

Organoids: noves oportunitats per l'estudi de les malalties intestinals (IBD)

Azucena Salas

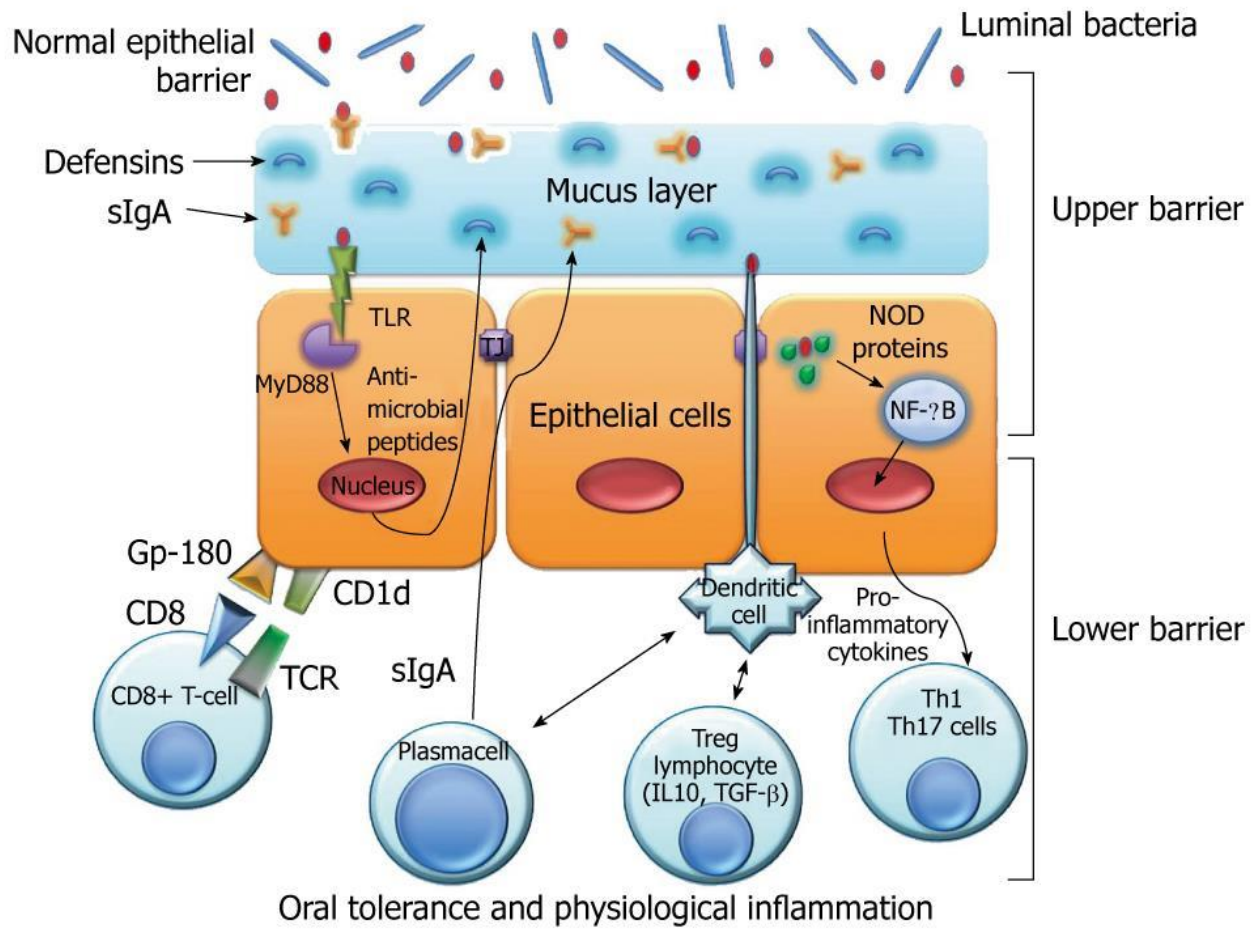
IDIBAPS-Hospital Clínic

Barcelona

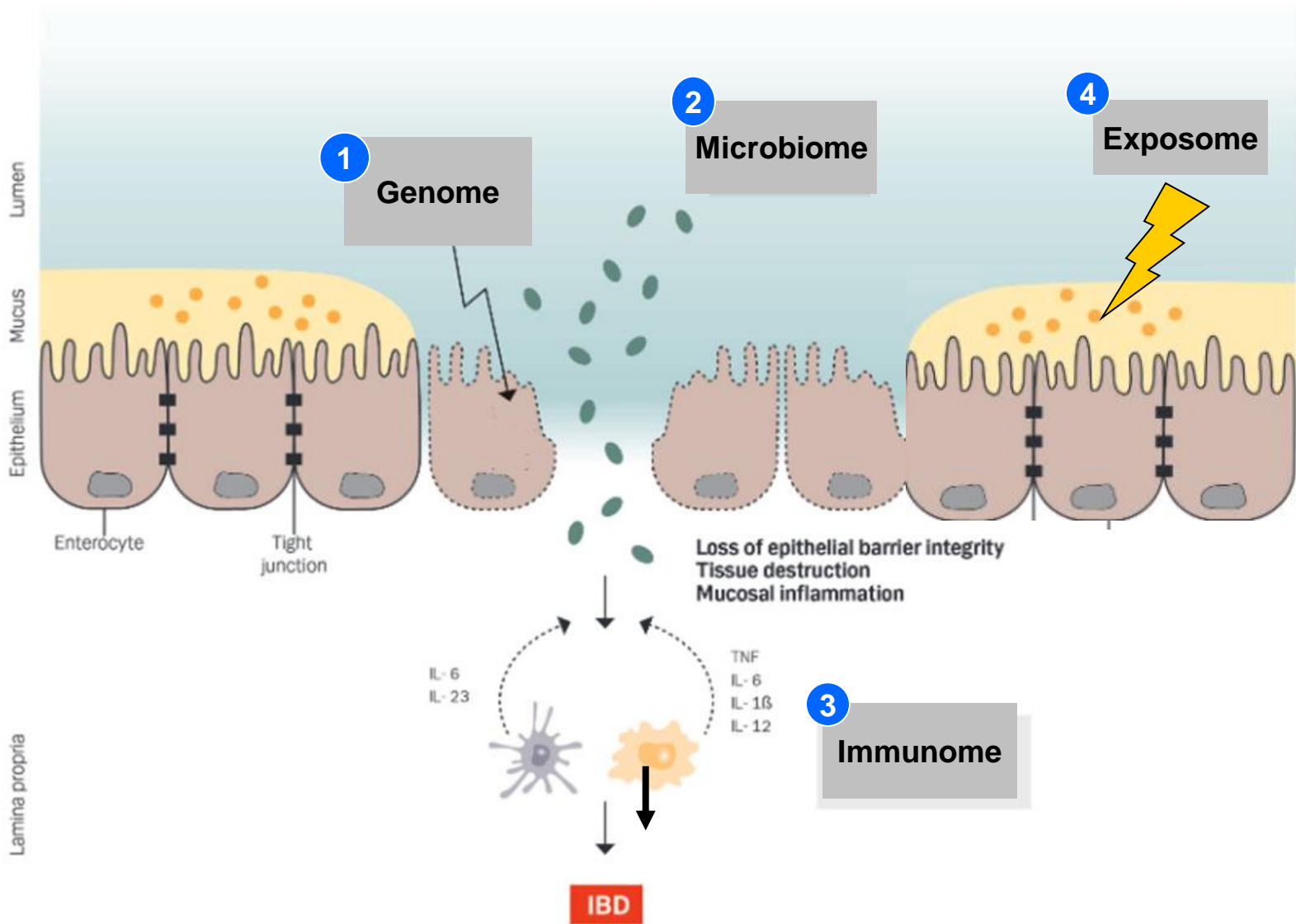
OUTLINE

- Importance of the epithelial compartment in tissue homeostasis and disease (Inflammatory bowel disease).
- Available tools to study the epithelium
- What are the organoids?
- How can I use organoids for my research?

