# Simulated microgravity promotes the formation of tridimensional cultures and stimulates pluripotency and a glycolytic metabolism in human hepatic and biliary tree stem/progenitor cells.

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## SUPPLEMENTARY TABLES

Supplementary Table 1. Table of human qPCR primer pairs for gene expression.

| Gene    | Gene Bank   | Forward Primer             | Reverse Primer             |
|---------|-------------|----------------------------|----------------------------|
| OCT4    | NM_002701   | TCGAGAACCGAGTGAGAGG        | GAACCACACTCGGACCACA        |
| SOX17   | NM_022454   | AAGATGCTGGGCAAGTCGTGG      | CTTGTAGTTGGGGTGGTCCTG      |
| PDX1    | NM_000209   | CATTGGAAGGCTCCCTAACA       | TTCCACTGGCATCAATTTCA       |
| SOX2    | NM_003106   | GGGAAATCCCAGGGGTGCAAAAGAGG | TTGCGTGAGTGTGGATGGGATTGGTG |
| ALB     | NM_000477.5 | AGAGGTCTCAAGAAACCTAGGAAA   | GGTTCAGGACCACGGATAGA       |
| CYP3A4  | NM_017460   | AAGTCGCCTCGAAGATACACA      | AAGGAGAGAACACTGCTCGTG      |
| β-Actin | NM_007393.5 | GGATGCAGAAGGAGATTACTGC     | CCACCGATCCACACAGAGTA       |

The ratio of concentration of gene of interest and the reference gene human  $\beta$ -Actin was assumed to be the gene of interest relative expression.

## Supplementary Table 2. List of used antibodies and their application(s)

| Name                 | Host / isotype | Source         | Catalog# | Dilution | Application |
|----------------------|----------------|----------------|----------|----------|-------------|
| Albumin              | Rabbit IgG     | Abcam          | Ab2406   | 1:200    | IF          |
| K19 (cytokeratin 19) | Mouse IgG1     | DAKO           | M0888    | 1:100    | IF          |
| MRP2                 | Mouse IgG2a    | Abcam          | ab3373   | 1:200    | IF          |
| OCT4A                | Rabbit IgG     | Cell Signaling | #2050    | 1:50     | IF          |
| PDX1                 | Rabbit IgG     | Santa Cruz     | SC-25403 | 1:50     | IF          |
| SOX2                 | Rabbit IgG     | Abcam          | AB97959  | 1:200    | IF          |
| SOX17                | Goat IgG       | R&D            | AF1924   | 1:50     | IF          |

## SUPPLEMENTARY VIDEO LEGEND

**Supplementary video 1.** This video showed the formation of a real organoid having a considerable size. The organoids were continuously in free fall and were affected by microgravity conditions.

**Supplementary video 2.** In the video it possible to observe smaller structures which are clearly suspended and for which the rotation speed had been correctly modulated to keep them in suspension.

#### SUPPLEMENTARY FIGURES

# **Supplementary Figure 1**



Supplementary Figure 1. Glucose levels into the grow medium in normogravity and microgravity conditions. Glucose levels of hBTSCs in microgravity analysed did not show significative difference (N=3; p > 0.05) when compared to glucose levels of hBTSCs cultured in normogravity.

#### **Supplementary Figure 2**



Supplementary Figure 2. Albumin gene expression into the in normogravity and simulated microgravity conditions. Graphs illustrating the gene expression of albumin (N=3, p <0.05) in HepG2 cells have been reported in different conditions, in normogravity (NG), simulated microgravity (SMG), and in cells maintained in NG (2D or 3D growth) for 15 days after being cultured in SMG (SGM $\rightarrow$ 2D NG and SGM $\rightarrow$ 3D NG).