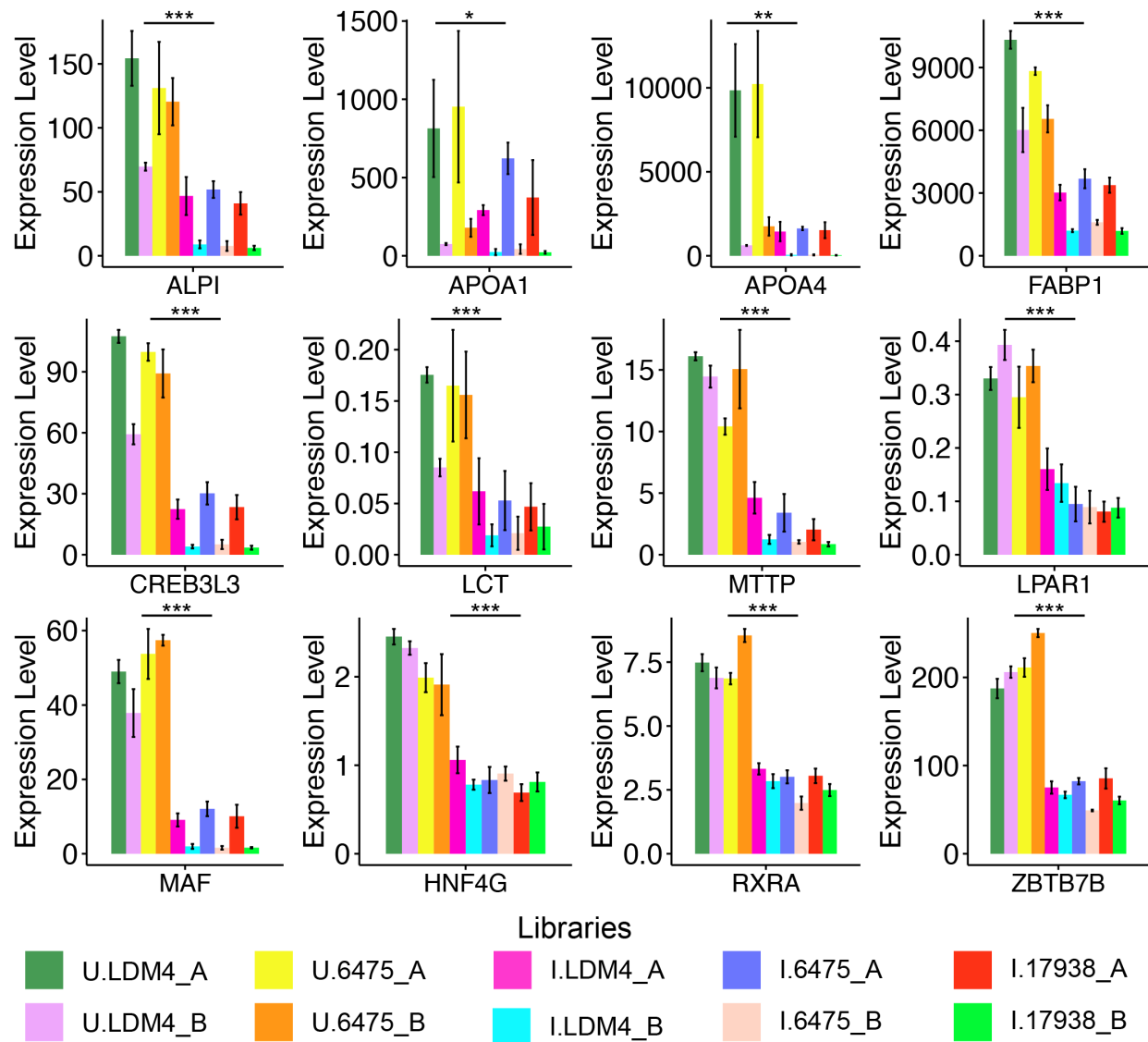


Supplemental Figure 1

Enterocyte markers

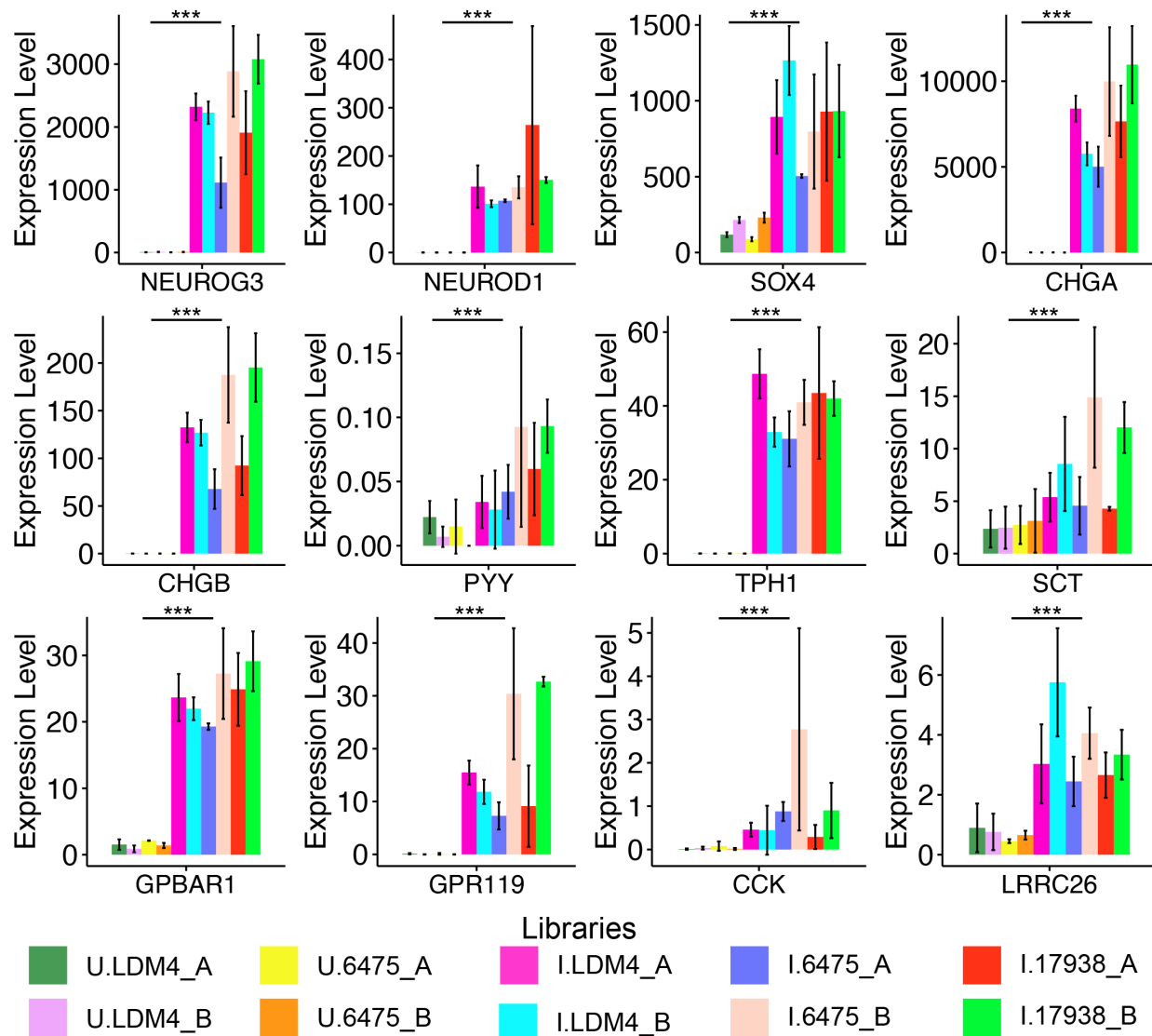


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Supplemental Figure S1. Expression levels of enterocyte cell markers (*ALPI*, *APOA1*, *APOA4*, *FABP1*, *CREB3L3*, *LCT*, *MTP*, *LPAR1*, *MAF*, *HNF4G*, *RXRA*, *ZBTB7B*) in uninduced and induced NGN3-HIOs. Libraries are labeled “U” for uninduced, “I” for induced” and “A” and “B” for the first and second batches of NGN3-HIOs. Expression levels shown are counts per million GeTMM transformed read counts. Significance of expression levels between the uninduced and induced libraries was calculated using a two-sample, two-sided, Mann-Whitney test. *, p < 0.05, **, p < 0.005, ***, p < 0.0005.