The many roles of the intestinal epithelial cell



Pathogenesis of IBD

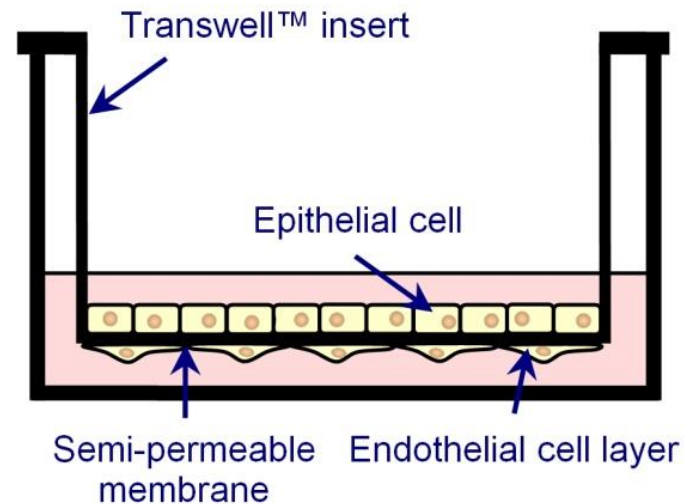
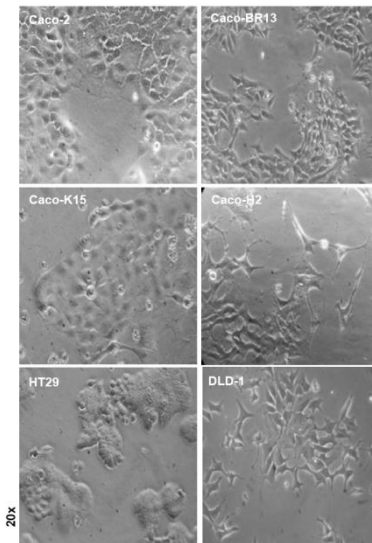


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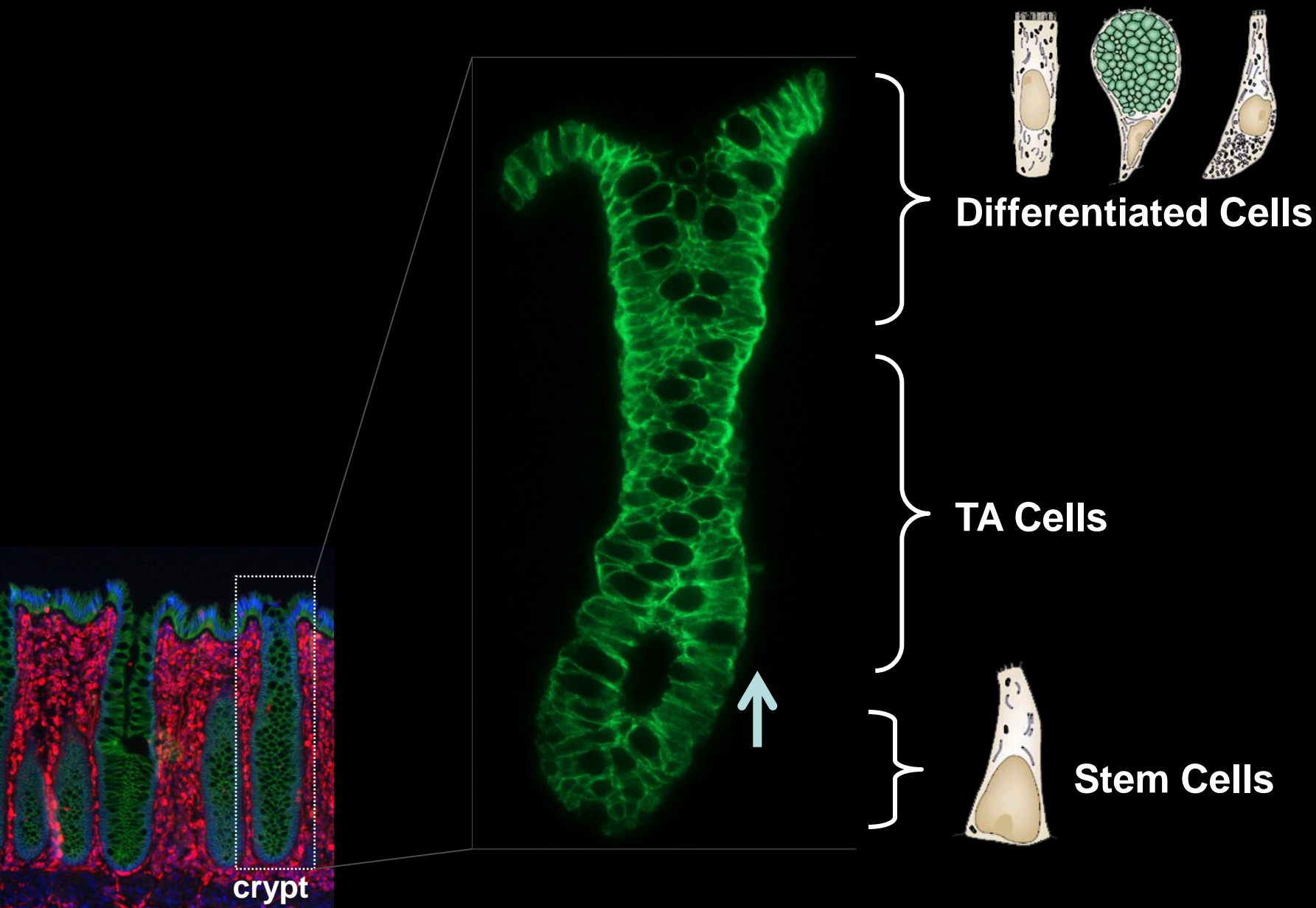
How can we study the epithelium function?

1) Epithelial cell lines



Limitations: they are immortalized cell lines and do not represent the behavior of primary cells; they do not reflect a disease phenotype.

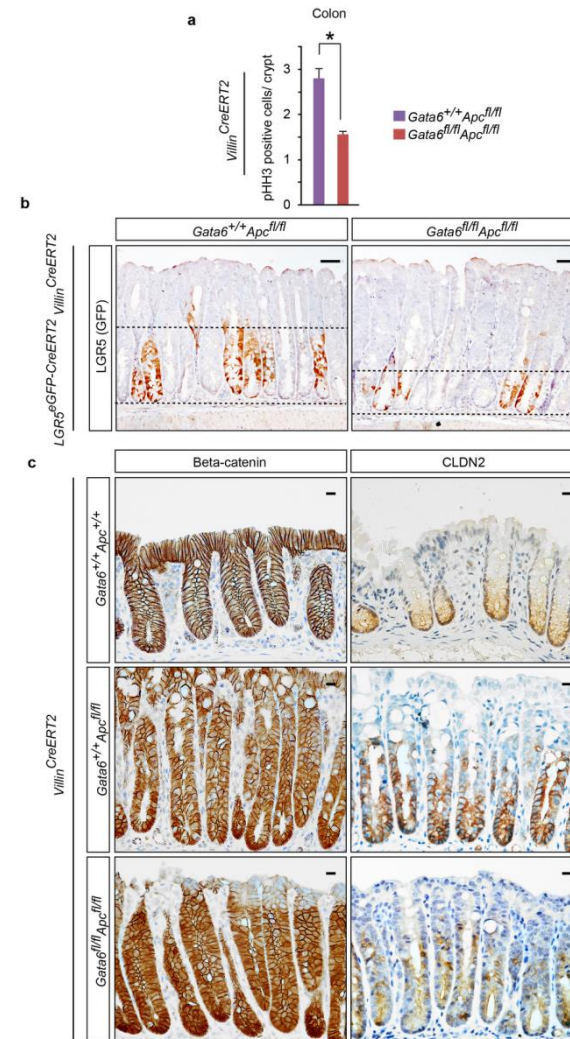
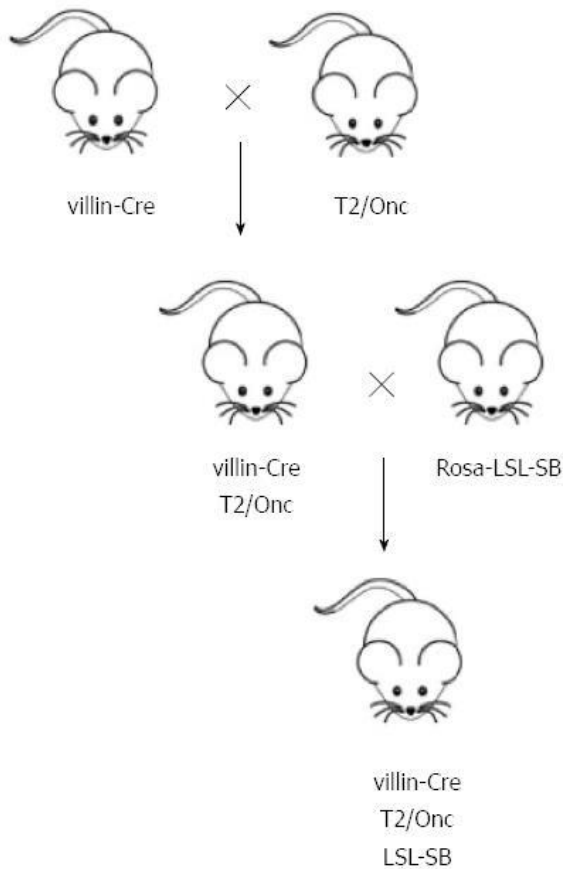
The Colonic Crypt



How can we study the epithelium function?

