



Transcriptomic analysis of intestine following administration of a transglutaminase 2 inhibitor to prevent gluten-induced intestinal damage in celiac disease

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Supplementary Files

Supplementary Table 1: Demographic Characteristics of the Patients in original cohort and in present study.

Supplementary Table 2: Effect of ZED1227 Treatment on the Ratio of villus height to crypt depth (VH:CrD) in original cohort and in present study.

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Supplementary Table 1: Demographic Characteristics of the Patients in original cohort and in present study.

Characteristic	Original cohort*		Present study cohort	
	ZED1227, 100 mg (N = 39)	Placebo (N = 38)	Drug (d, n=34)	Placebo (p, n=24)
Age — yr (mean ± sd)	41.0±14.8	42.5±14.4	40.7±15.1	43.2±14.9
Female sex — no. (%)	24 (62)	28 (74)	22 (64.7)	17 (70.8)
White race — no. (%)	39 (100)	38 (100)	34 (100)	24 (100)
Weight — kg (mean ± sd)	73.2±13.7	68.4±14.7	74.1±13.9	71.4±17.0

*Original cohort Demographic Characteristics is published in Schuppan, D. et al. A Randomized Trial of a Transglutaminase 2 Inhibitor for Celiac Disease. N. Engl. J. Med. 385, 35–45 (2021).

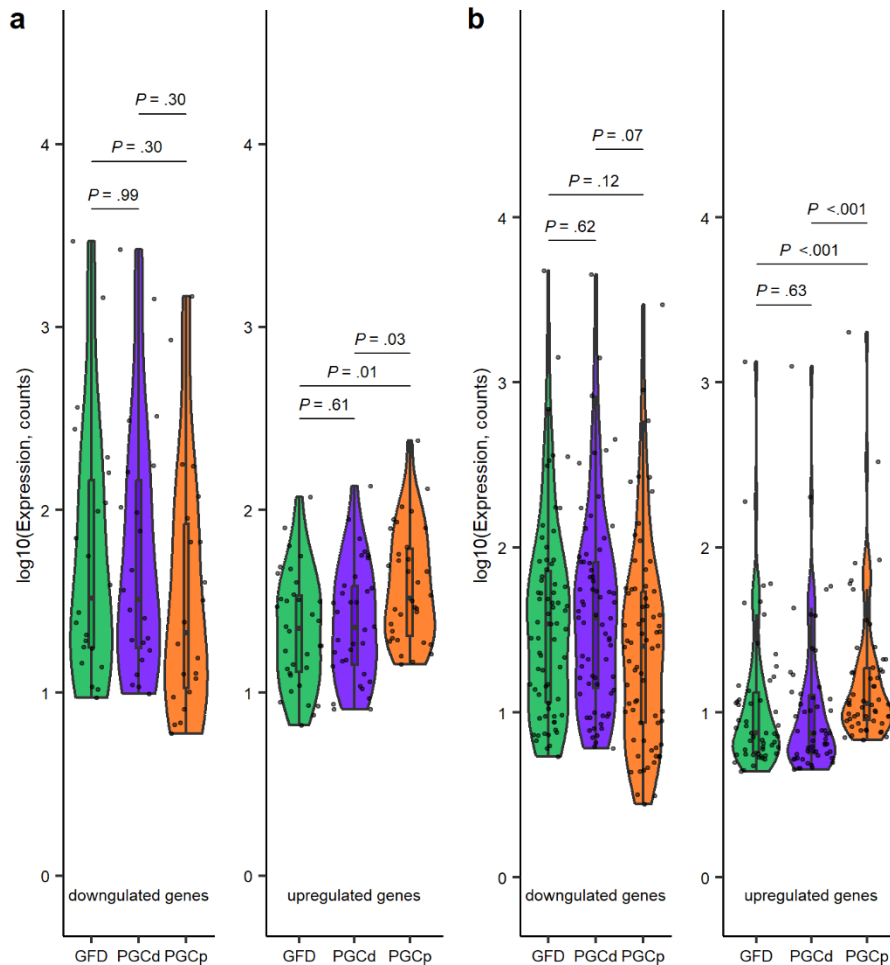
Supplementary Table 2: Effect of ZED1227 Treatment on the Ratio of villus height to crypt depth (VH:CrD) in original cohort and in present study.