Supplemental Figure 2

Enteroendocrine markers

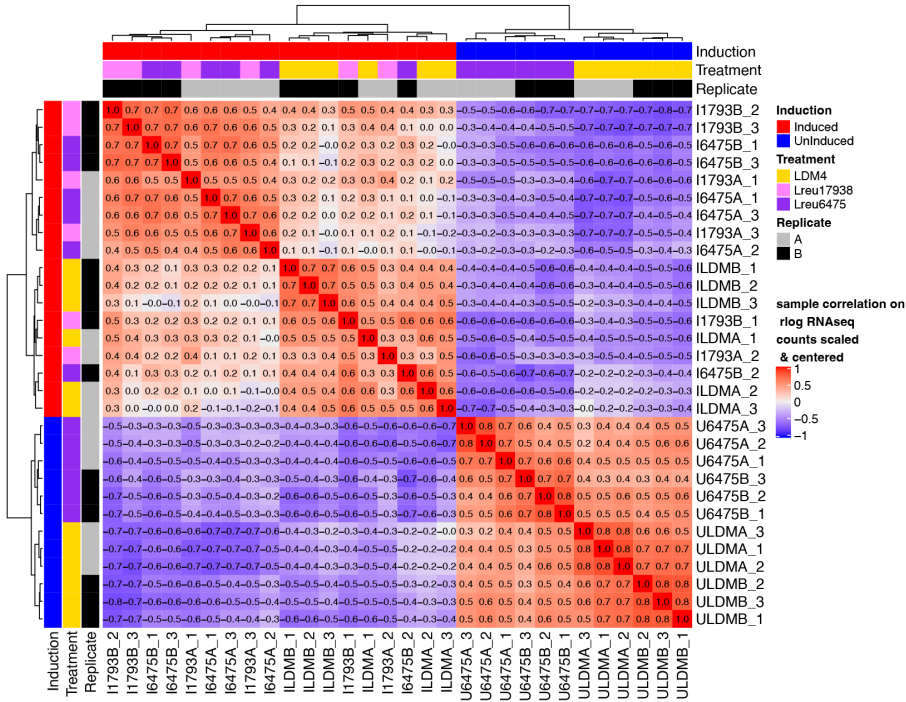


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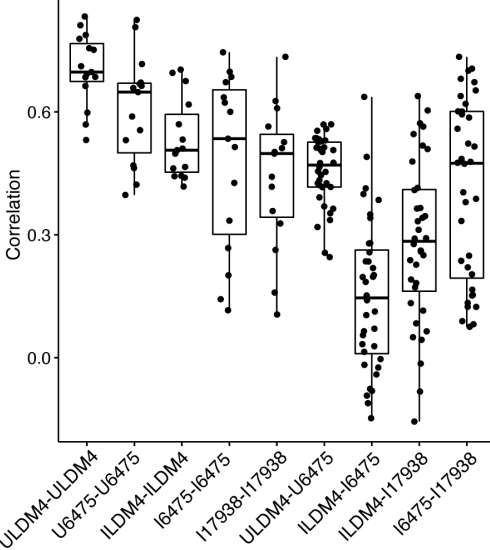
Supplemental Figure S2. Expression levels of enteroendocrine cell precursor markers (*NEUROG3*, *NEUROD1*, *SOX4*) and cell markers (*CHGA*, *CHGB*, *PYY*, *TPH1*, *SCT*, *GPBAR1*, *GPR119*, *CCK*, *LRRC26*) in uninduced and induced NGN3-HIOs. Libraries are labeled “U” for uninduced, “I” for induced” and “A” and “B” for the first and second batches of NGN3-HIOs. Expression levels shown are counts per million GeTMM transformed read counts. Significance of expression levels between the uninduced and induced libraries was calculated using a two-sample, two-sided, Mann-Whitney test. *, $p < 0.05$, **, $p < 0.005$, ***, $p < 0.0005$.