1) Epithelial cell lines

2) Animal models



How can we study the epithelium function?

- 1) Epithelial cell lines
- 2) Animal models
- 3) Intestinal stem cell organoids

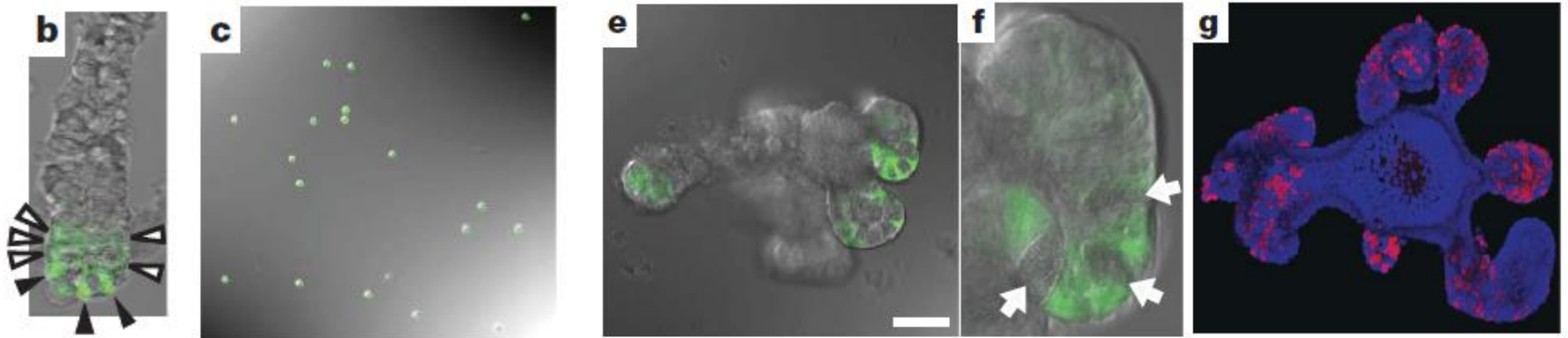
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- **What are the organoids?**
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Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche

Toshiro Sato¹, Robert G. Vries¹, Hugo J. Snippert¹, Marc van de Wetering¹, Nick Barker¹, Daniel E. Stange¹, Johan H. van Es¹, Arie Abo², Pekka Kujala³, Peter J. Peters³ & Hans Clevers¹

Lgr5-GFP mice



Establishment of Organoid Cultures from Human Intestinal Epithelium

2011

Long-term Expansion of Epithelial Organoids From Human Colon, Adenoma, Adenocarcinoma, and Barrett's Epithelium

TOSHIRO SATO,* DANIEL E. STANGE,* MARC FERRANTE,*†,§ ROBERT G. J. VRIES,* JOHAN H. VAN ES,* STIENEKE VAN DEN BRINK,* WINAN J. VAN HOUDT,||,¶ APOLLO PRONK,|| JOOST VAN GORP,* PETER D. SIERSEMA,* and HANS CLEVERS*

2012

Isolation and *in vitro* expansion of human colonic stem cells

Peter Jung^{1,9}, Toshiro Sato^{2,3,9}, Anna Merlos-Suárez¹, Francisco M Barriga¹, Mar Iglesias⁴, David Rossell⁵, Herbert Auer⁶, Mercedes Gallardo⁷, Maria A Blasco⁷, Elena Sancho¹, Hans Clevers² & Eduard Batlle^{1,8}

2013

A functional CFTR assay using primary cystic fibrosis intestinal organoids

Johanna F Dekkers¹⁻³, Caroline L Wiegerinck^{2,4}, Hugo R de Jonge⁵, Inez Bronsveld⁶, Hettie M Janssens⁷, Karin M de Winter-de Groot¹, Arianne M Brandsma^{1,3}, Nienke W M de Jong^{1,3}, Marcel J C Bijvelds⁵, Bob J Scholte⁸, Edward E S Nieuwenhuis⁴, Stieneke van den Brink^{9,10}, Hans Clevers^{9,10}, Cornelis K van der Ent¹, Sabine Middendorp^{2,4} & Jeffrey M Beekman¹⁻³

2014

Development of an enhanced human gastrointestinal epithelial culture system to facilitate patient-based assays

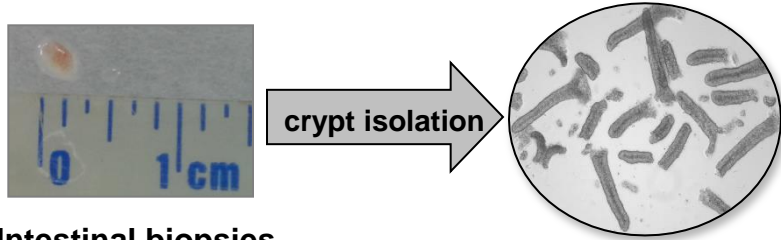
Kelli L VanDussen,¹ Jeffrey M Marinshaw,² Nurmohammad Shaikh,³ Hiroyuki Miyoshi,¹ Clara Moon,¹ Phillip I Tarr,^{3,4} Matthew A Ciorba,² Thaddeus S Stappenbeck¹

2015

Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids

Mami Matano^{1,4}, Shoichi Date^{1,2,4}, Mariko Shimokawa^{1,4}, Ai Takano^{1,4}, Masayuki Fujii^{1,3}, Yuki Ohta¹, Toshiaki Watanabe³, Takanori Kanai¹ & Toshiro Sato¹

Epithelial Organoid Culture (EpOC) Generation



Intestinal biopsies
(or surgical piece)

Available online protocols (Journal of Visualized Experiments)