Variable	Original cohort*		Present study cohort	
	ZED1227, 100 mg (N = 38)	Placebo (N = 30)	Drug (d, n=34)	Placebo (p, n=24)
VH:CrD				
GFD	2.09±0.35	1.98±0.33	2.11±0.34	1.95±0.36
PGC	1.94±0.48	1.39±0.61	1.89±0.40	1.35±0.65
Change from GFD (95% CI)	-0.13 (-0.28 to 0.03)	-0.61 (-0.78 to -0.44)	-0.21 (-0.36 to -0.07)	-0.59 (-0.83 to -0.35)

*Original cohort VH:CrD descriptive statistics is published in Schuppan, D. et al. A Randomized Trial of a Transglutaminase 2 Inhibitor for Celiac Disease. N. Engl. J. Med. 385, 35–45 (2021).

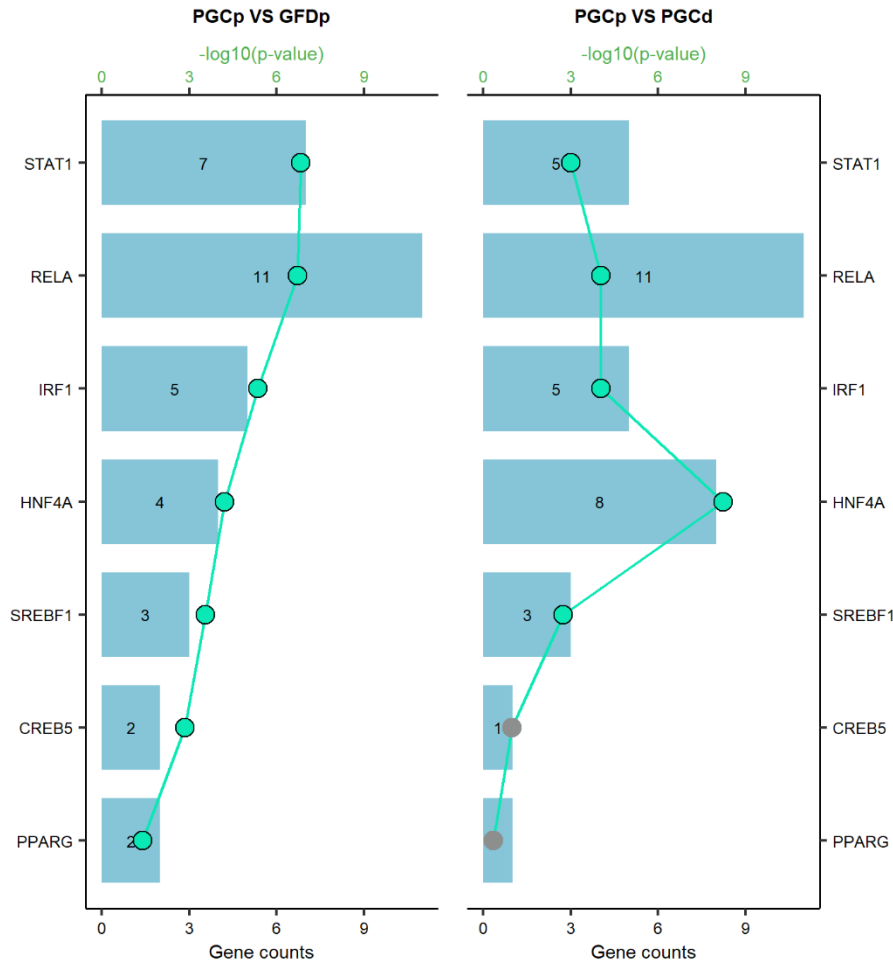
Plus-minus values are means ±SD. The change from GFD is presented as a least-squares means estimate.

