# Supplementary information

**Manuscript title:**
Intestinal organoids for assessing nutrient transport, sensing and incretin secretion

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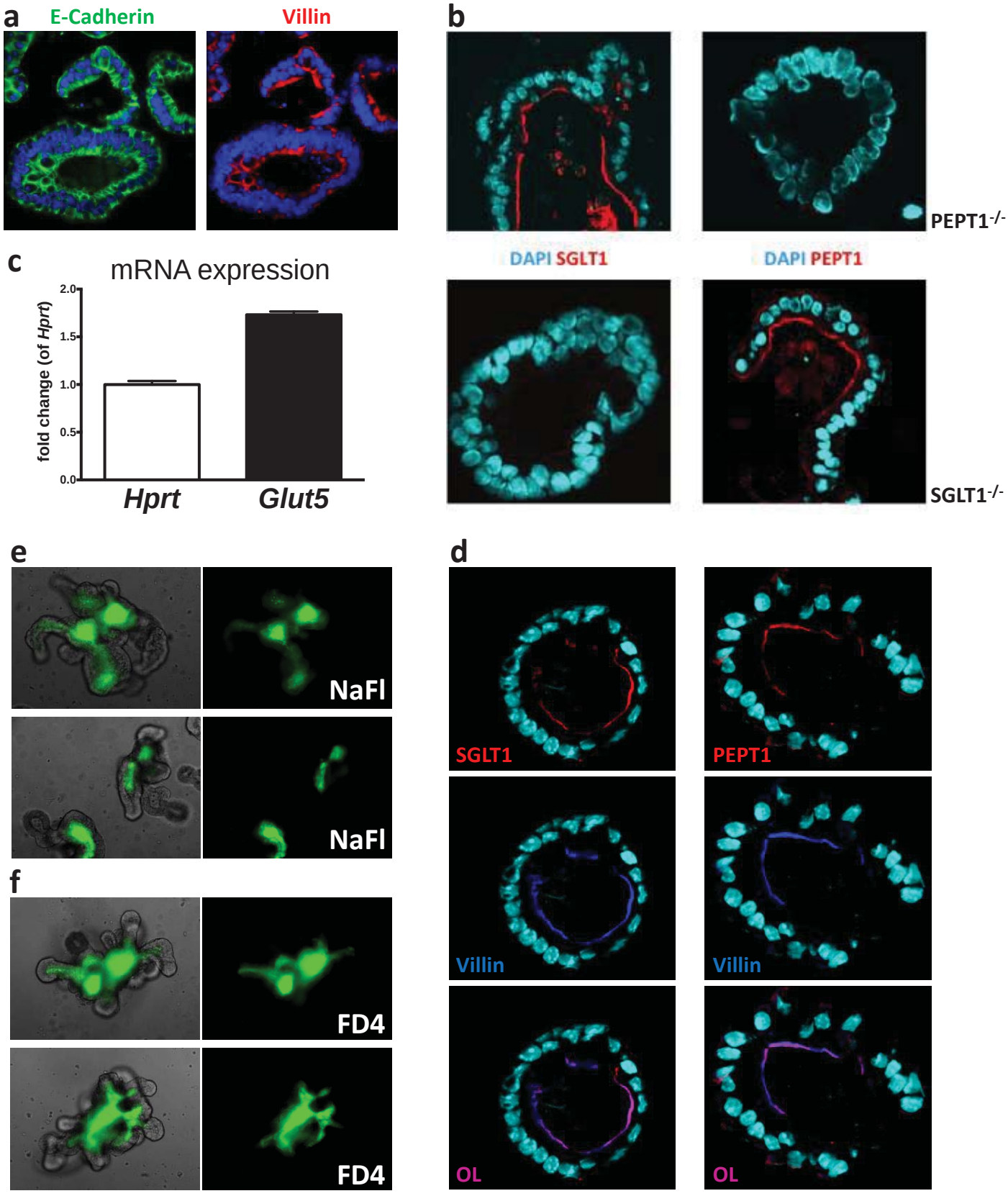
# Supplementary Table S1

**Supplementary Table S1: Statistical analyses of transport and hormone secretion studies.** Overview of statistical analyses and calculated p-values for the data depicted in **Fig. 1c-e**, **Fig. 2b-d** and **Fig. 2f,g**.