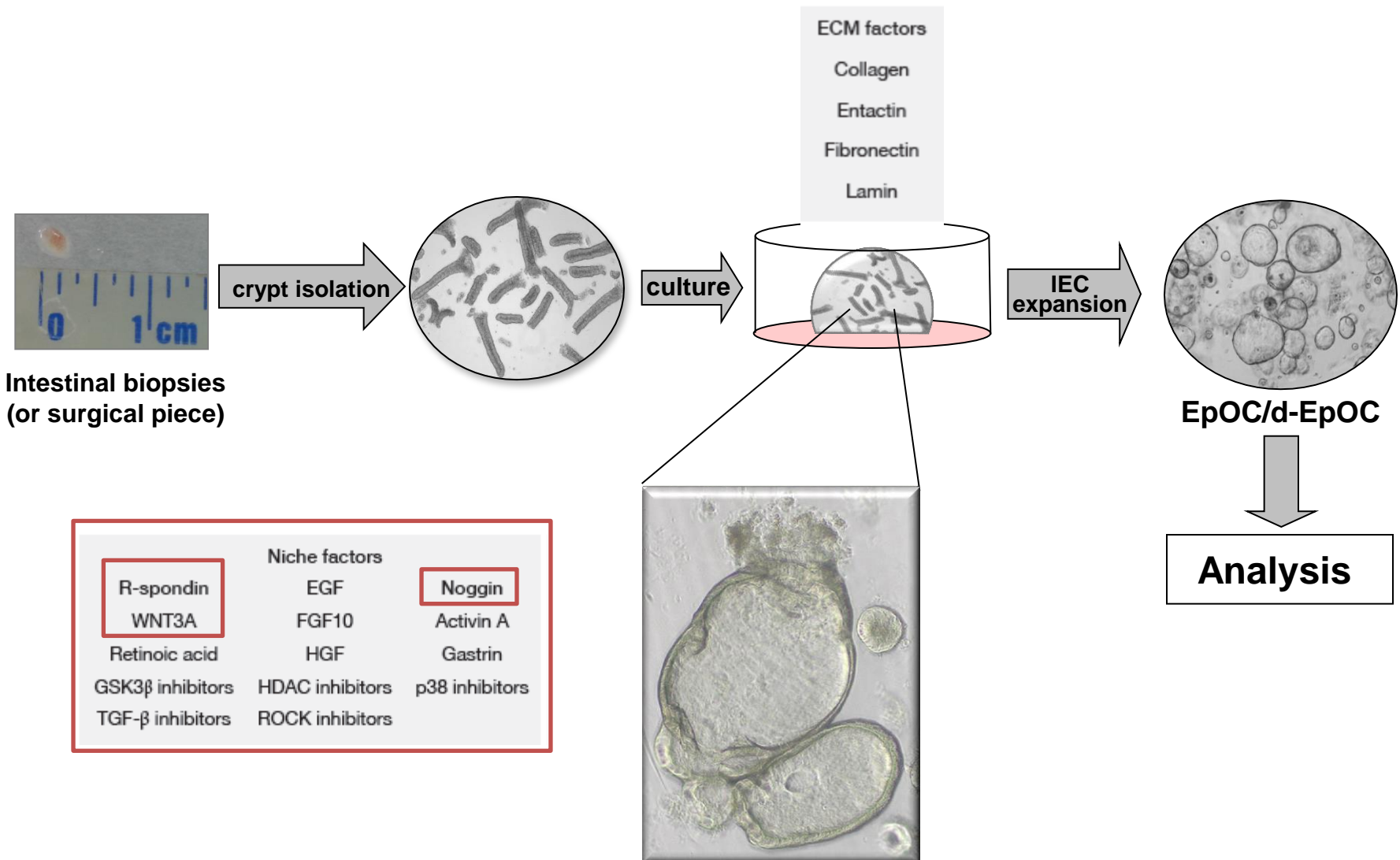
Helmrath Lab

<http://www.jove.com.sire.ub.edu/video/52483/establishment-human-epithelial-enteroids-colonoids-from-whole-tissue>

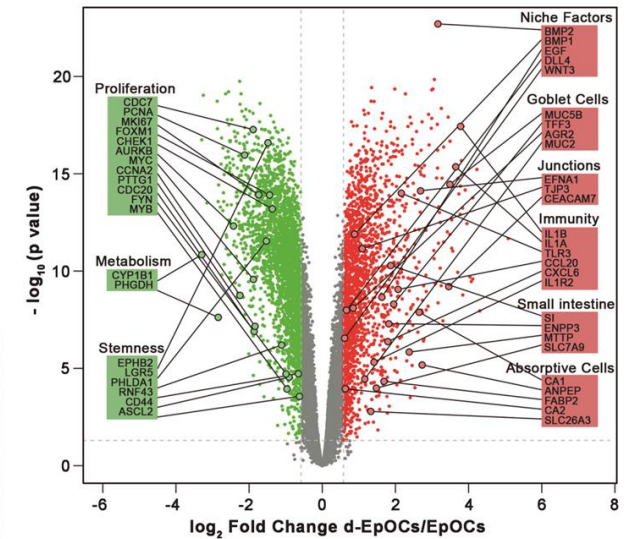
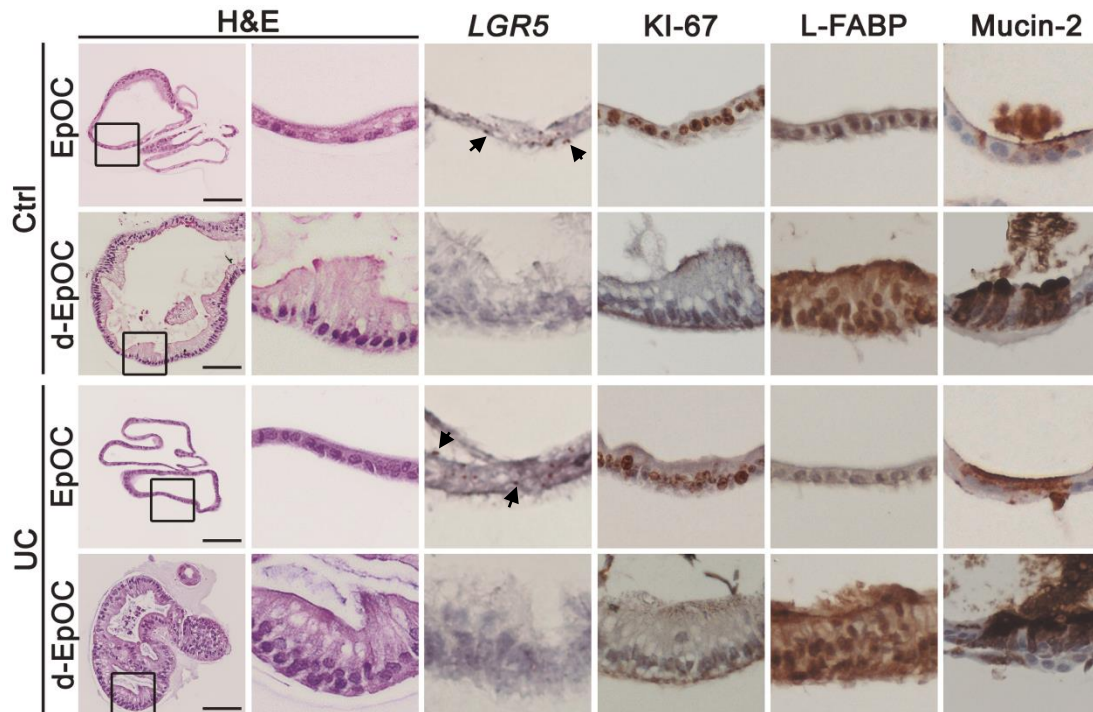
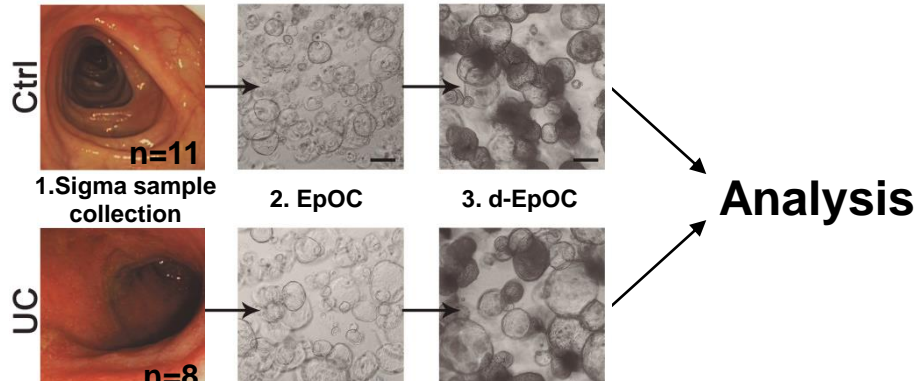
Clevers Lab

<http://www.jove.com.sire.ub.edu/video/53359/organoids-as-model-for-infectious-diseases-culture-human-murine>

Epithelial Organoid Culture (EpOC) Generation



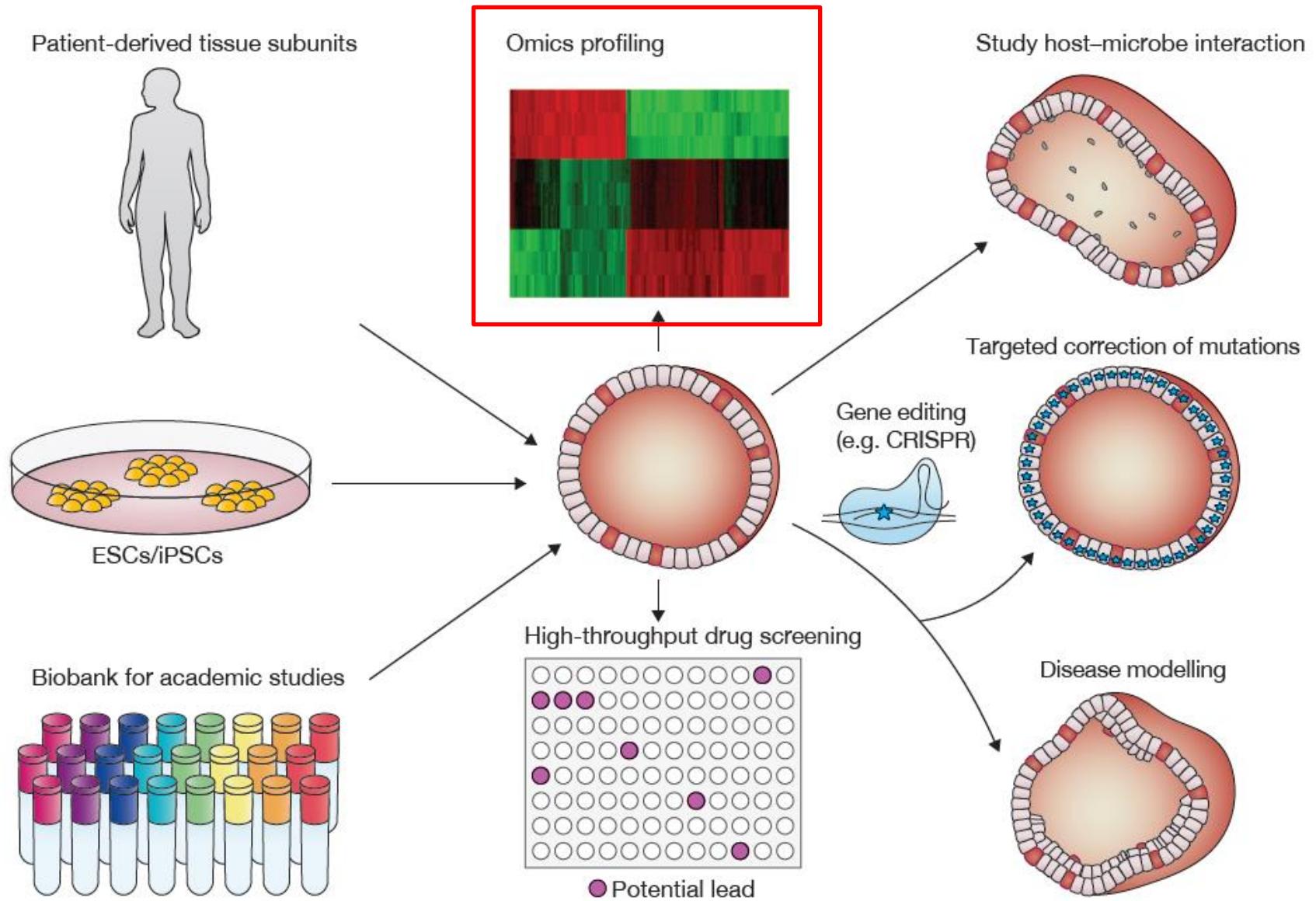
Differentiation of stem cells into intestinal epithelium in vitro using EpOCs



