IPSC-DERIVED ORGANOID BASED MODEL OF INTESTINAL FILOVIRUS INFECTION

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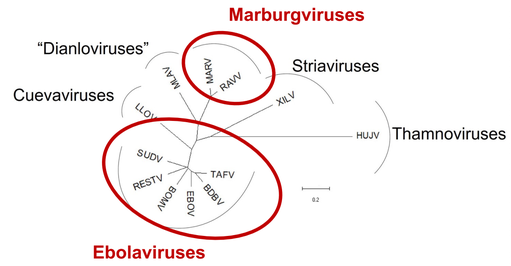
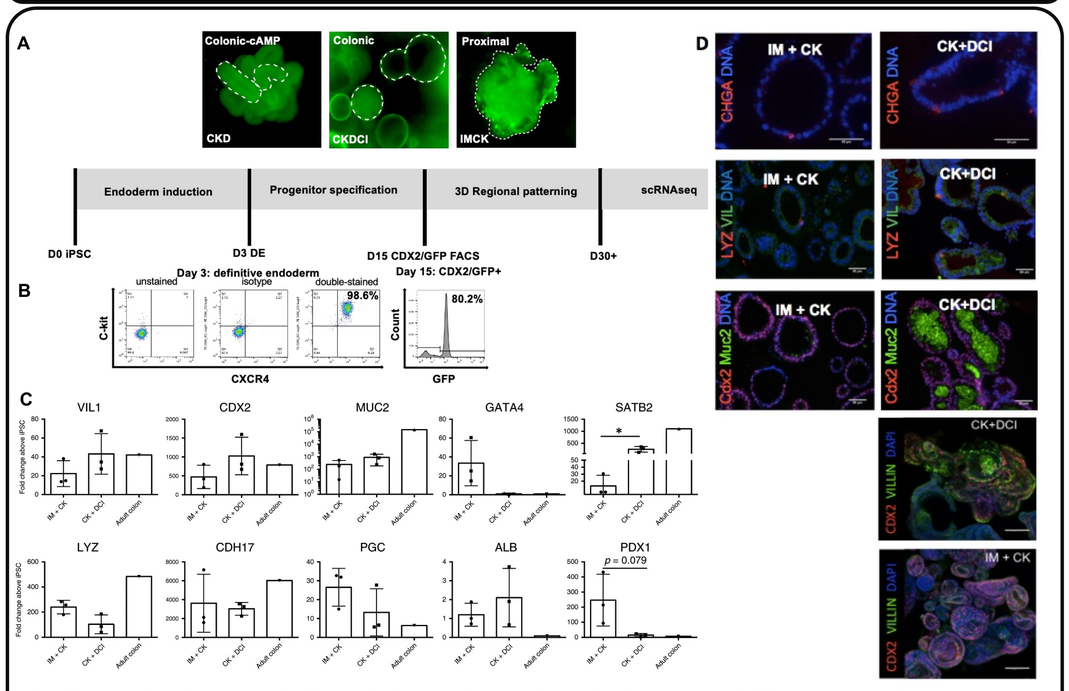
INTRODUCTION

The devastating 2014 Ebola virus (EBOV) disease outbreak in West Africa has sparked the development and regulatory approval of antiviral countermeasures, including an emergency-use vaccine and therapeutic monoclonal antibodies. While these countermeasures are highly beneficial to block EBOV infection and interfere with disease progression at early stages, they are less suitable for the treatment of late-stage EBOV disease (EVD), which is less studied. This includes gastrointestinal symptoms, and diarrhea, in particular. Affected EVD patients lose copious amounts of fluids in a matter of days, rapidly deteriorating into hypovolemic shock and death. Similar intestinal manifestations were also reported for MARV disease, another filovirus. At present, no available animal models, including non-human primates, can recapitulate the gastrointestinal symptoms of EVD patients. **To fill this gap, we proposed to establish a human intestinal infection model to study the effects of filovirus infection on intestinal epithelial integrity.**

