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Review

Synergizing Organoid and CRISPR Technologies: Pioneering a New Era in Biomedical Research

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Organoids have emerged as a groundbreaking technique in biomedical research. Leveraging clinical samples, they enable the rapid creation of in vitro models that faithfully preserve the pathological, genetic, and epigenetic features of patient samples, thereby illuminating preclinical research. CRISPR, a well-established technique, empowers precise genetic manipulation on a large scale. The combination of these techniques enables researchers to simultaneously manipulate thousands of genes and assess their impact on phenotypes. In this study, we analyze several papers that integrate organoids and CRISPR techniques to identify key factors in development or pathogenesis.

Murine Trophoblast Organoids as a Model for Trophoblast Development and CRISPR-Cas9 Screening.

The placenta plays the role of a bridge connecting the mother and the fetus during pregnancy, providing essential support and protection for the fetus. The developmental defects of the placenta can have a significant impact on the growth and development of the embryo, making high-throughput screening of regulators of mouse trophoblast development necessary. However, previous studies have found limited strategies for the directed differentiation of trophoblast cells under two-dimensional conditions. Here, for the first time, a mouse trophoblast organoid was constructed. Further analysis through chimeric analysis, immunostaining, in situ hybridization, single-cell sequencing, and other technologies demonstrated the similarity between the trophoblast organoid and primary placental trophoblast cells. Multiple transcription factors and cell-cell communication factors that may regulate trophoblast cell differentiation were identified through analysis.

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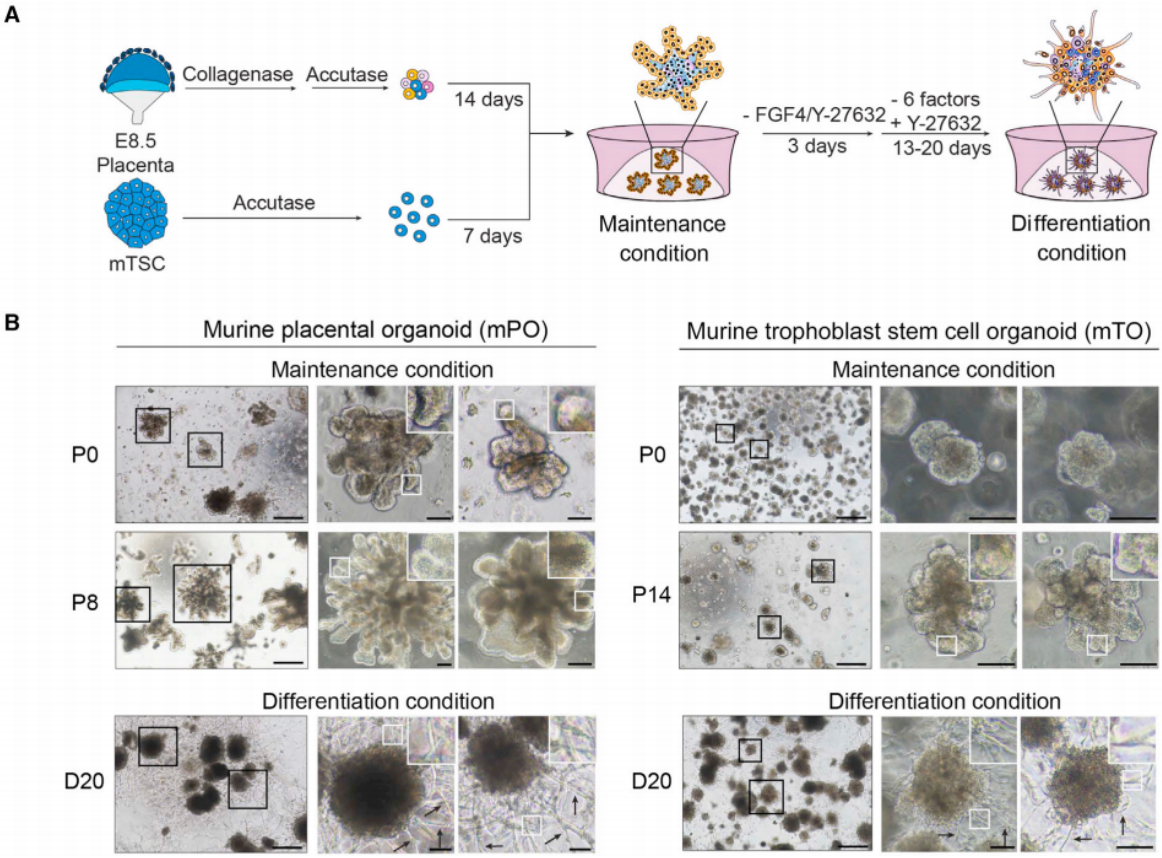

Figure 1. Creation of organoids derived from mouse trophoblasts.

