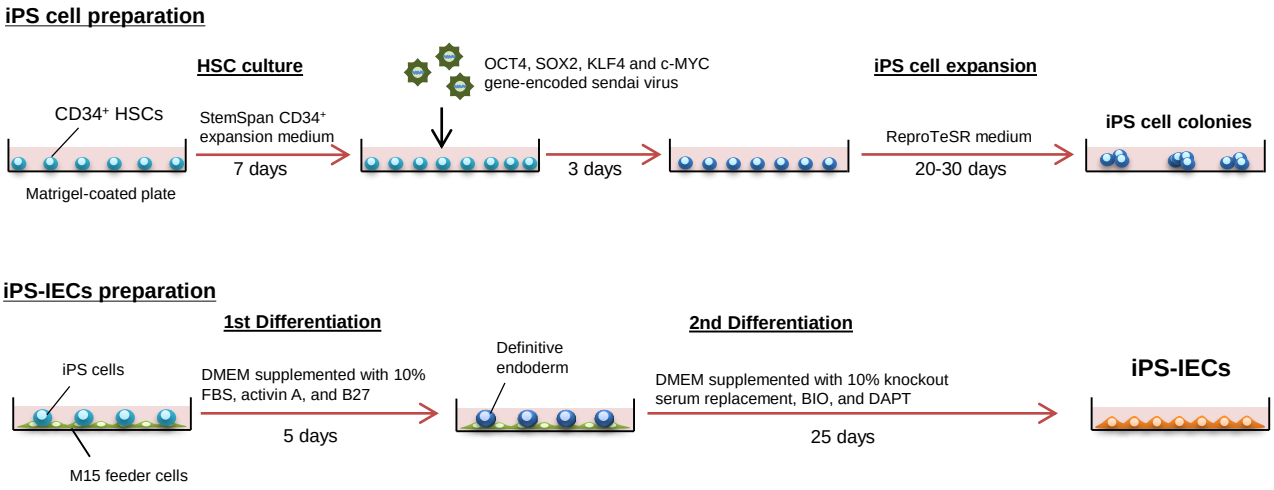
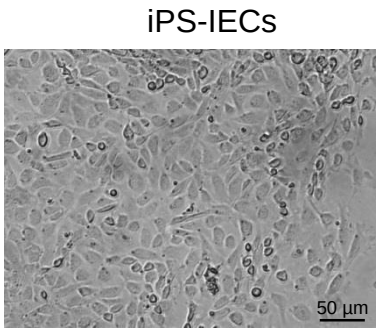
Supplement Chart 1

