Supplemental Data **#DMD-AR-2020-000281**

Drug Metabolism and Disposition

Evaluation of tissue stem cell derived human intestinal organoids, a physiologically relevant model to evaluate cytochrome P450 induction in gut

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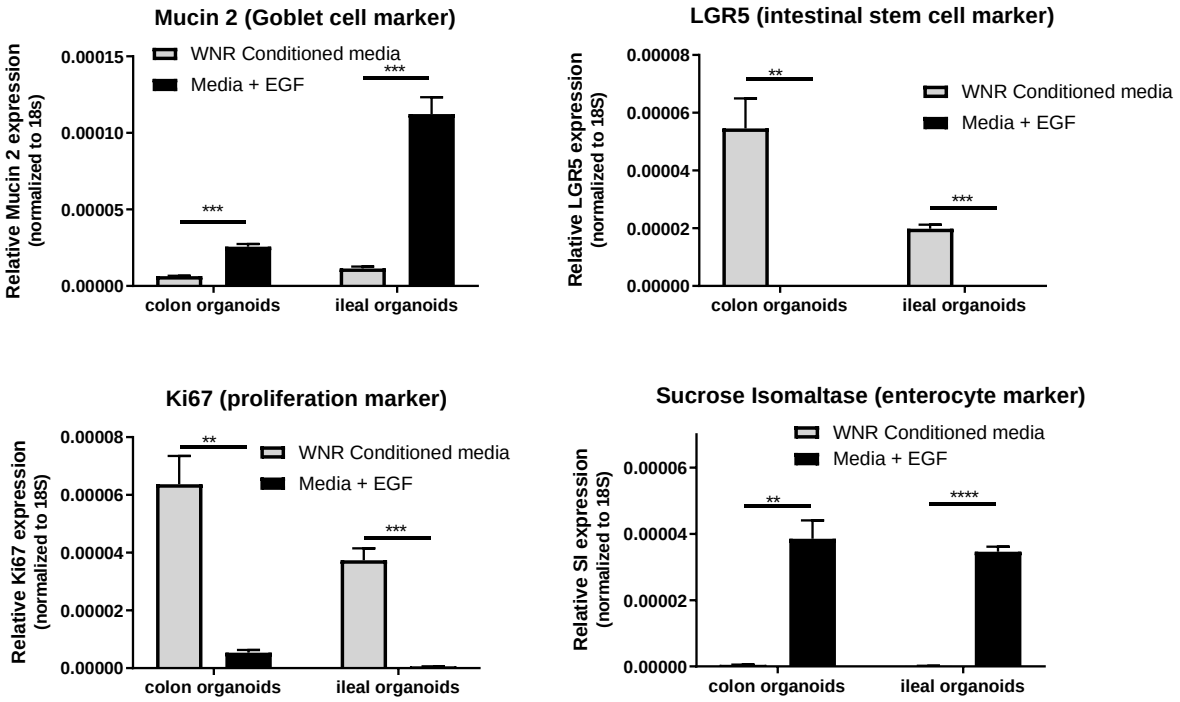

Supplemental Figure legend. Ileal and colon organoids were grown for 1 day in WNR conditioned media followed by a further 3 days of growth in WNR conditioned media or Media + EGF. Media was then removed and matrigel plugs were resuspended in RNAlater® (Thermo Fisher Scientific) and kept frozen (-80°C) for subsequent RNA isolation. The mRNA from organoids was extracted with MagMax™ – 96 Total RNA isolation kit. A fixed volume of total RNA was reverse transcribed into cDNA (SuperScript™ VILO™ cDNA-synthesis kit). RTPCR was performed on ViiA7 or QuantStudio real-time PCR system using TaqMan Assays-on-Demand™ Gene Expression assays to assess expression of target genes and normalized to the housekeeping gene 18S (Hs99999901_s1). LGR5 (Hs00969422_m1), Ki67 (HS04260396_g1), Mucin2 (Hs00159374_m1) and SI (Hs00356112_m1).

For analysis, media was removed from wells and total RNA extracted from organoids within the Matrigel plug. Figure data demonstrate alteration in cell type composition upon treatment of ileal

organoids and colon organoids from donor 1 with media containing EGF. Specifically, growth in media + EGF results in an increase in the goblet cell marker Mucin2 and the enterocyte marker sucrose isomaltose in organoids compared to organoids grown in WRN conditioned media. This is accompanied by a loss of expression of the stem cell marker LGR5 and the proliferation marker Ki67 in organoids grown in media + EGF compared to those grown in WRN conditioned media. Statistical analysis was by unpaired T-test (**, $p < 0.01$; ***, $p < 0.001$, ****, $p < 0.0001$).