Moreover, by employing mouse trophoblast organoids expressing Cas9 protein, efficient CRISPR/Cas9 screening was conducted using a library of sgRNAs targeting G-protein-coupled receptors (GPCRs). This approach successfully identified the crucial gene Adgrf5 (adhesion GPCRF5/Gpr116) that influences trophoblast differentiation.

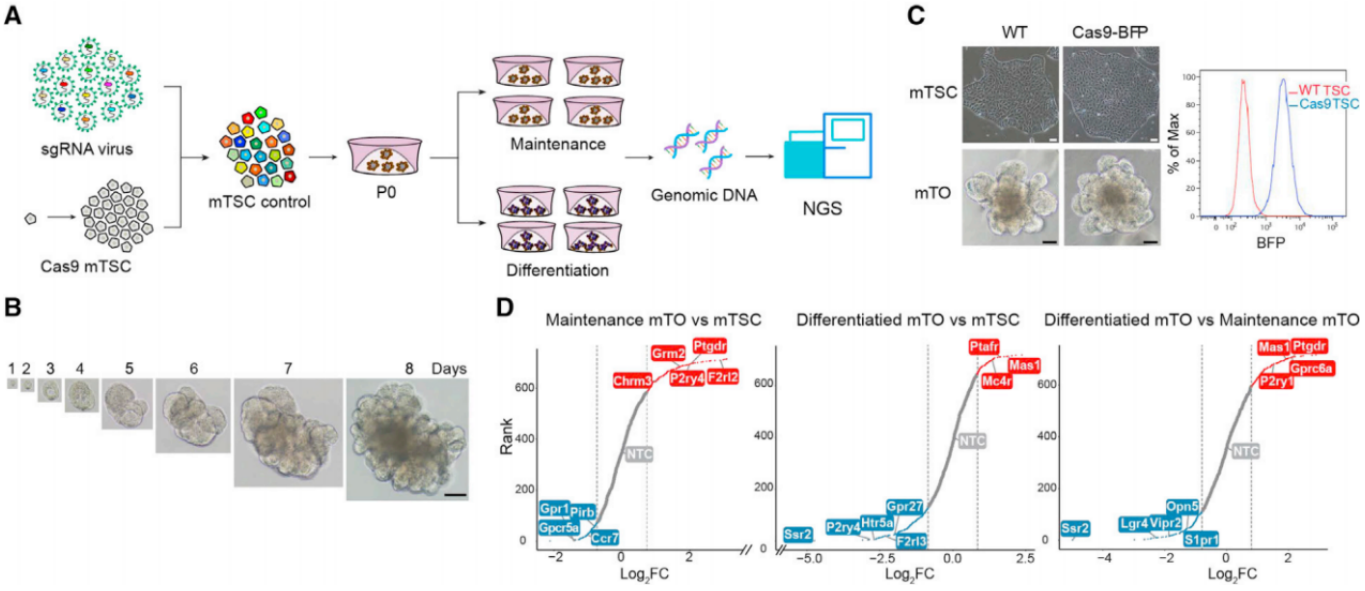

Figure 2. Targeted CRISPR-Cas9 screening within trophoblast organoids.

Mouse trophoblast organoids under maintenance conditions contain a rich population of stem cell-like

groups, while differentiated organoids exhibit various trophoblast cell types similar to the placenta. Trophoblast organoids with gene knockouts mimic in vivo defective phenotypes, indicating that these organoids serve as effective in vitro models for studying trophoblast development. Importantly, this study utilized mouse trophoblast organoids as a platform for efficient CRISPR/Cas9 screening, providing a feasible method for high-throughput screening of trophoblast lineage regulatory factors in vitro.

Engineered human hepatocyte organoids enable CRISPR-based target discovery and drug screening for steatosis.

By establishing a CRISPR drug screening system for human liver organoids, three different triggering factors—free fatty acid load, interindividual genetic variation (PNPLA3 I148M), and monogenic lipid disorders (APOB and MTTP mutations)—were utilized. Human fetal liver cell organoids were employed to simulate the initial stage of non-alcoholic fatty liver disease (NAFLD), namely, hepatic steatosis. Screening of candidate drugs revealed compounds capable of effectively inhibiting hepatic steatosis. Mechanistic evaluation of effective drugs unveiled molecular pathways inhibiting de novo lipogenesis (DNL). A total of 35 genes associated with lipid metabolism and/or NAFLD risk were screened, with FADS2 (fatty acid desaturase 2) identified as a crucial determinant in liver steatosis. Enhanced expression of FADS2 increased the abundance of polyunsaturated fatty acids, thereby reducing de novo lipogenesis of fat.