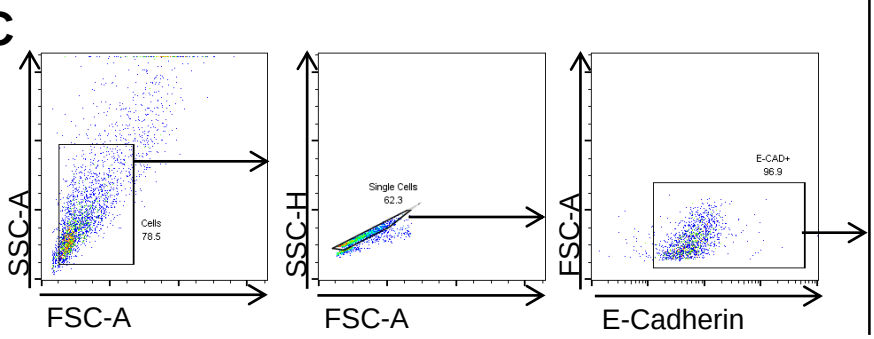
A

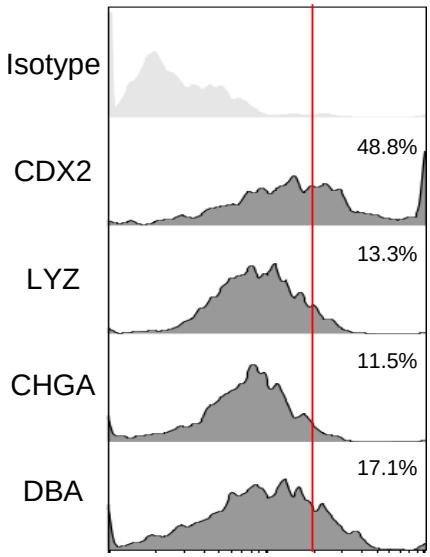


B



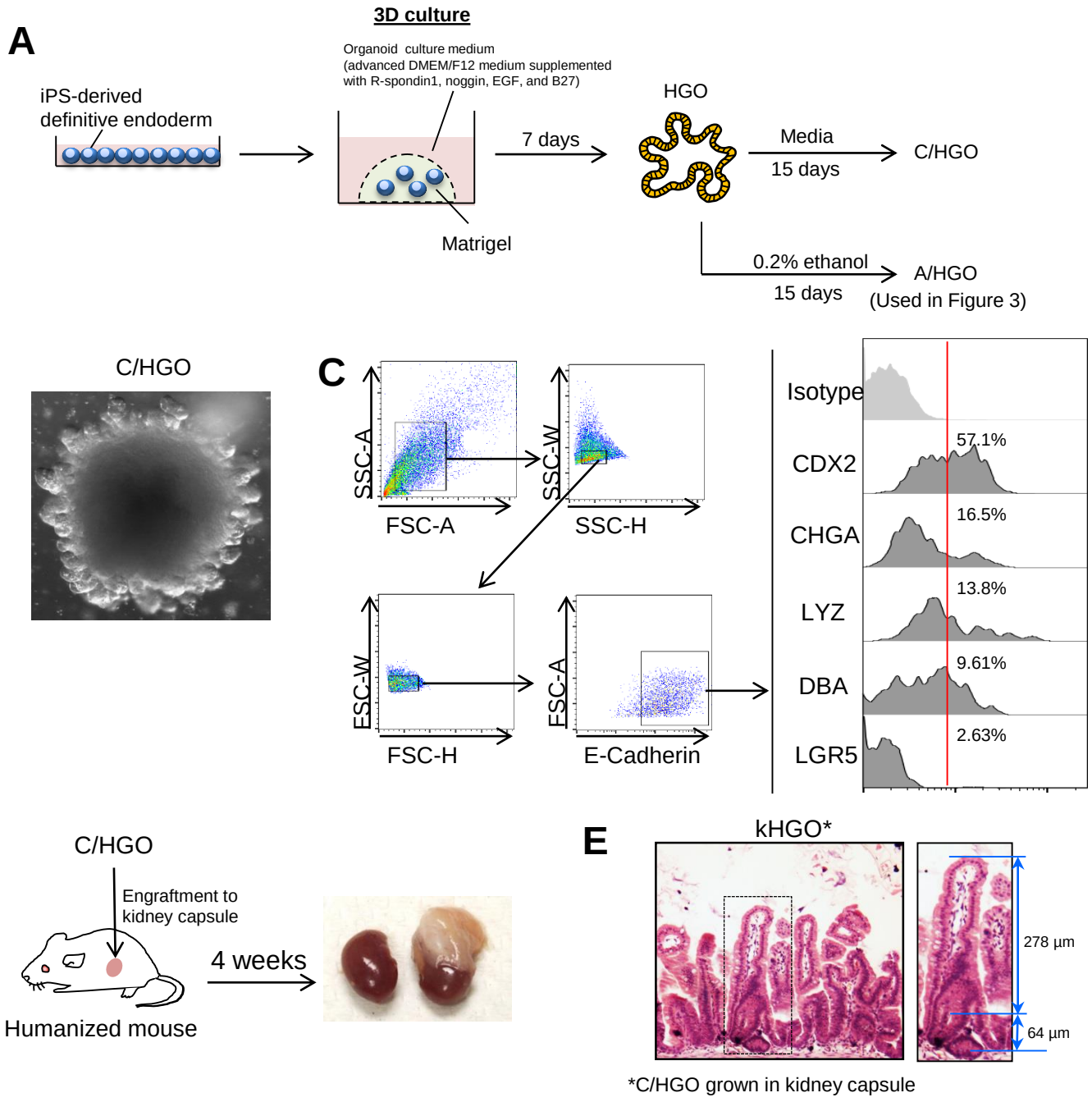
C





iPS-IECs are a mixture of intestinal epithelial cells.
A: Procedures for the preparation of iPS-IECs. **B:** iPS-IECs differentiated from iPS cell-derived definitive endoderm on the 25th day cultivation was observed under a microscope (200x magnification). **C:** iPS-IECs were stained with antibodies specific for E-cadherin, CDX2, LYZ, CHGA, and DBA and analyzed by flow cytometry. The percentages of CDX2^{+}, LYZ^{+}, CHGA^{+}, and DBA^{+} cells in E-cadherin^{+} intestinal epithelial cells are shown.