Supplemental Figure 3

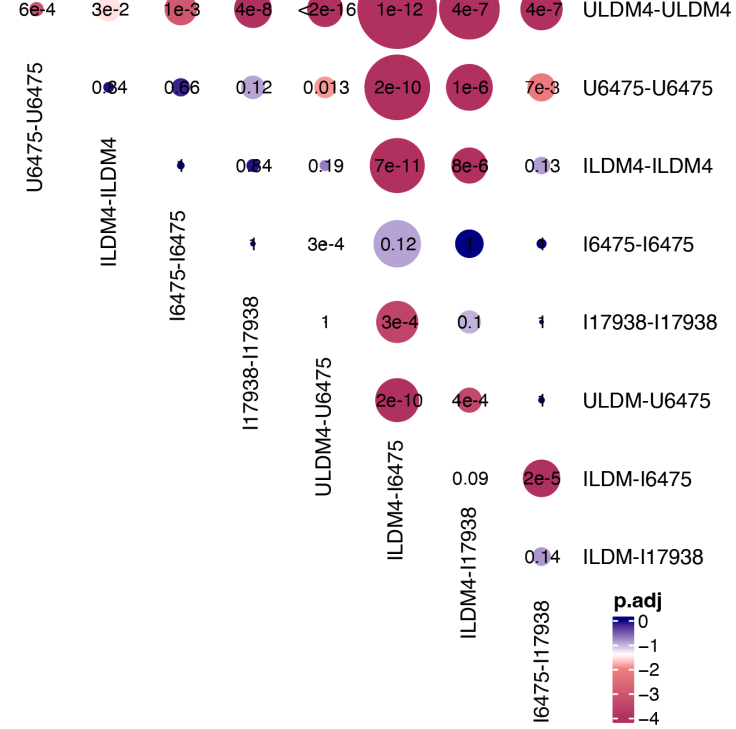
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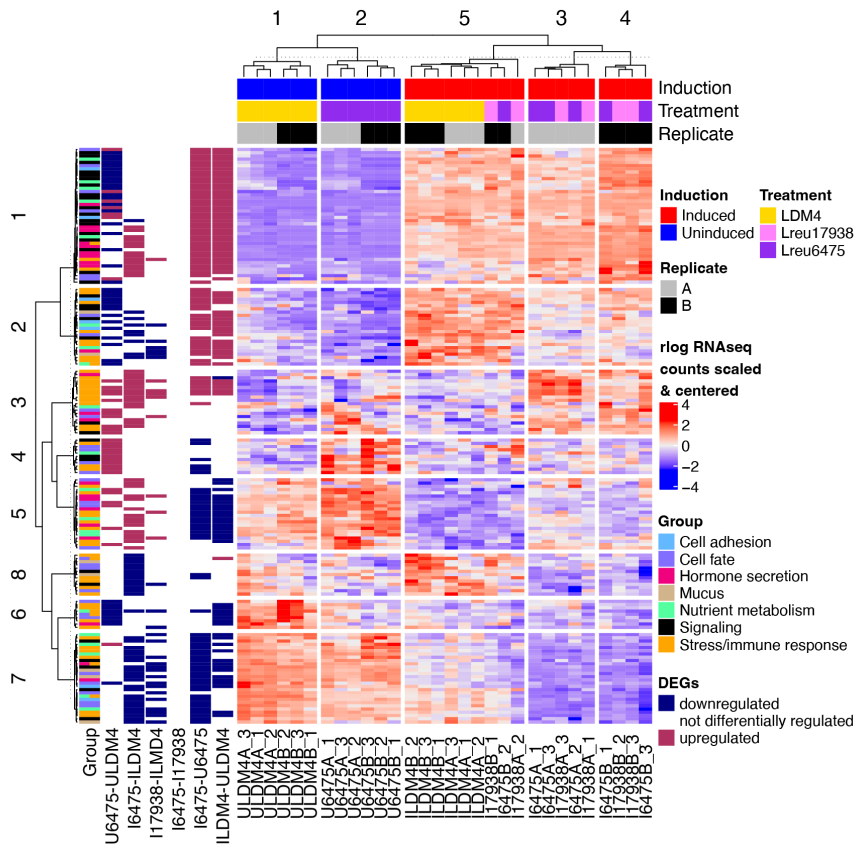