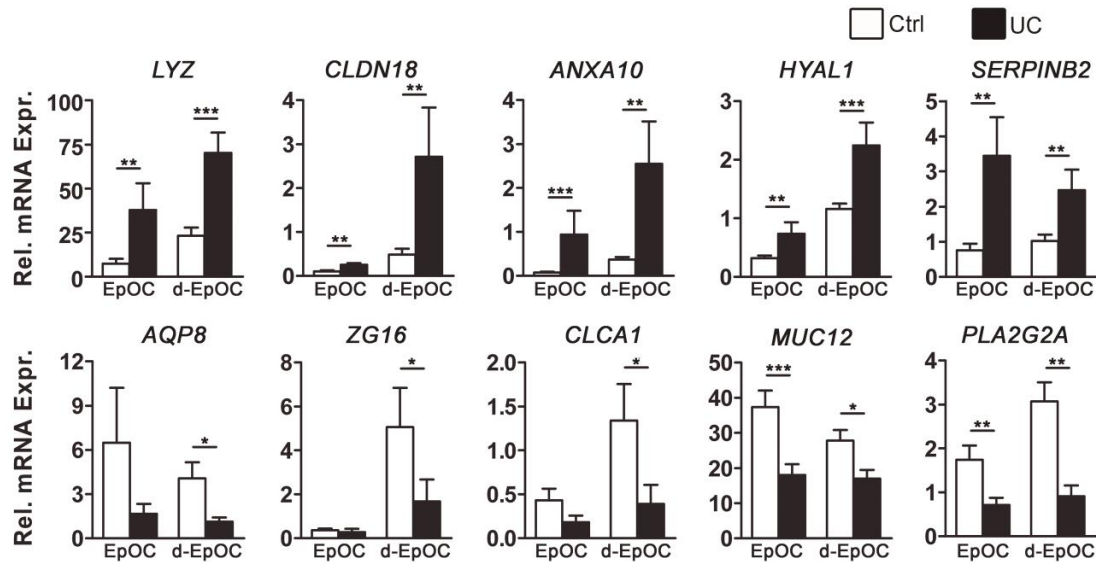
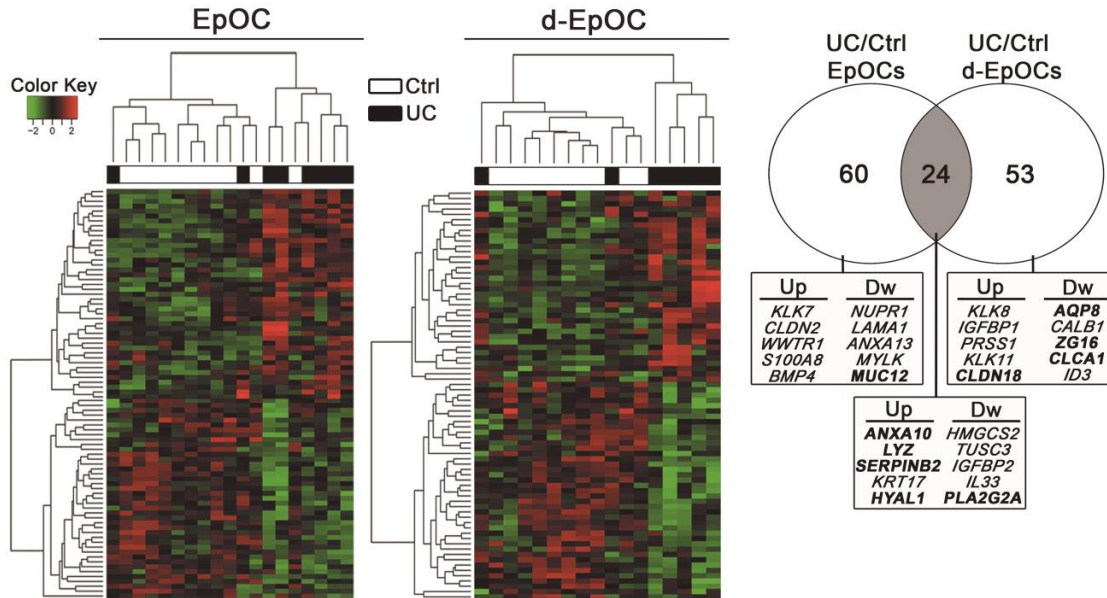
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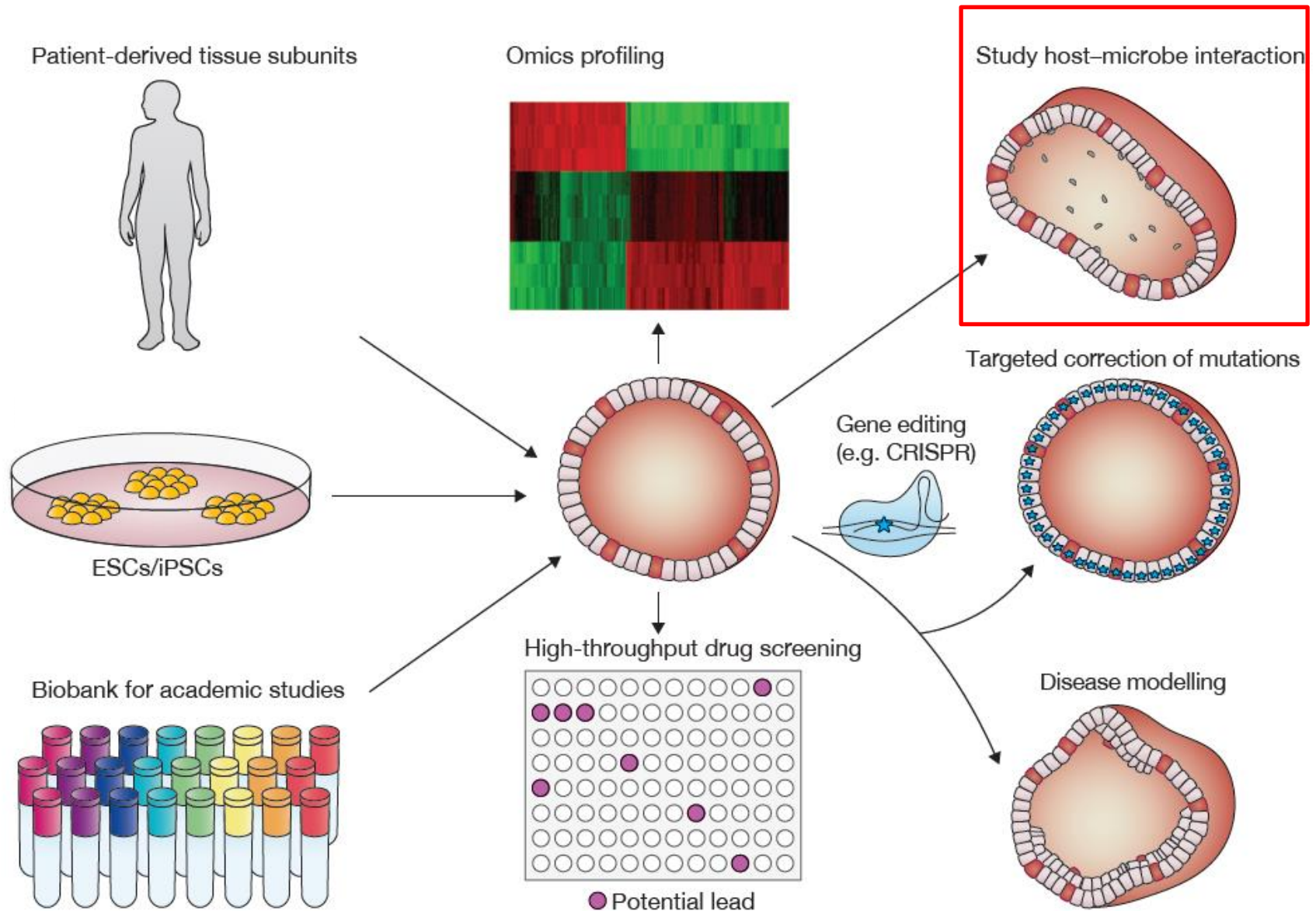
Applications of organoids for studying development, homeostasis and diseases