| Figure | Comparison | Test | P-Value |
|---|---|---|---|
| **1c left** | WT Glucose vs. WT Glucose + Phloridzin | Two Way ANOVA followed by Tukey test | <0.001 |
| | WT Glucose vs. SGLT1$^{-/-}$ Glucose | | <0.001 |
| | SGLT1$^{-/-}$ Glucose vs. SGLT1$^{-/-}$ Glucose + Phloretin | | 0.040 |
| **1c mid** | WT α-MDG vs. WT α-MDG + Phloridzin | Two Way ANOVA followed by Tukey test | <0.001 |
| | WT α-MDG vs. SGLT1$^{-/-}$ α-MDG | | <0.001 |
| **1c right** | WT Fructose vs. GLUT5$^{-/-}$ Fructose | Two Way ANOVA followed by Tukey test | <0.001 |
| | GLUT5$^{-/-}$ Fructose vs. GLUT5$^{-/-}$ Fructose + Phloretin | | 0.018 |
| **1d** | WT Gly-Sar vs. PEPT1$^{-/-}$ Gly-Sar | Student´s t test (unpaired) | 0.008 |
| **1e** | WT Gly-Sar vs. WT Gly-Sar + Gly-Gly | One Way ANOVA followed by Holm-Sidak method | 0.009 |
| | WT Gly-Sar vs. WT Gly-Sar + Cefadroxil | | <0.001 |
| **2b** | Duodenum vs. Jejunum | One Way ANOVA followed by Holm-Sidak method | 0.034 |
| | Duodenum vs. Ileum | | <0.001 |
| | Jejunum vs. Ileum | | <0.001 |
| **2c** | Basal vs. Glucose | One Way ANOVA followed by Holm-Sidak method | <0.001 |
| | Basal vs. Gly-Sar | | 0.013 |
| | Basal vs. DCA | | <0.001 |
| **2d left** | WT Basal vs. Gly-Sar | One Way ANOVA followed by Holm-Sidak method | 0.006 |
| | WT Basal vs. F/I | | <0.001 |
| | PEPT1$^{-/-}$ Basal vs. Gly-Sar | One Way ANOVA followed by Holm-Sidak method | **0.876** |
| | PEPT1$^{-/-}$ Basal vs. F/I | | <0.001 |
| | WT F/I vs. PEPT1$^{-/-}$ F/I | Two Way ANOVA followed by Tukey test | **0.961** |
| **2d right** | WT Basal vs. Glucose | One Way ANOVA followed by Holm-Sidak method | 0.003 |
| | WT Basal vs. F/I | | <0.001 |
| | SGLT1$^{-/-}$ Basal vs. Glucose | One Way ANOVA followed by Holm-Sidak method | **0.964** |
| | SGLT1$^{-/-}$ Basal vs. F/I | | <0.001 |
| | WT F/I vs. SGLT1$^{-/-}$ F/I | Two Way ANOVA followed by Tukey test | **0.743** |
| **2f** | Duodenum vs. Jejunum | One Way ANOVA followed by Holm-Sidak method | <0.001 |
| | Duodenum vs. Ileum | | <0.001 |
| | Jejunum vs. Ileum | | <0.001 |
| **2g** | WT Basal vs. Glucose | One Way ANOVA followed by Holm-Sidak method | 0.043 |
| | WT Basal vs. F/I | | <0.001 |
| | SGLT1$^{-/-}$ Basal vs. Glucose | One Way ANOVA followed by Holm-Sidak method | **0.746** |
| | SGLT1$^{-/-}$ Basal vs. F/I | | <0.001 |
| | WT F/I vs. SGLT1$^{-/-}$ F/I | Two Way ANOVA followed by Tukey test | **0.438** |