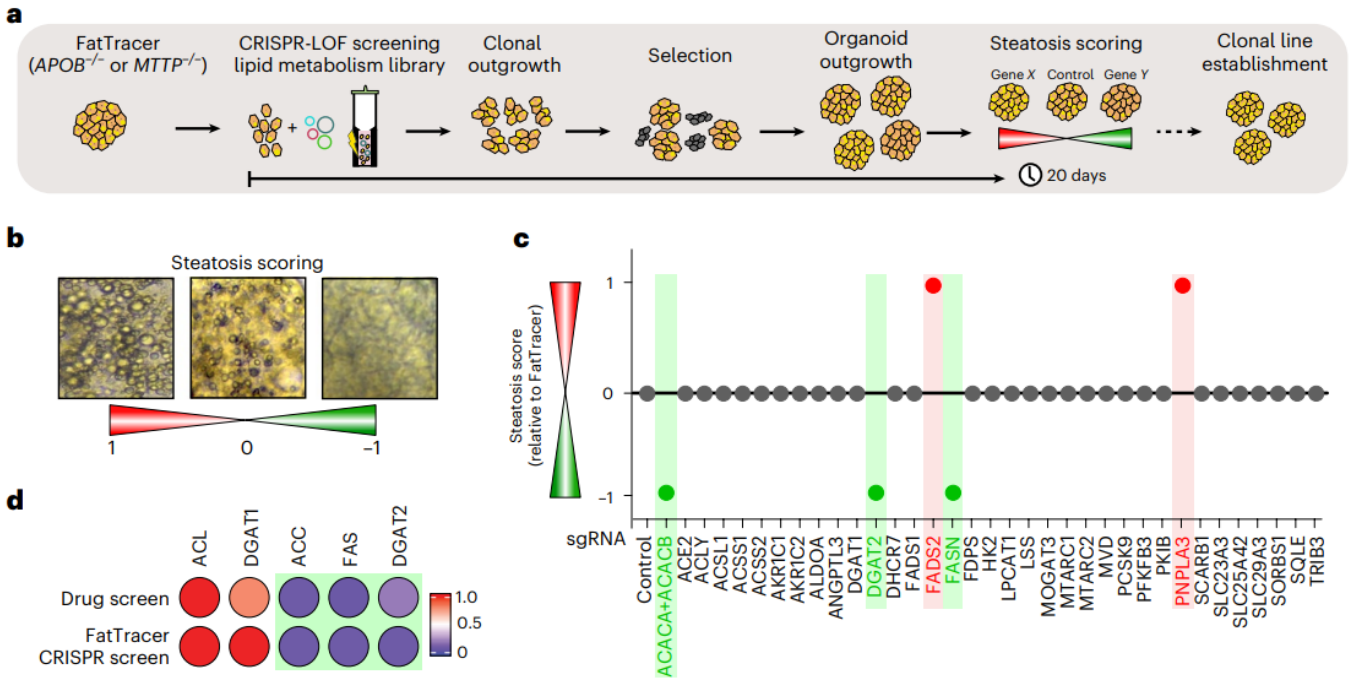

Figure 3. Establishment of liver organoids for CRISPR screening.

By establishing organoids that simulate hepatic steatosis, it is possible to mimic the lipidomic features of non-alcoholic fatty liver disease. Utilizing CRISPR technology enables large-scale, in vitro research on the etiology of hepatic steatosis and the development of drug targets.

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Unbiased transcription factor CRISPR screen identifies ZNF800 as master repressor of enteroendocrine differentiation.

Enteroendocrine cells (EECs) are hormone-secreting cells found in the epithelium of the stomach, small intestine, and colon. The differentiation process from stem cells to various subtypes of enteroendocrine cells (EECs) is a tightly regulated process involving numerous specific transcription factors (TFs). These TFs form a regulatory network to modulate the expression of downstream genes. The transcription factor NEUROG3 is crucial for the differentiation and development of gastrointestinal pancreatic enteroendocrine cells (EECs). By establishing an intestinal organoid model for CRISPR screening, we aim to investigate the upstream regulatory mechanisms of NEUROG3 and endogenous inhibitory factors that regulate EEC differentiation.