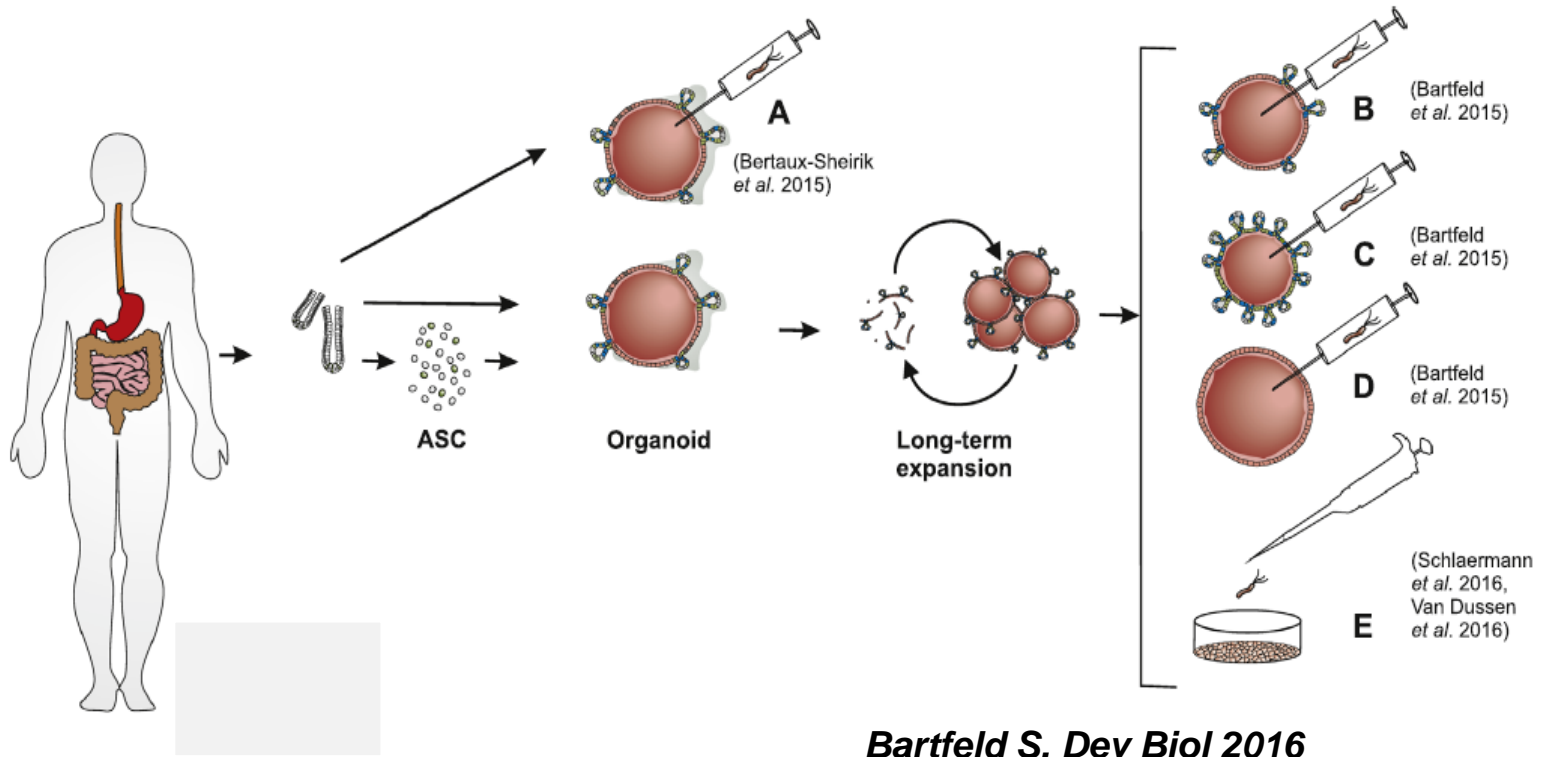
OMICS profiling of EpOCs



Applications of organoids for studying development, homeostasis and diseases



Organoids to model host-microbiome interactions

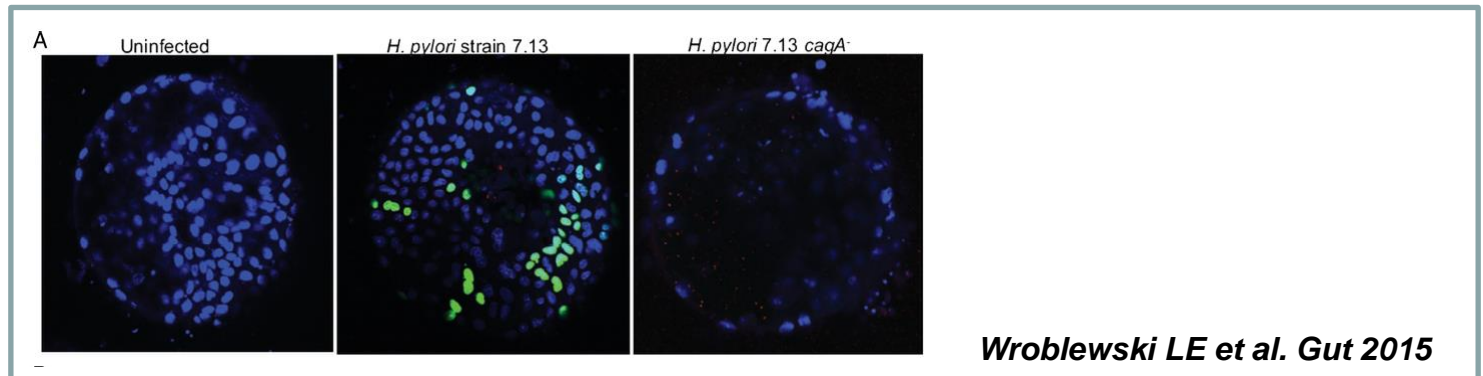
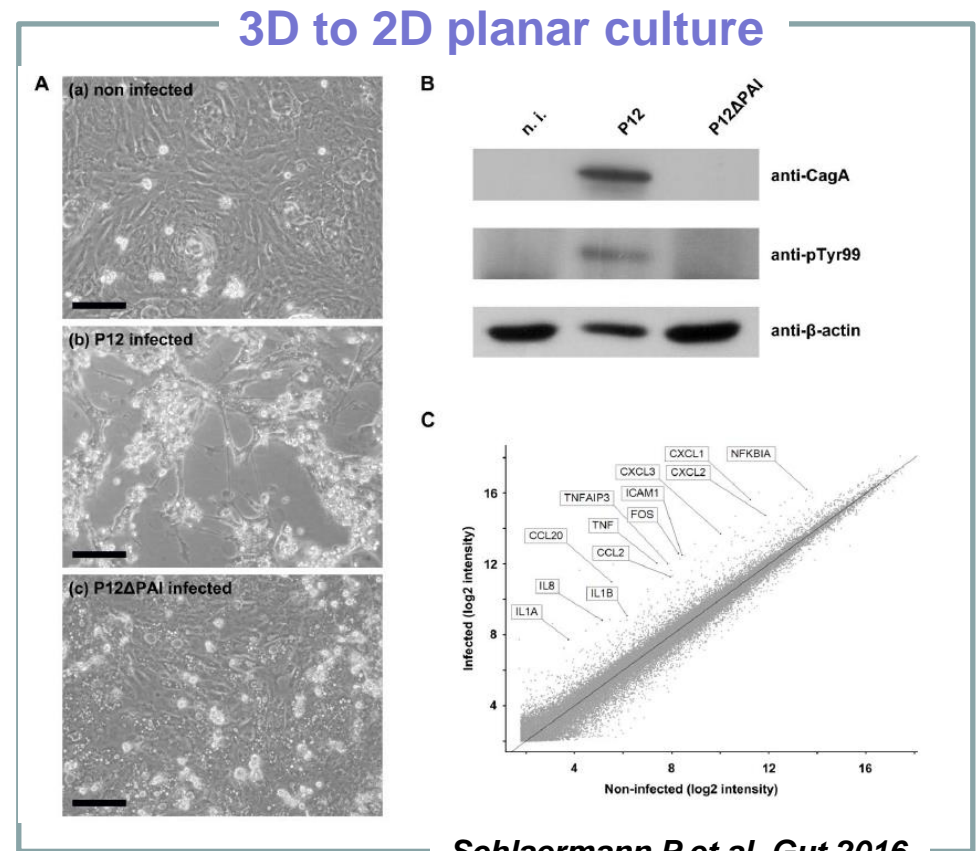
