to allow organoids to settle toward the coverslip.
4. Tip: This orientation ensures optimal focus during imaging.
5. Image using a CReM Nikon widefield microscope or Zeiss LSM 700 confocal microscope.

Expected Results

- 29 Organoids should display clear cytoplasmic GFP or specific immunostaining signals for targeted antigens (Villin, MUC2, EBOV NP, MARV NP, MARV NC), with distinct nuclear

staining from Hoechst. Alexa Fluor signals should be well-defined and evenly distributed without edge artifacts.

Quality Control and Reproducibility

30

	Problem	Possible cause	Solution
	Organoids disintegrate	Pipette shear stresses	Use cut tips and gentle pipetting
	Weak signal	Insufficient antibody penetration	Extend primary incubation or increase Triton X-100 to 0.7%
	Edge fluorescence	DAPI artifacts	Use Hoechst instead of DAPI

Protocol 5: Forskolin-Induced Functional Assay in iPSC- and Primary-Derived Gut Organoids with Ebola and Marburg Virus

31 **Authors:** Elizabeth Yvonne Flores et al.

Abstract

32 This protocol outlines a forskolin-induced stimulation assay in iPSC-derived and primary human gut organoids. It is designed to evaluate dynamic changes in organoid morphology and functional responses both under baseline conditions and following infection with Ebola virus (EBOV) or Marburg virus (MARV). Organoids are dissociated from Matrigel, replated in 3D culture at low density to prevent spheroid overlap, and treated with forskolin or vehicle control. High-resolution imaging is performed at defined time points post-treatment, and organoid cross-sectional area (CSA) is quantified using OrganoSeg, an open-source MATLAB plug-in. Normalized CSA measurements allow for the assessment of forskolin-induced organoid swelling relative to baseline and control conditions.

Materials and Reagents

33

Component	Specification / Source	Purpose / Notes
Human iPSC-derived and primary-derived gut organoids	Distal colonic organoids (HCOs), proximal intestinal organoids (HIOs), primary-derived gut organoids	Maintained in 3D Matrigel prior to dissociation
3D Matrigel	Basement membrane matrix (Corning, pre-chilled)	3D scaffold for organoid replating
Phosphate-buffered saline (PBS)	Sterile	For washing and rinsing organoids
CKDCI or IMCK medium	Defined organoid culture medium	Used for plating and treatment recovery
Forskolin	Sigma, Cat# F3917	Stimulating agent (5 μ M)
DMSO	Vehicle control for forskolin	Same final concentration as in forskolin-treated wells
Tissue culture plates	37°C pre-warmed	For droplet plating of organoids
EVOS M5000 Imaging System	High-resolution imaging	For acquisition of organoid morphology
OrganoSeg MATLAB plug-in	Open-source software	Quantification of organoid cross-sectional area (CSA)

Methods

34 Step 1 — Organoid Dissociation and Replating

1. Dissociate HIOs as described in the viral infection protocol.
2. Wash dissociated organoids with PBS and centrifuge at $200 \times g$ for 3 minutes.
3. Resuspend the resulting pellet in fresh 3D Matrigel at 37°C at low density to prevent spheroid overlap.
4. Plate organoid-Matrigel droplets onto pre-warmed tissue culture plates coated with medium.
5. Allow organoids to recover for 48–72 hours prior to forskolin treatment.

35 Step 2 — Forskolin Treatment

1. Supplement organoid culture medium (CKDC1 or IMCK) with either 5 μ M forskolin or vehicle (DMSO).
2. Apply treatments to the organoid cultures and incubate under standard culture conditions.
3. Capture baseline high-resolution images prior to treatment using the EVOS M5000 Imaging System.
4. Refresh medium containing forskolin or vehicle at 24, 48, and 72 hours post-treatment.
5. Acquire images at each designated time point using identical imaging parameters to baseline.

36 **Step 3 — Image Acquisition and Quantification**

1. Save all images in .tif format for analysis.
2. Segment organoid images using OrganoSeg with the following parameters:
 - Intensity threshold: 0.5
 - Window size: 100
 - Size threshold: 100
3. Manually review post-segmentation images to exclude spheroids at the edge of the field, burst or flattened organoids, and organoids absent in either pre- or post-treatment images.
4. Extract output metrics from OrganoSeg, including total organoid number and cross-sectional area (CSA).
5. Calculate normalized whole-well CSA using the formula:
$$\text{Normalized CSA (\%)} = (\text{CSA post-treatment} / \text{CSA pre-treatment}) \times 100$$
6. Subtract CSA values from control wells to correct for baseline changes unrelated to forskolin.

37 **Step 4 — Experimental Design and Replication**

1. Include control wells treated with DMSO and/or organoid culture medium in each experiment.
2. Perform at least three replicate wells per treatment condition.
3. Repeat imaging and analysis at each designated time point (24, 48, 72 hours).

38 **Step 5 — Statistical Analysis**

1. Conduct statistical analyses using GraphPad Prism or equivalent software.
2. Compare treatment groups using two-way ANOVA followed by Tukey's multiple comparisons test. Present data as mean $\pm$ SEM.
3. Consider $p \leq 0.05$ statistically significant.

Quality control and reproducibility

39

	Problem	Possible Cause	Recommended Solution
	Organoids overlap or fuse	High plating density	Reduce Matrigel/organoid concentration for droplet plating
	Inconsistent organoid swelling	Variable imaging focus or segmentation	Standardize imaging parameters and verify OrganoSeg settings
	Bursting or flattened spheroids	Mechanical stress during handling	Handle droplets gently; minimize pipetting
	Low forskolin response	Inaccurate concentration or degraded compound	Verify stock concentration and storage conditions

Expected Results

40 Following stimulation with forskolin (commonly 5 μ M), organoids derived from healthy or functional intestinal epithelia exhibit a robust, time-dependent luminal swelling within 24 hours. This is driven by cAMP-mediated activation of CFTR channels, leading to chloride and fluid secretion into the lumen.

Qualitative observation:

Organoids appear visibly larger and more translucent under brightfield microscopy. The central lumen expands, often by 2–3 $\times$ the initial diameter, depending on differentiation state and donor source.

Quantitative results:

Swelling kinetics: Volume increase detectable within 24 hours, plateauing around 72 hours.

Fold-change: Typically 2 $\times$ increase in cross-sectional area relative to baseline (vehicle-treated controls).

Infection effects: Pathogen-infected organoids exhibit significantly reduced or absent swelling in response to forskolin. Morphologically, these organoids may retain dense, compact structures with limited luminal expansion, indicating impaired epithelial ion/fluid transport.