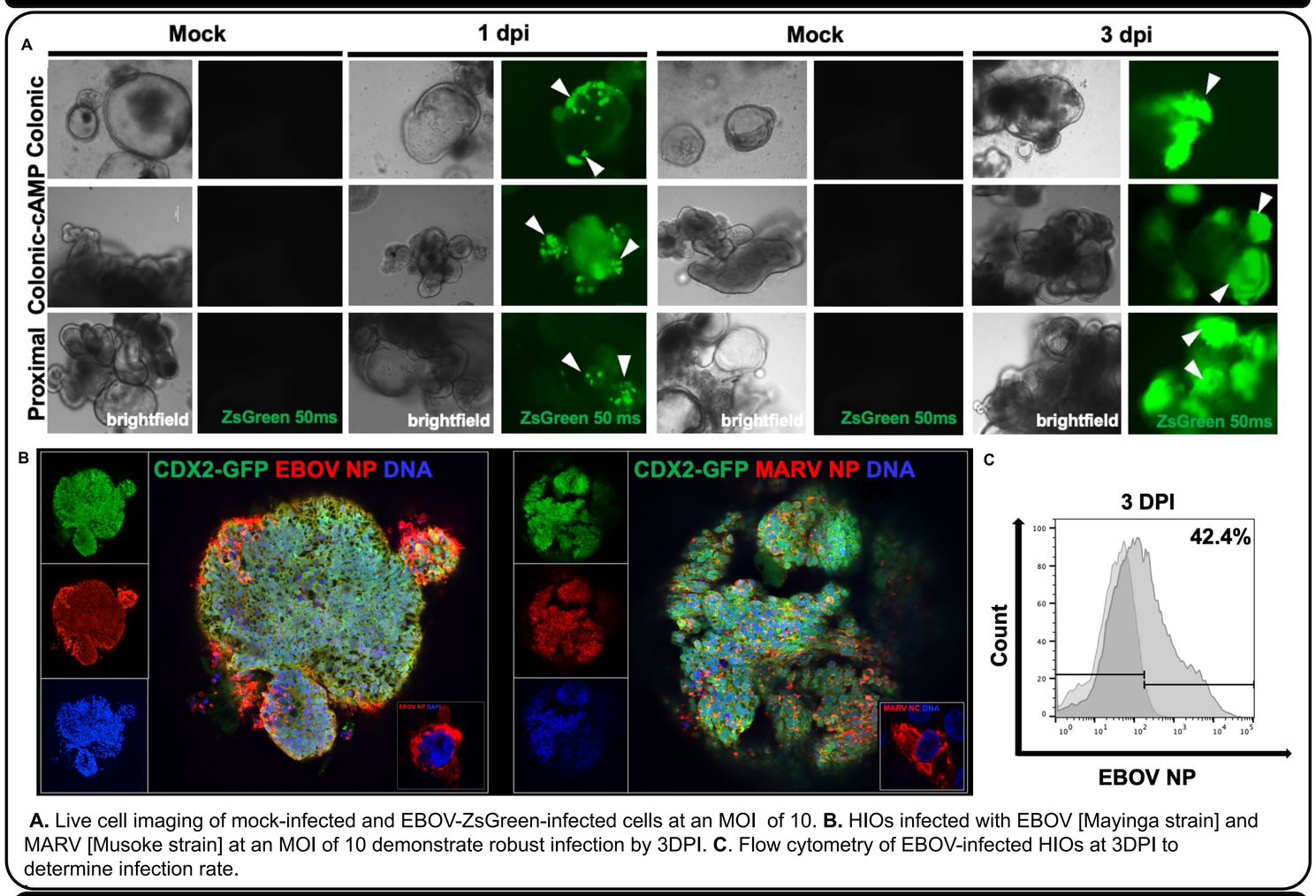


CDX2^{GFP+} sorted iPSC-derived intestinal organoids show conserved regional specificity