Supplementary Fig. 1



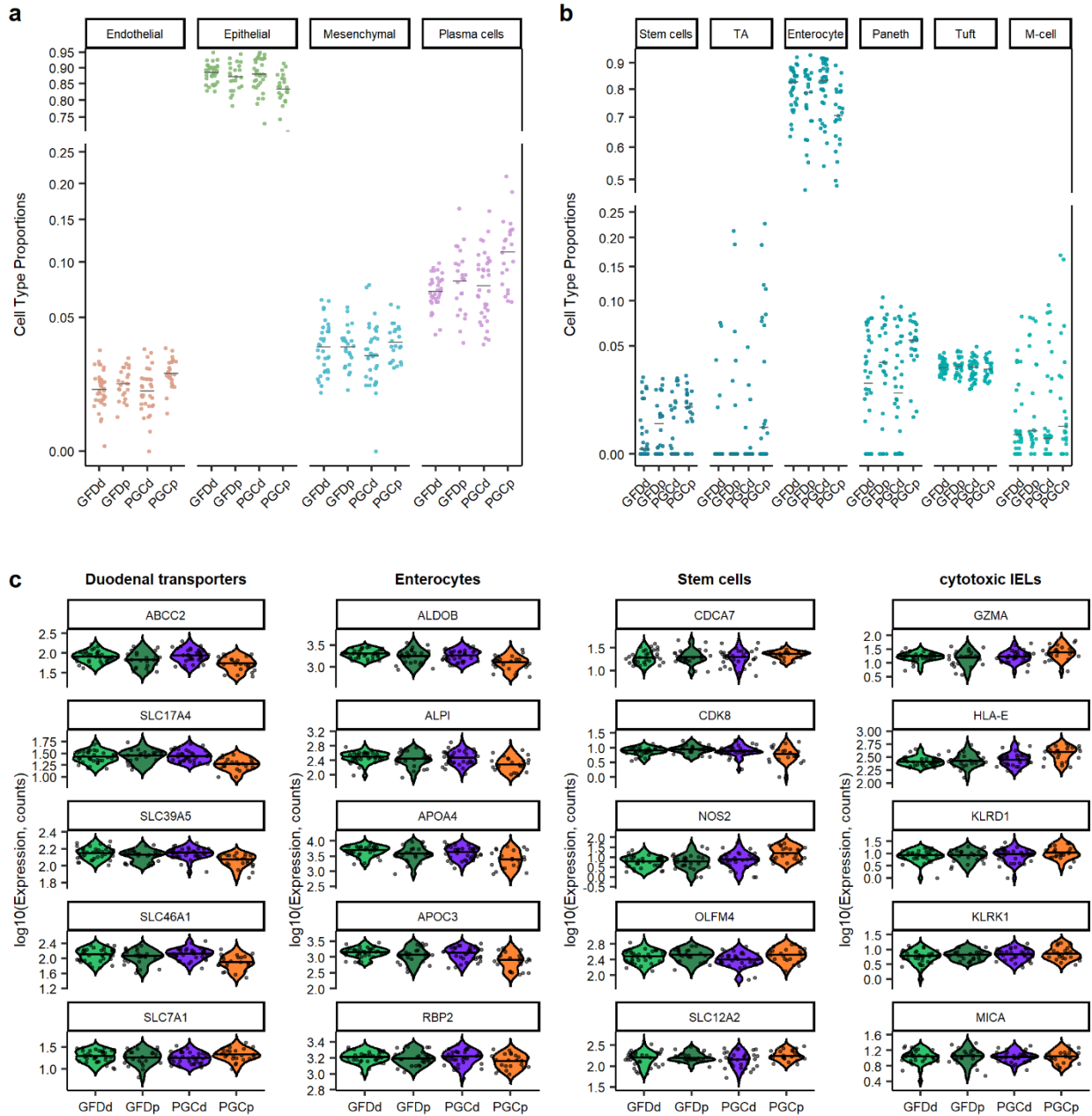
Supplementary Fig. 1: Expression of common genes. a, Violin plots showing the log-transformed expression of 56 common (in PGCp VS PGCd and PGCp VS GFDp comparisons) DEGs down-regulated (left panel) and up-regulated (right panel). **b,** Violin plot of 124 uniquely differentially expressed genes in PGCp VS PGCd comparison'. Kruskal-Wallis test followed by the Dunn's post hoc test was used for group comparisons. Statistical significance was defined as a $P < .05$. **a:** downregulated genes: GFD-PGCd $P = 0.99$, PGCp-GFD $P = 0.30$, PGCd-PGCp $P = 0.30$; upregulated genes: GFD-PGCd $P = 0.61$, PGCp-GFD $P = 0.01$, PGCd-PGCp $P = 0.03$; **b:** downregulated genes: GFD-PGCd $P = 0.62$, PGCp-GFD $P = 0.12$, PGCd-PGCp $P = 0.07$; upregulated genes: GFD-PGCd $P = 0.63$, PGCp-GFD $P = 6.68 \times 10^{-4}$, PGCd-PGCp $P = 1.98 \times 10^{-4}$. The box plot center lines represent the median, the box boundaries represent IQR and the whisker length minimum and maximum range. Values from individual patients are shown. GFD ($n = 58$), PGCd ($n = 34$), and PGCp ($n = 23$)