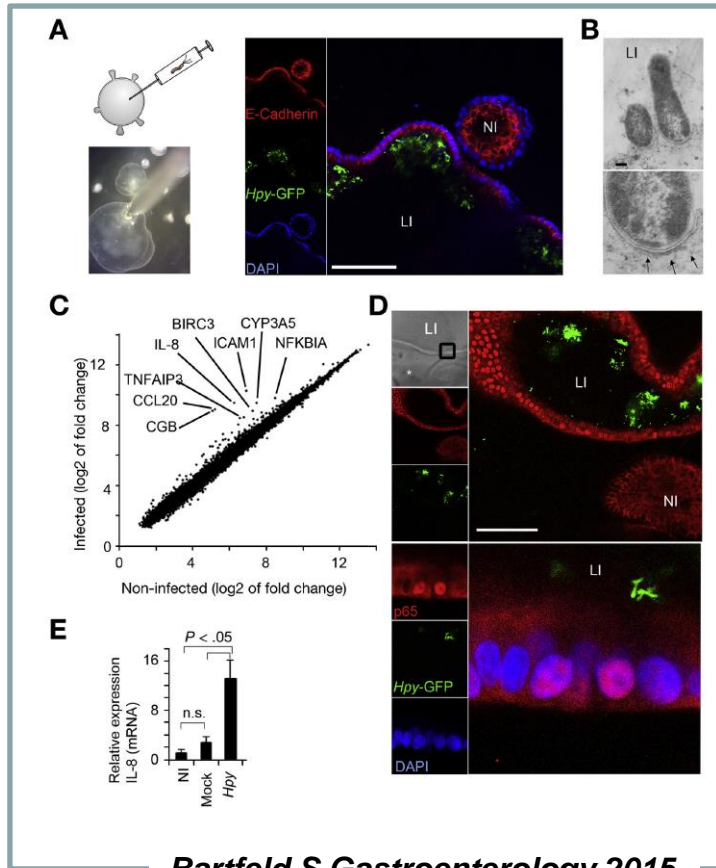


Intestinal Organoids as a Novel Tool to Study Microbes–Epithelium Interactions

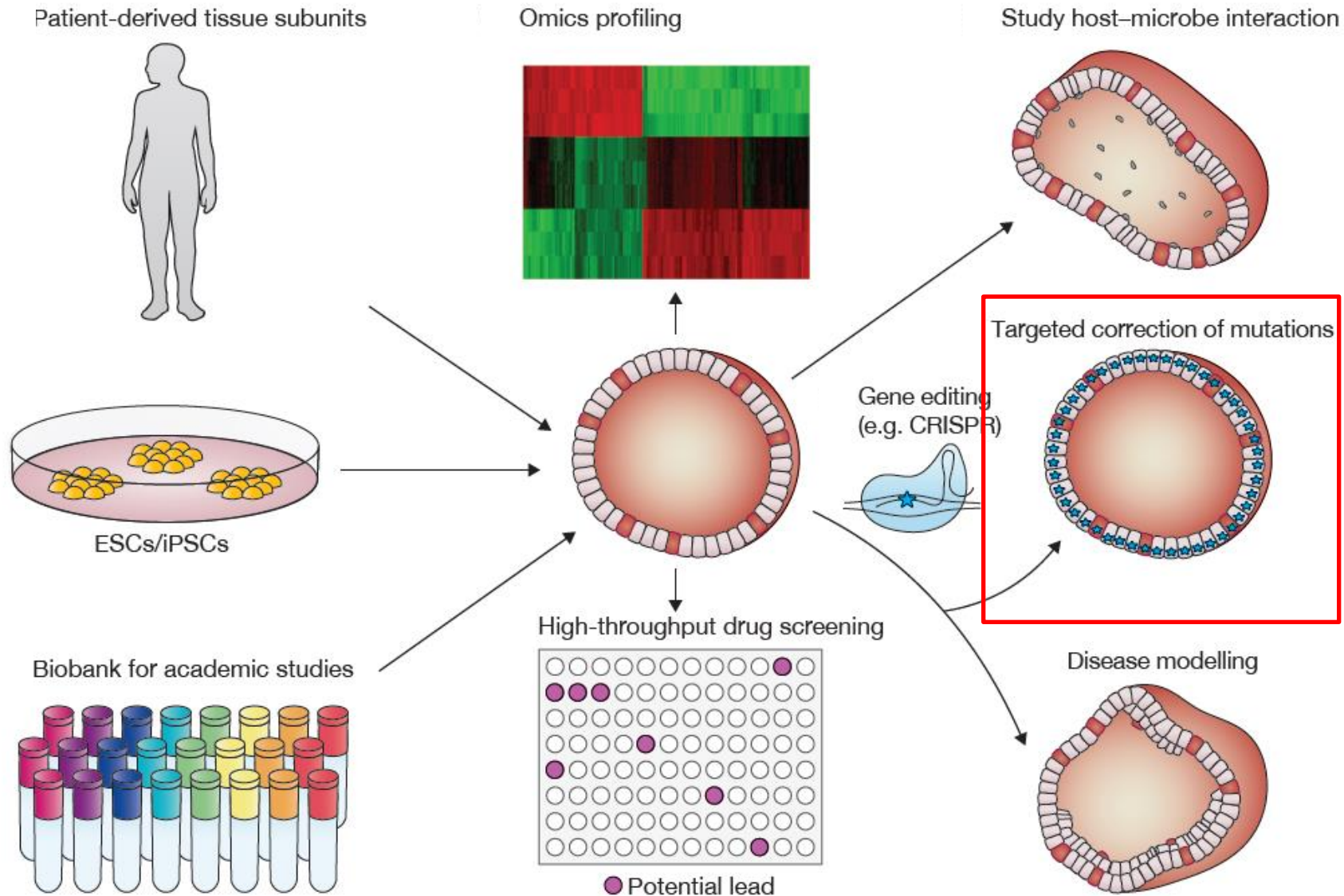
Giulia Nigro, Melissa Hanson, Cindy Fevre, Marc Lecuit, and Philippe J. Sansonetti