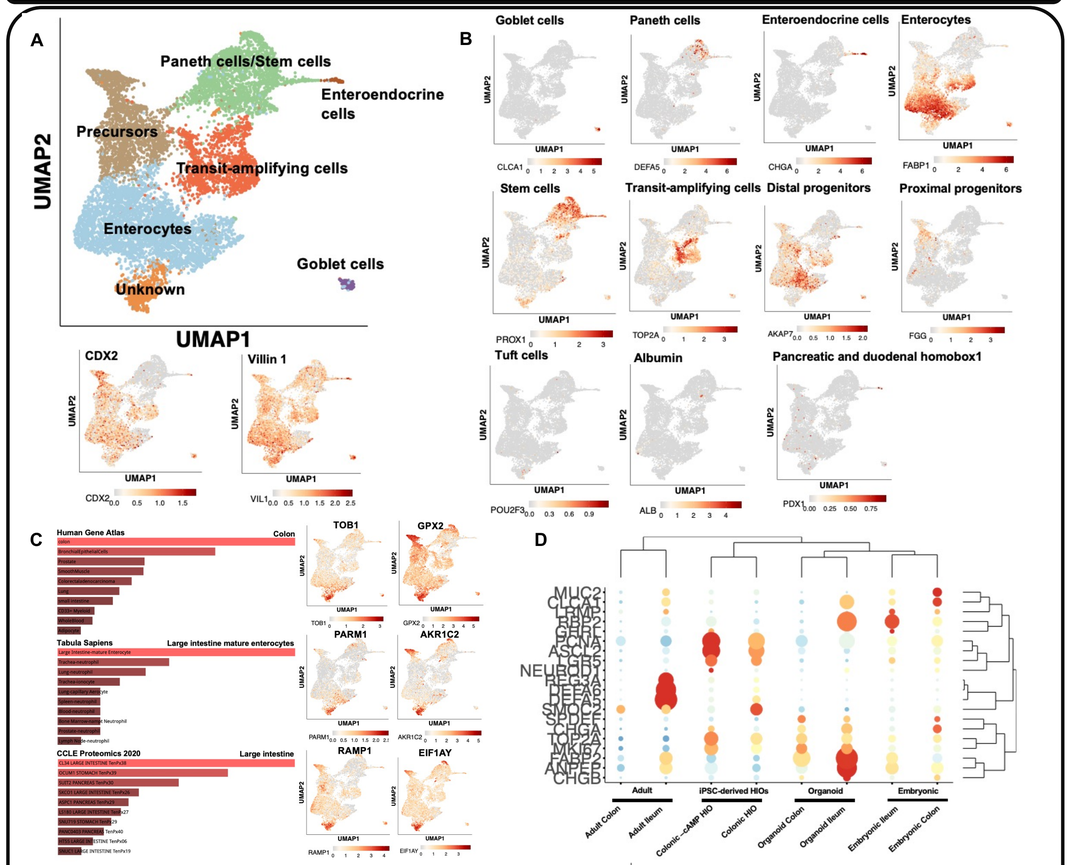
A. Schematic diagram of differentiation protocol into distal (colonic-cAMP and colonic) vs proximal (small intestine) HIOs. **B.** Flow cytometry of single-cell suspensions from HIOs using C-kit and CXCR4 for definitive endoderm and FITC for GFP percentage. Representative micrographs of HIOs at day 45 of differentiation using different media conditions, scale bar=100um. **C.** qRT-PCR confirms the distal vs proximal identity of organoids cultured in CK-DCI vs IM-CK conditions, respectively. **D.** Immunofluorescence analysis confirms the presence of Paneth cells expressing Lysozyme (LYZ), enteroendocrine cells expressing Chromogranin A (CHGA) and secretion of Mucin 2 (Muc2) into the lumen of the colonic HIOs and robust expression of CDX2 and Villin.

HIOs are permissive to filovirus infection



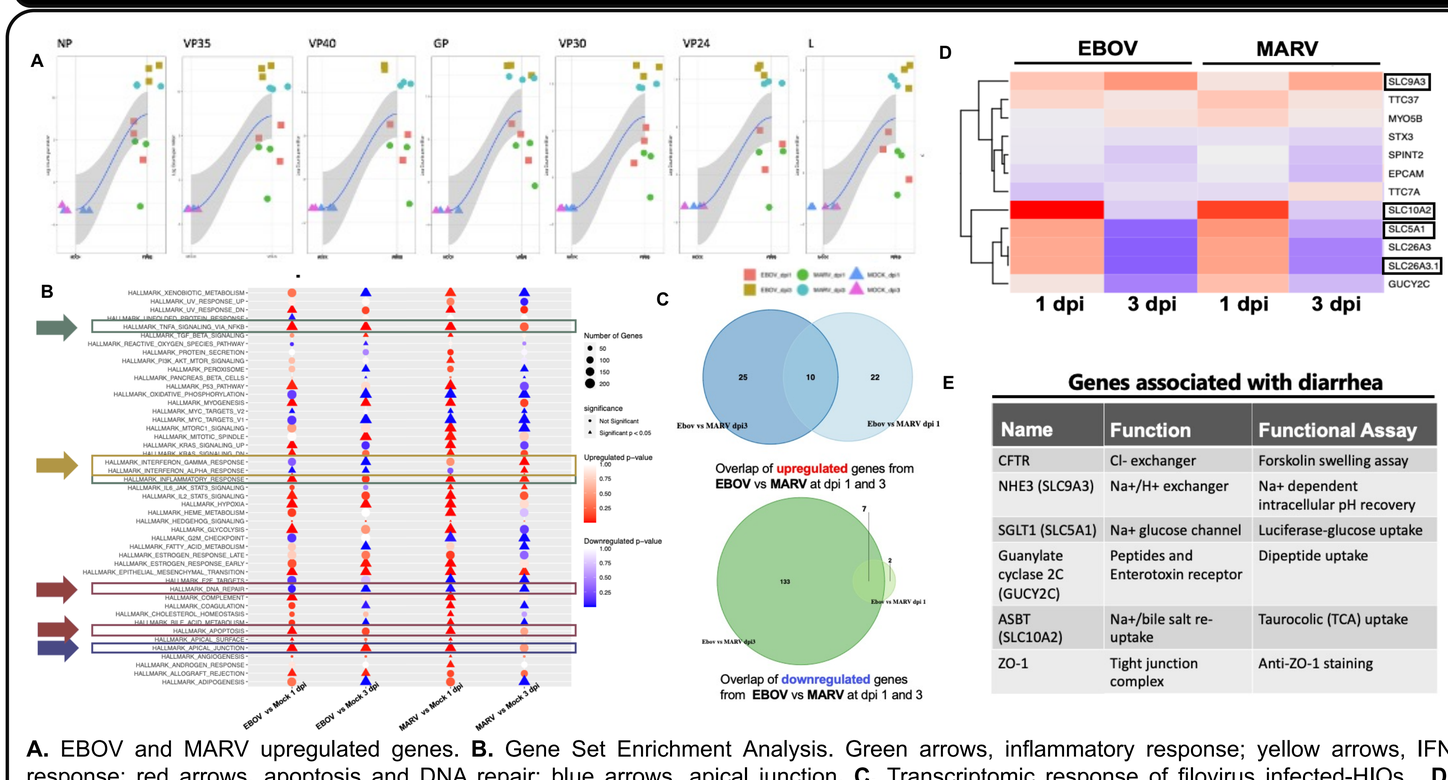
A. Live cell imaging of mock-infected and EBOV-ZsGreen-infected cells at an MOI of 10. **B.** HIOs infected with EBOV [Mayinga strain] and MARV [Musoke strain] at an MOI of 10 demonstrate robust infection by 3DPI. **C**. Flow cytometry of EBOV-infected HIOs at 3DPI to determine infection rate.