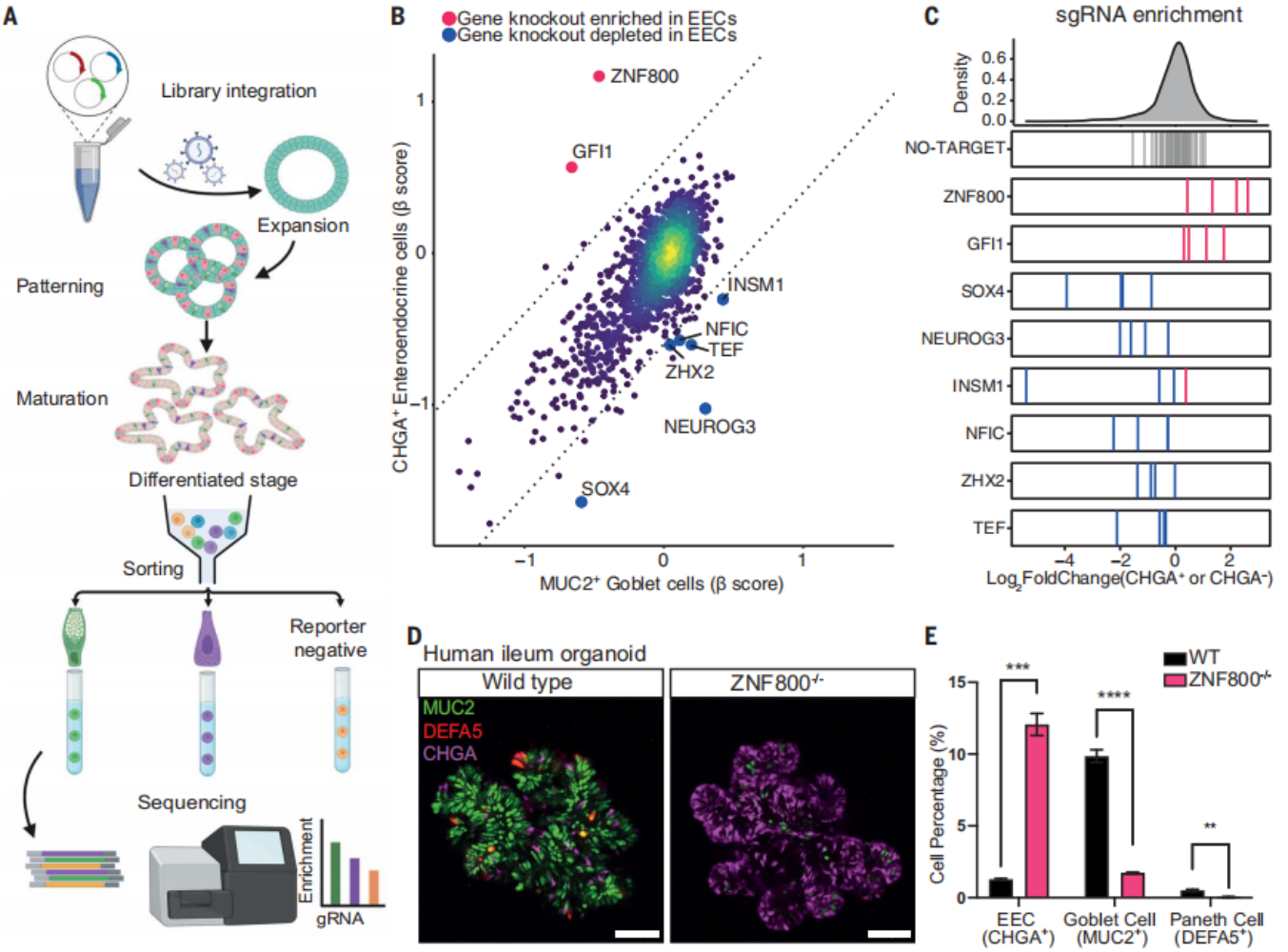

Figure 4. CRISPR screen across the TF landscape for inducing endocrine differentiation in human small intestine organoids.

Exploiting the high resemblance between small intestinal organoids and human organs, a CRISPR screen was conducted to identify regulatory factors in enteroendocrine cell (EEC) lineage differentiation. This revealed ZNF800 as a novel transcription factor that inhibits EEC differentiation, elucidating the significant role of its downstream target genes in the process of EEC differentiation.

In conclusion, organoid models can better replicate the structure and function of human organs, while CRISPR technology enables precise editing of genes within organoid models, facilitating the study of these genes' functions in organ development, maintenance, and disease processes. By knocking in, knocking out, or editing specific genes, researchers can gain in-depth insights into the roles of genes in various biological processes. This provides a powerful tool for investigating the impact of specific gene mutations on organ development and function. Furthermore, organoid models combined with CRISPR can be employed in high-throughput drug screening, expediting the drug development process. Personalized organoid models, based on individual genetic variations and integrated with CRISPR technology, allow for customized studies on the impact of genetic variations on organ structure and function. This provides a robust tool for the development of personalized medicine, aiding in a better understanding of individual differences and the customization of treatment plans.

Reference

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