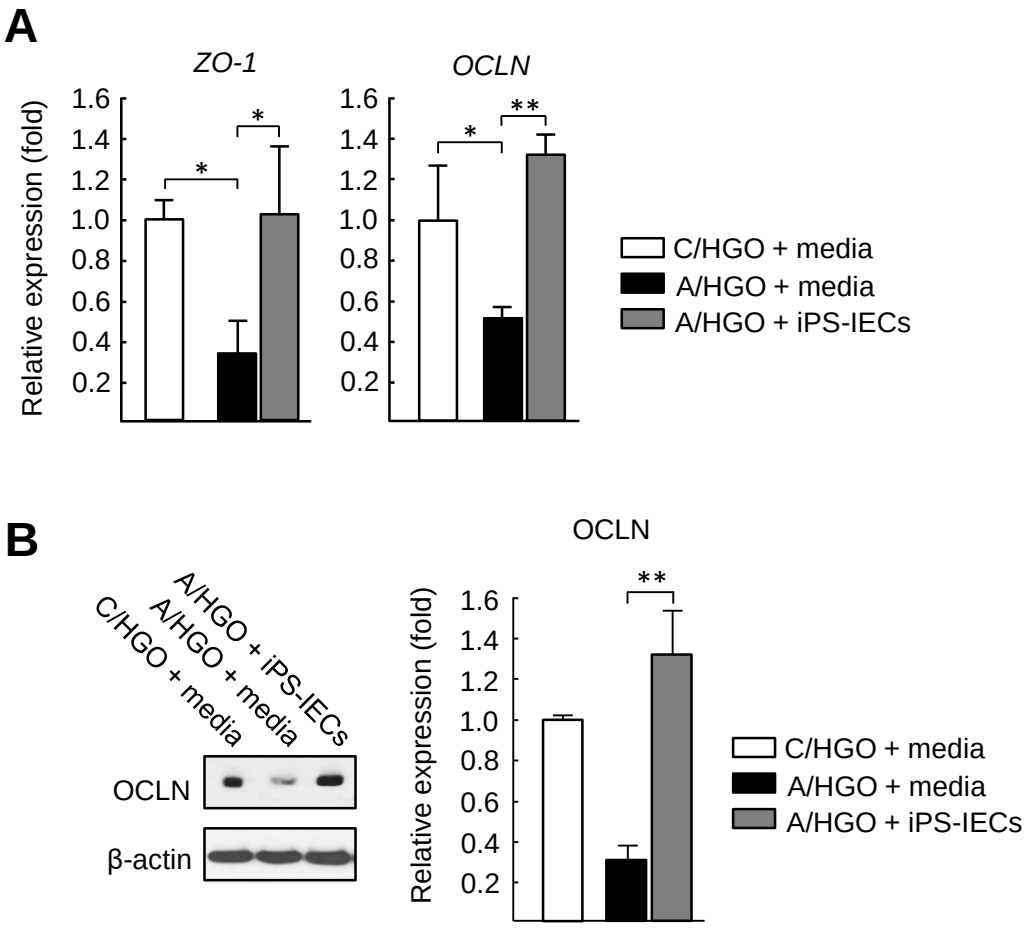
Supplement Chart 2



*C/HGO grown in kidney capsule

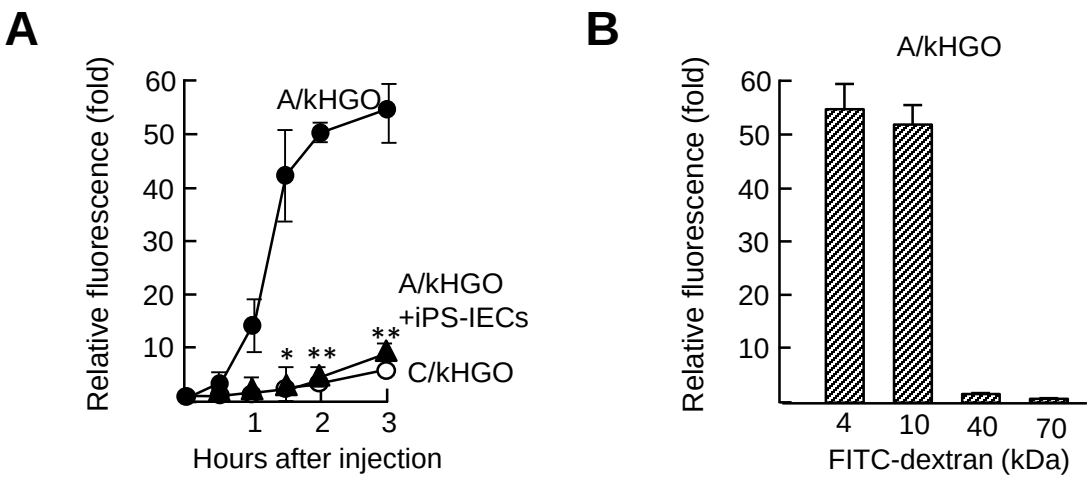
Human gut organoids. A: iPS-IECs-derived definitive endoderm was 3D cultured for 7 days (HGOs). The growth of organoids was tested in HGO cultures added with or without alcohol. In the majority of experiments, the HGOs were additionally cultured with (A/HGOs) or without (C/HGOs) 0.2% ethanol for 15 days. **B:** The microscopic morphology of the C/HGO. Bar: 200 µm. **C:** The cellular compositions of C/HGOs. C/HGOs were analyzed flow cytometrically for the percentages of $CDX2^+$ (enterocytes), $CHGA^+$ (enteroendocrine cells), LYZ^+ (Paneth cells), DBA^+ (goblet cells), and $LGR5^+$ (stem cells) in E-cadherin$^+$ intestinal epithelial cells. **D:** C/HGO was enlarged by the grafting to the kidney capsule of a humanized mouse (kHGO). kHGOs cultured with or without 0.2% alcohol were used for the microinjection of *E. faecalis*. **E:** The section of the kHGO, removed from the mouse kidney 4 weeks after the grafting, was stained with H&E (light microscopic images, 200x magnification). Data are representative at least two independent experiments.

Supplement Chart 3



Tight junction protein expression in A/HGOs co-cultured with or without iPS-IECs. After washing with media, C/HGOs and A/HGOs were co-cultured with or without iPS-IECs in alcohol-free fresh media for 15 days. These organoids were analyzed for *ZO-1* and *OCLN* mRNA expression by real-time PCR (**A)** and OCLN protein level by Western blotting (**B**). Protein level of OCLN was quantified by densitometric analysis. **P* < 0.05, ***P* < 0.01.

Supplement Chart 4



iPS-IECs reduce leakage of FITC-dextran from A/kHGO.
A/kHGO and C/kHGO were co-cultured with iPS-IECs (1 x 10^5 cells) for 12 days. **A:** Each organoid was microinjected with 4 kDa FITC-dextran (0.5 μM, 10 μL). Thirty minutes to 3 hours after the cultivation, the FITC-dextran in culture fluids of the organoids was measured by a fluorometer. **B:** A/kHGO was microinjected with 4, 10, 40, or 70 kDa FITC-dextran (0.5 μM, 10 μL). Three h after cultivation, the FITC-dextran in the culture fluids was measured. *$P < 0.05$, **$P < 0.01$.