C



38 **Supplemental Figure S3.** Similarity and differences among *NGN3*-HIO transcriptomes. **A)** Boxplots of
39 Pearson correlation values within and between transcriptomes. **B)** Correlogram of mean differences
40 (circle size) and adjusted p-values (circle fill) between comparisons shown in A. Samples are listed,
41 whereby “U” refers to uninduced *NGN3*-HIOs, “I” for induced *NGN3*-HIOs, “LDM4” for media only
42 treatment, “6475” for *L. reuteri* 6475 treatment, “17938” for *L. reuteri* 17938 treatment, “A” or “B” refers
43 to the biological replicate, and “1”, “2”, or “3” refers to the technical replicate within each biological
44 replicate. **C)** Genes differentially regulated between *L. reuteri* 6475 and 17938 on induced *NGN3*-HIOs.
45 The graph shows the \log_2 fold change expression of the gene for the indicated comparison. The bars are
46 colored using the \log_{10} scaled mean GeTMM counts to illustrate how abundantly expressed the gene is.
47 Transparent overlays are used on genes not differentially expressed for the given comparison.
48 Comparisons shown: U6475-ULDM4, *L. reuteri* 6475 on uninduced HIOs compared to LDM4 media
49 control; I6475-ILDM4, *L. reuteri* 6475 on induced HIOs compared to LDM4 media control; I17938-
50 ILDM4, *L. reuteri* 17938 on induced HIOs compared to LDM4 media control; I6475-I17938 *L. reuteri*
51 6475 compared to *L. reuteri* 17938 on induced HIOs; ILDM4-ULDM4, LDM4 media control on induced
52 versus uninduced HIOs; I6475-U6475, *L. reuteri* 6475 on induced versus uninduced HIOs. For each,
53 positive fold changes indicate genes upregulated by the condition listed first.
54