scRNAseq of Colonic and Colonic-cAMP HIOs



A. HIO at day 66 of differentiation were processed for scRNA-seq using the Chromium 10X Genomics platform. Two independent differentiations from each lineage, colonic and colonic-cAMP, were analyzed. **B.** UMAP visualization of expression of specific intestinal epithelial cell lineages. **C.** Gene set enrichment analysis using Enrichr analyzing the top tissue types when references to Human Gene Atlas. **D.** Bubble plot comparison of iPSC-derived HIOs to adult tissue, embryonic tissue and resected HIOs. **E.** Proportional graph of different cell types.

Filovirus-infected HIOs display an early inflammatory mediated response and dysregulation of diarrheal-associated genes



Name	Function	Functional Assay
CFTR	Cl- exchanger	Forskolin swelling assay
NHE3 (SLC9A3)	Na+/H+ exchanger	Na+ dependent intracellular pH recovery
SGLT1 (SLC5A1)	Na+ glucose channel	Luciferase-glucose uptake
Guanylate cyclase 2C (GUCY2C)	Peptides and Enterotoxin receptor	Dipeptide uptake
ASBT (SLC10A2)	Na+/bile salt re-uptake	Taurocolic (TCA) uptake
ZO-1	Tight junction complex	Anti-ZO-1 staining

A. EBOV and MARV upregulated genes. **B.** Gene Set Enrichment Analysis. Green arrows, inflammatory response; yellow arrows, IFN response; red arrows, apoptosis and DNA repair; blue arrows, apical junction. **C.** Transcriptomic response of filovirus infected-HIOs. **D.** Unsupervised hierarchical clustering heat map detailing the expression of genes associated with diarrheal illness in EBOV and MARV infected HIOs at 1 and 3 dpi. **E.** Genes associated with diarrhea and their corresponding functional assays.

CONCLUSIONS

- Successful robust EBOV and MARV infections of iPSC-derived HIOs, affecting mostly epithelial CDX2+ enterocytes was achieved.
- The infected cells showed signs of cell damage, and transcriptomics analysis indicated the modulation of cell junction pathways and a set of ion transporters known to play a role in the induction of diarrhea.
- Taken together, these data suggest **EBOV and MARV compromise barrier integrity of the intestinal epithelium and cause abnormal ion flux as the basis for gastrointestinal dysfunction and diarrhea**.

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Mithal, A., Capilla, A., Heinze, D. *et al.* Generation of mesenchyme free intestinal organoids from human induced pluripotent stem cells. *Nat Commun* **11,** 215 (2020). https://doi.org/10.1038/s41467-019-13916-6