Methods Mol Biol 2016

Response of gastric organoids to H pylori infection

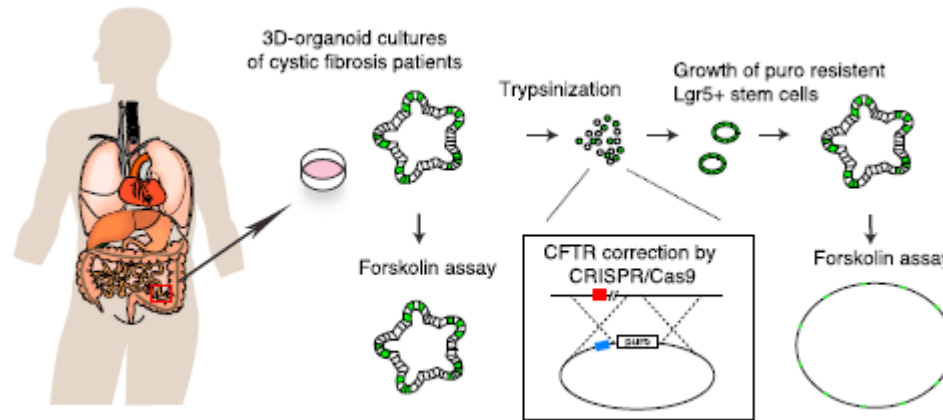


Applications of organoids for studying development, homeostasis and diseases



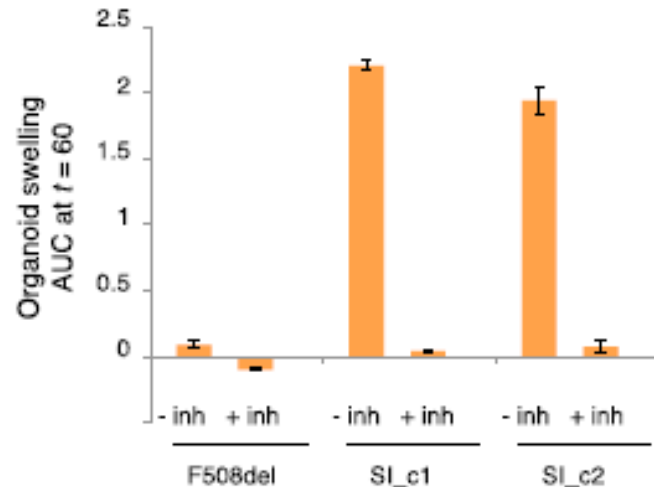
Gene editing in organoids

Cystic fibrosis patients with mutation in the CFTR gene (F508 mutations) a cAMP- and cGMP-regulated epithelial chloride channel.

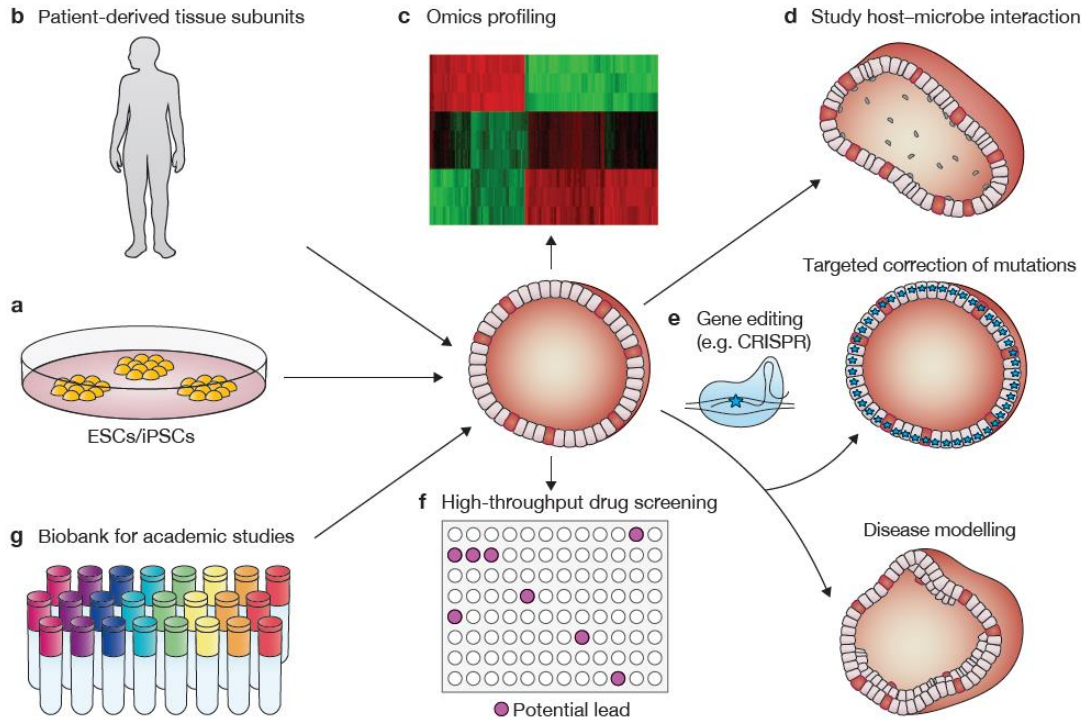


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Forskolin-induced swelling assay



Forskolin activates CFTR by raising the amount of i.c. cAMP, leading to fluid secretion into the lumen and swelling of organoids.



Limitations of the organoid use:

- **High cost**
- **Technical challenges**
- **Growth outside the Matrigel environment**

Acknowledgements

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Anna María Corraliza	

Gastroenterology Unit-Hospital Clinic

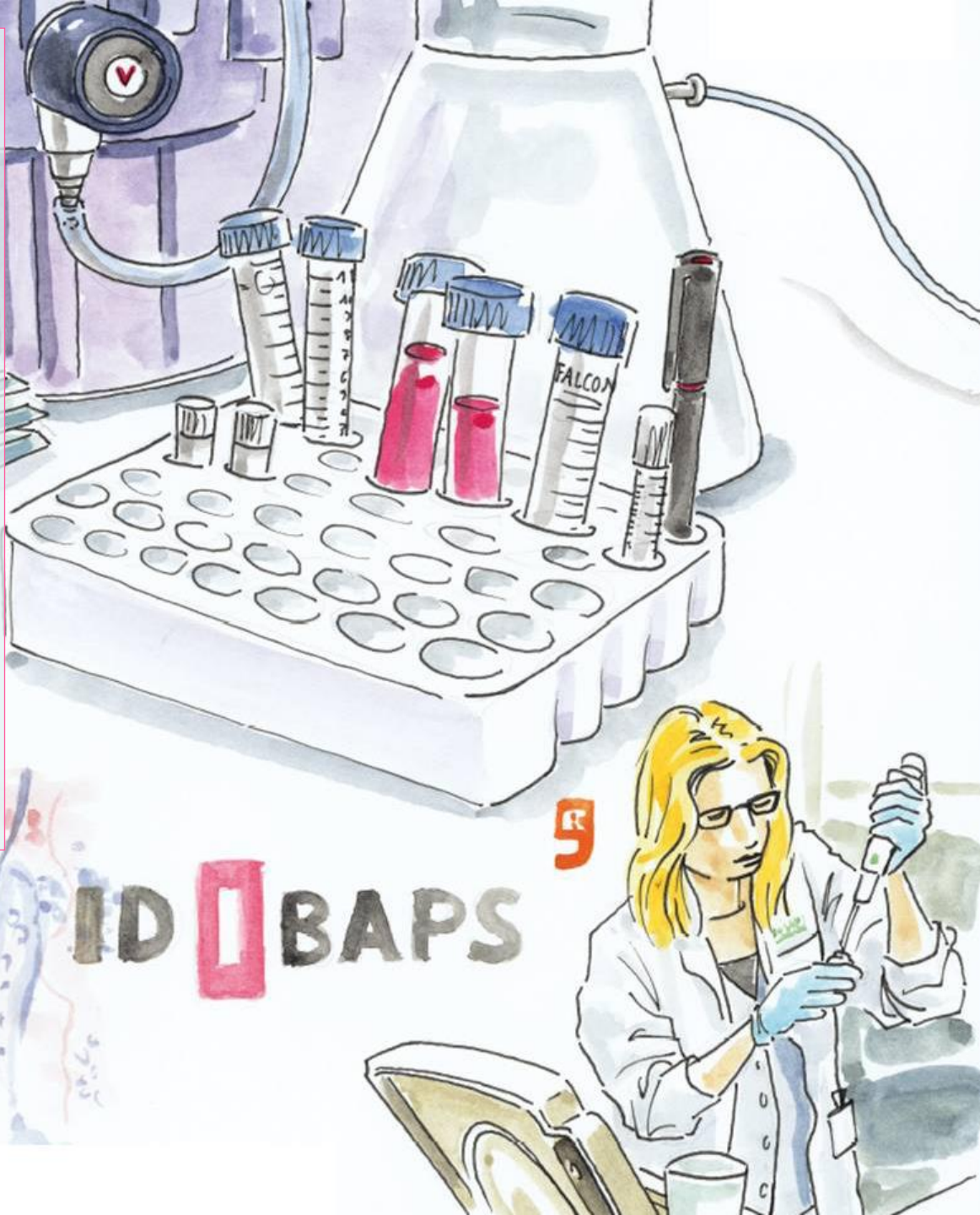
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Josep Llach	Elena Ricart
Maica Masamunt	The patients

CRC Laboratory-IRB

Eduard Batlle	Peter Jung
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Gastroenterology Unit-Mútua de Terrassa

Anna Carrasco	Maria Esteve
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