Supplemental Figure 4



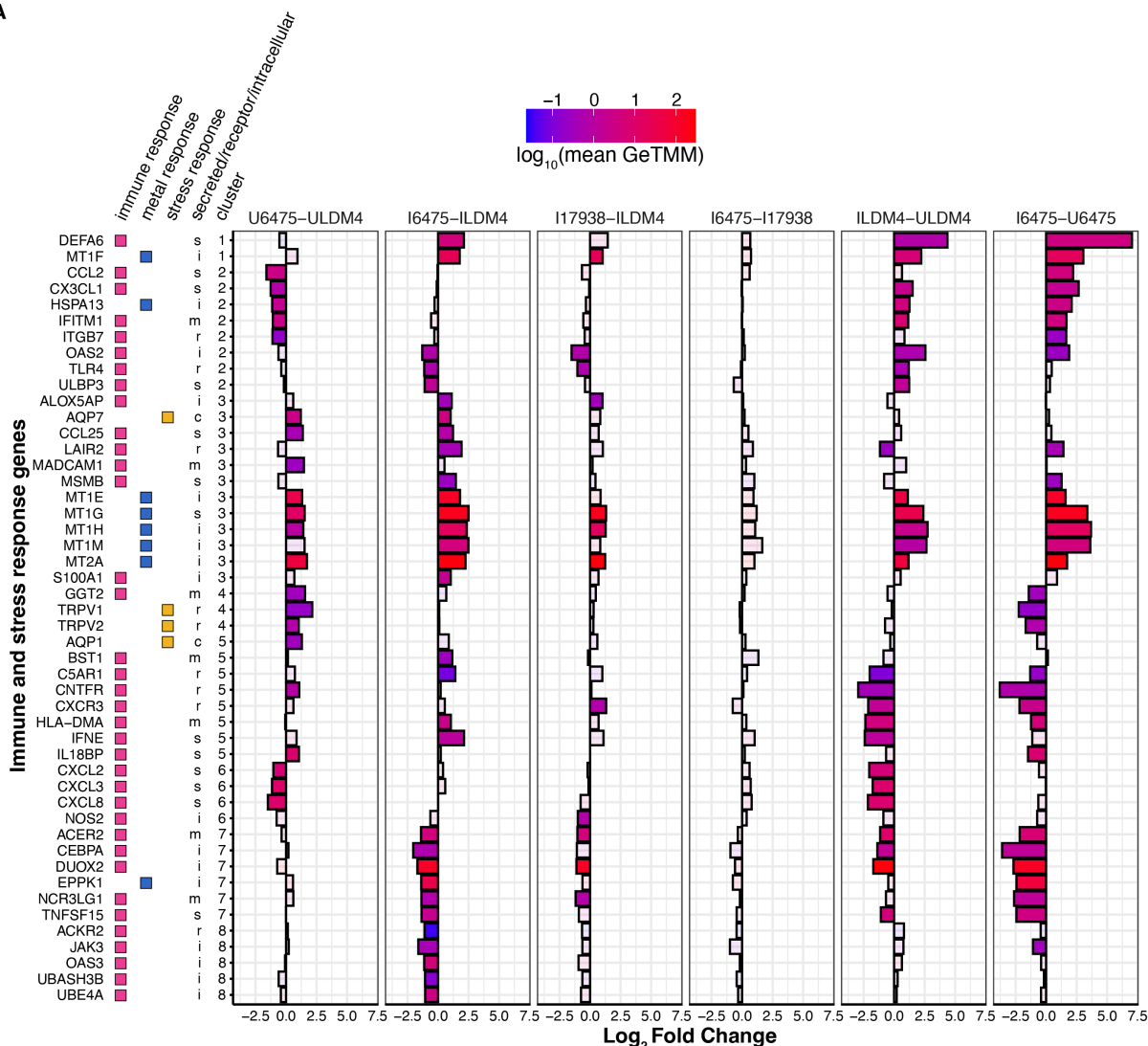
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56 **Supplemental Figure S4.** Cluster analysis of DEGs belonging to functionally enriched groups. The
 57 heatmap shows gene expression values as rlog counts that were scaled and centered. Samples (the
 58 columns) along the bottom of the heatmap are labeled as “U” for uninduced *NGN3*-HIOs, “I” for induced
 59 *NGN3*-HIOs, “LDM4” for media only treatment, “6475” for *L. reuteri* 6475 treatment, “17938” for *L.*
 60 *reuteri* 17938 treatment, “A” or “B” for the biological replicate, and “1”, “2”, or “3” for the technical
 61 replicate within each biological replicate. Samples are annotated above the heatmap as shown in the
 62 legend. Genes (rows) were arranged by K-means clustering and annotated into groups as shown in the
 63 legend. For each sample comparison (e.g. U6475-ULDM4), if the gene was down or upregulated (e.g.
 64 higher in U6475 than ULDM4), a color is given as shown in the legend.

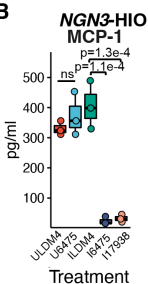
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Supplemental Figure 5

