**Supplementary Material**

**Cell isolation and organoid culture from mouse liver and small intestine**

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**MATERIALS**

**Table S1.** **Reagents of organoids culture and analysis.**

|  |  |  |
| --- | --- | --- |
| Reagents and Materials | | |
| PBS 1× | RuiSenBiotech | - |
| FBS | Gibico | 10099-141C |
| 4% (wt/vol) paraformaldehyde | Absin | abs9179-500mL |
| RNeasy Mini Kit | Qiagen | 74104 |
| DNeasy Blood & Tissue kit | Qiagen | 69506 |
| BSA | Sangon Biotech | A0332 |
| Histowax (58-60 ℃) | Hualingpai | A-11001 |

**Table S2.** **General Instruments of organoids culture and analysis.**

|  |  |  |
| --- | --- | --- |
| General Instruments | | |
| Biological safety cabinet | Baker company | MODEL: SG403A-HE-INT |
| Cell culture incubator | Thermo | STERI-CYCLE i160 |
| Bright-field microscope | Nikon | TS100 |
| High-speed centrifuge | Eppendorf | 5810 R |
| Water bath | Thermo Fisher Scientific | ISOTEMP 215 |
| Temperature-controlled shaker | SZ | ZHSY-50S |
| Low temperature shaker | Thermo | 888-213-1790 |
| Fine scissors | Suzhou Medical Equipment | MR-S231 |
| Two fine forceps | Shanghai Medical instrument | 180801 |
| Glass Pasteur pipette | Thermo | 10006021 |
| Centrifuge tubes | Thermo, Corning | 339652, CLS430791 |
| 1.5 mL Microcentrifuge tubes | Corning | MCT-150C |
| Soft Microcentrifuge tubes | Corning | MCT-200-C |
| 10-mm Petri dish | Corning | 353003 |
| 24-Well adherent culture plate | Corning | 353047 |
| Disposable sterilized syringe | Nantong Tiansheng Experimental Equipment | LTD, 10 mL and 1 mL |
| Sterile swabs | Huabao | Sterile type 80 mm |
| Ice box | Esky | 1257470 |
| 5 mL Flat-bottom glass tubes | Thermo Fisher Scientific | 11563542 |
| Tissue cassette | VWR | 720-0228 |
| Glass slides | Nantong Tiansheng Experimental Equipment | TS-PNSO23 |
| Coverslips | CITOTEST | 10212424C |

**Table S3. Antibodies of organoids analysis.**

|  |  |  |
| --- | --- | --- |
| Antibodies | | |
| Goat Anti-Rabbit IgG H&L (HRP) | Abcam | ab6721 |
| Rabbit Anti-Mouse IgG H&L (HRP) | Abcam | ab6728 |
| Recombinant Anti-Cytokeratin 7 antibody [EPR17078] | Abcam | ab181598 |
| Anti-Ki67 antibody [SP6] | Abcam | ab16667 |
| Anti-Cytokeratin 19 antibody [A53-B] | Abcam | ab194399 |
| Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) | Abcam | ab150083 |
| Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488 | Invitrogen | A-11001 |
| DAPI | BD Pharmingen | 564907 |

**Experimental procedures**

**I. Analysis of organoids: Isolation of DNA**

1. Perform the procedure in main text III 2) ~3) to remove the Matrigel.
2. Discard the supernatant, add ~1 mL precooled PBS 1× and pipette up and down three times. Transfer the mixture to 1.5 mL EP, and centrifuge at 250 g and 4°C for 4 min.
3. Resuspend organoids in PBS 1×, transfer them to a 1.5 mL EP tube, and centrifuge at 300 g and 4°C for 4 min.
4. Discard the supernatant and add 30 µL Direct-PCR solution and 0.5 µL proteinase K per sample.
5. Incubate at 60°C overnight.
6. Centrifuge at ≥ 8000 g at RT for 10 min.
7. DNA can be stored at 4°C.
8. If necessary, purify DNA using a DNA purification kit.

**II. Analysis of organoids: Isolation of RNA**

1. To collect organoids, follow steps I 1) ~3) to collect cell mass.
2. Remove the supernatant, add 350 µL RLT to each 1.5 mL microcentrifuge tube, and vortex-mix for 30 s.
3. To promote cell mass lysis, draw the mixtures at least 5 times through a sterile and without RNase syringe of No. 20 needle.
4. The mixtures can be stored at –80°C for 1 month.
5. RNA can be extracted following the RNeasy Mini Kit (Qiagen, Hilden, Germany) protocol guides.