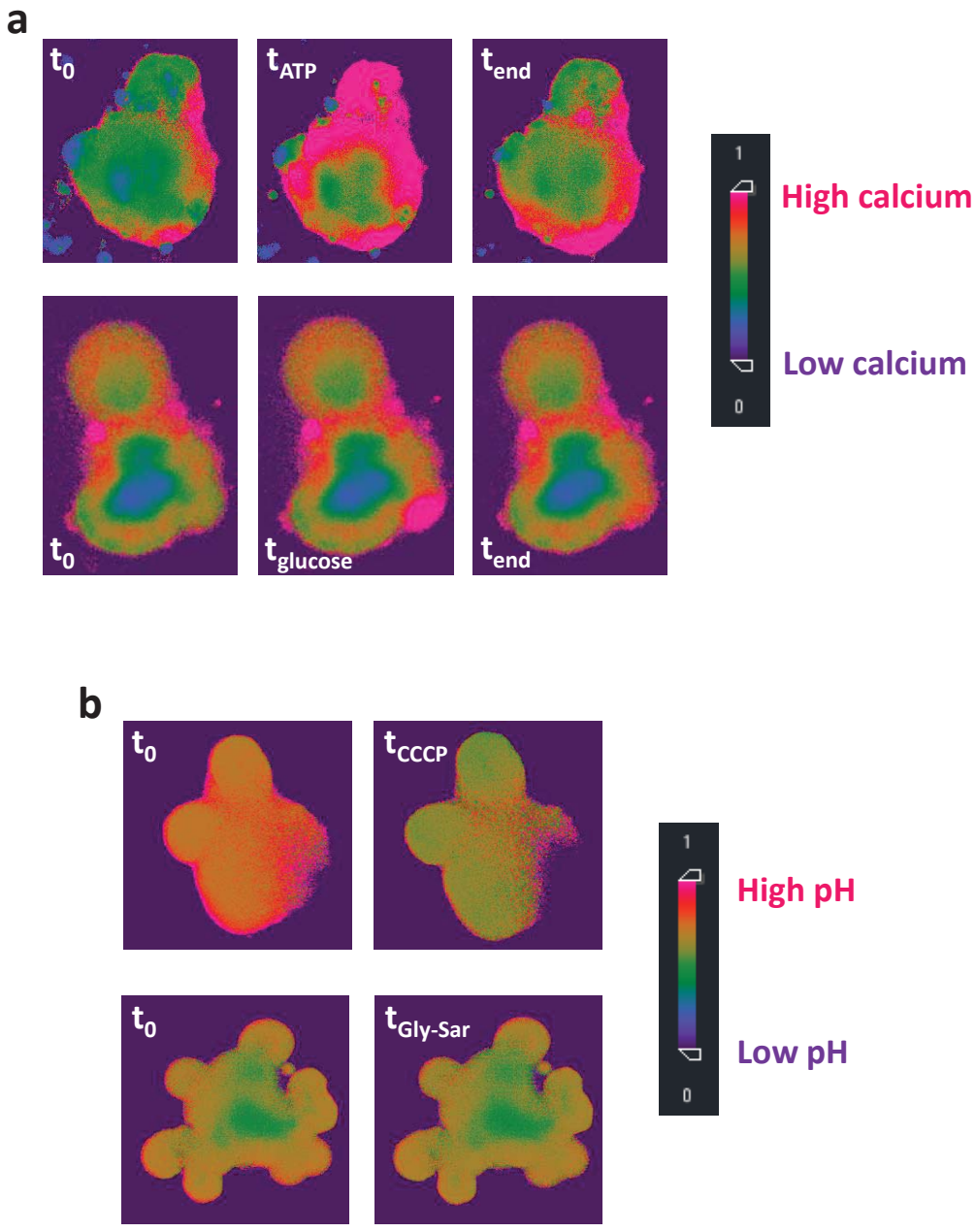
# Supplementary Figure S1




**Supplementary Figure S1: Cell polarization, specificity of PEPT1 and SGLT1 antibodies, *Glut5* expression, and permeability of the organoid epithelium**.

**(a)** Immunofluorescent staining of villin (red) and E-Cadherin (green) shows polarized intestinal epithelial cells; nuclei (blue). **(b)** Organoids derived from PEPT1$^{-/-}$ (upper panel) and SGLT1$^{-/-}$ (lower panel) mice demonstrate no staining to the corresponding PEPT1 or SGLT1 antibodies, respectively. As a control, PEPT1$^{-/-}$ organoids show specific SGLT1 staining and vice versa. **(c)** *Glut5* mRNA expression in SI organoids derived from WT mice. mRNA levels of the housekeeping gene *Hprt* serve as control. **(d)** Apical colocalization of villin and transporters. **(e, f)** Permeability of sodium fluorescein (NaFl) and FITC-Dextran 4 kDa (FD4), both accumulating in the organoid lumen.

# Supplementary Figure S2




**Supplementary Figure S2: Live-cell imaging of intracellular calcium and acidification in WT organoids. (a)** Calcium responses to 100 µM ATP (upper panel) and 50 mM glucose (lower panel) in WT organoids. Ratio of fluorescence intensities F ($\lambda_{ex}$340 nm)/F ($\lambda_{ex}$380 nm) display changes in intracellular calcium levels according to the color legend. **(b)** Intracellular acidification of organoid epithelial cells upon treatment with the protonophore CCCP (upper panel) and 50 mM Gly-Sar (lower panel), ratio images. Ratio of fluorescence intensities F ($\lambda_{ex}$490 nm)/F ($\lambda_{ex}$450 nm) display changes in intracellular calcium levels according to the color legend.