A



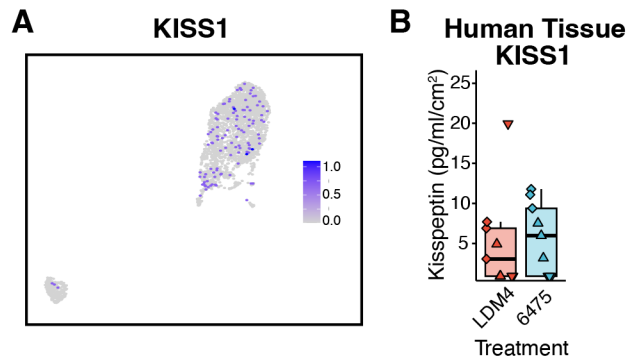
B



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 67 **Supplemental Figure S5.** *L. reuteri* regulates immune, metal, and stress response. A) Immune, metal,
 68 and stress genes differentially regulated by *L. reuteri*. The genes are annotated with their function,
 69 whether they are secreted, a receptor, or intercellular, and what cluster they belong to relative to
 70 Supplemental Figure S4. The graph shows the \log_2 fold change expression of the gene for the indicated
 71 comparison. The bars are colored using the \log_{10} scaled mean GeTMM counts to illustrate how
 72 abundantly expressed the gene is. Transparent overlays are used on genes not differentially expressed for
 73 the given comparison. Comparisons shown: U6475-ULDM4, *L. reuteri* 6475 on uninduced HIOs

74 compared to LDM4 media control; I6475-ILDm4, *L. reuteri* 6475 on induced HIOs compared to LDM4
75 media control; I17938-ILDm4, *L. reuteri* 17938 on induced HIOs compared to LDM4 media control;
76 I6475-I17938 *L. reuteri* 6475 compared to *L. reuteri* 17938 on induced HIOs; ILDM4-ULDm4, LDM4
77 media control on induced versus uninduced HIOs; I6475-U6475, *L. reuteri* 6475 on induced versus
78 uninduced HIOs. For each, positive fold changes indicate genes upregulated by the condition listed first.
79 **B)** MCP-1 protein levels measured by Luminex on uninduced (U) or induced (I) HIOs treated with *L.*
80 *reuteri* 6475 or 17938.
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Supplemental Figure 6



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Supplemental Figure S6: KISS1 may be produced in the intestinal epithelium. **A)** UMAP of KISS1 using the Gut Cell Atlas adult jejunum data. **B)** Lack of secretion of kisspeptin in response to bacterial media control (LDM4) and *L. reuteri* 6475 conditioned media from *ex vivo* human jejunal intestinal tissue. Shape represents unique human intestinal donors. Significance was determined using a linear mixed model with $p < 0.05$ considered as significant.

Supplemental Tables available in Excel document

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131
132 **Supplemental Table 1:** Sequencing reads per library. Number of sequencing reads for each sample after
133 filtering and aligning to the reference human genome (see Methods).

134
135 **Supplemental Table 2:** Genes differentially regulated between *L. reuteri* strains 6475 and 17938 in
136 induced and uninduced HIEs. Libraries are labeled “U” for uninduced, “I” for induced” and “A” and “B”
137 for the first and second batches of organoids. For each comparison column, e.g. U6475-ULDM, “0”
138 means no difference, “-1” means ULDM has higher expression values than U6475, “1” means U6475 has
139 higher expression values than ULDM. Expression levels shown are computed using the rlog. Output from
140 DESeq2 (base mean (average of count values post normalization for size factors), log₂ fold change, log₂
141 fold change standard error, test statistic from a Wald test, p-value, and adjusted p-value using the
142 Benjamini-Hochberg procedure) for each comparison are given.

143
144 **Supplemental Table 3:** Enriched functional groups in *L. reuteri* over media alone DEGs. Annotations
145 were taken from the indicated annotation group as annotated by the PANTHER classification system and
146 Reactome annotated pathways. Groupings were manually assigned with the intention of generalizing the
147 types of functional groups among the data. Enriched refers to whether the functional group is enriched in
148 the set of DEGs or depleted. DEG Up-regulated (+) or Down-regulated (-) displays if the genes within the
149 functional group were up or down-regulated by the respective *L. reuteri* strain compared to the media
150 alone control.

151
152 **Supplemental Table 4:** Functional DEGs in *L. reuteri* over media alone annotations. DEGs belonging to
153 a functional group are annotated at three levels, upper, middle, and final, with increasing levels of
154 resolution. As well, the DEGs are classified by a subtype giving information about their cellular location.
155 DEG up- or downregulation information, gene information, and output from DESeq2 are given as in
156 Supplemental Table 2. GeTMM transformed read counts are given as well.

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