Supplementary Fig. 2



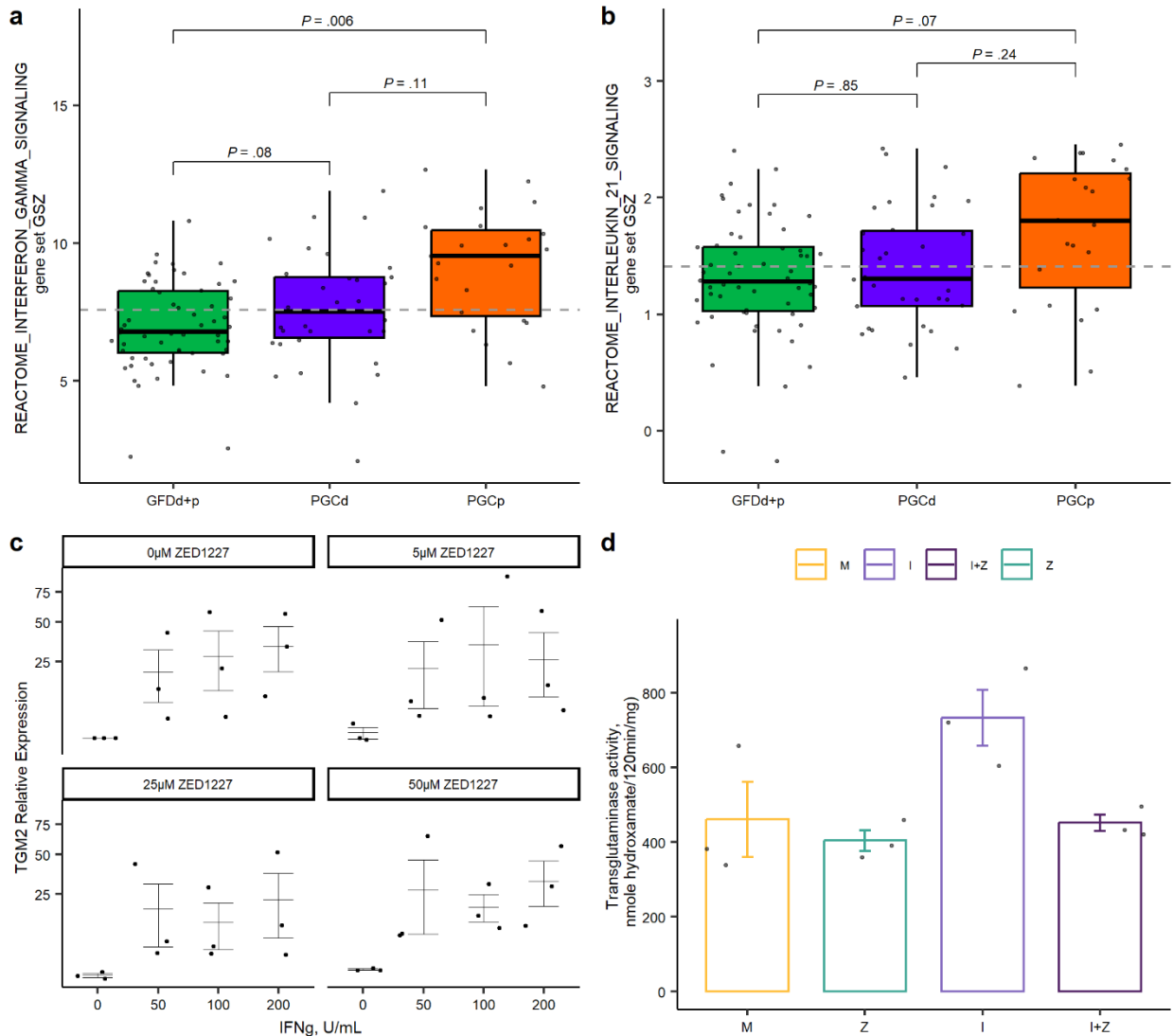
Supplementary Fig. 2: Transcriptional factors enrichment. Barplot of enriched transcriptional factors in PGCp vs GFDp (left) and PGCp vs PGCD (right) comparisons. Fisher's exact test was used for p-values calculation. Green and gray dots denote significant and non-significant adjusted p-values, respectively. Statistical significance was defined as a $P < .05$ ($-\log_{10}(p\text{-value}) > 1.3$).

Supplementary Fig. 3



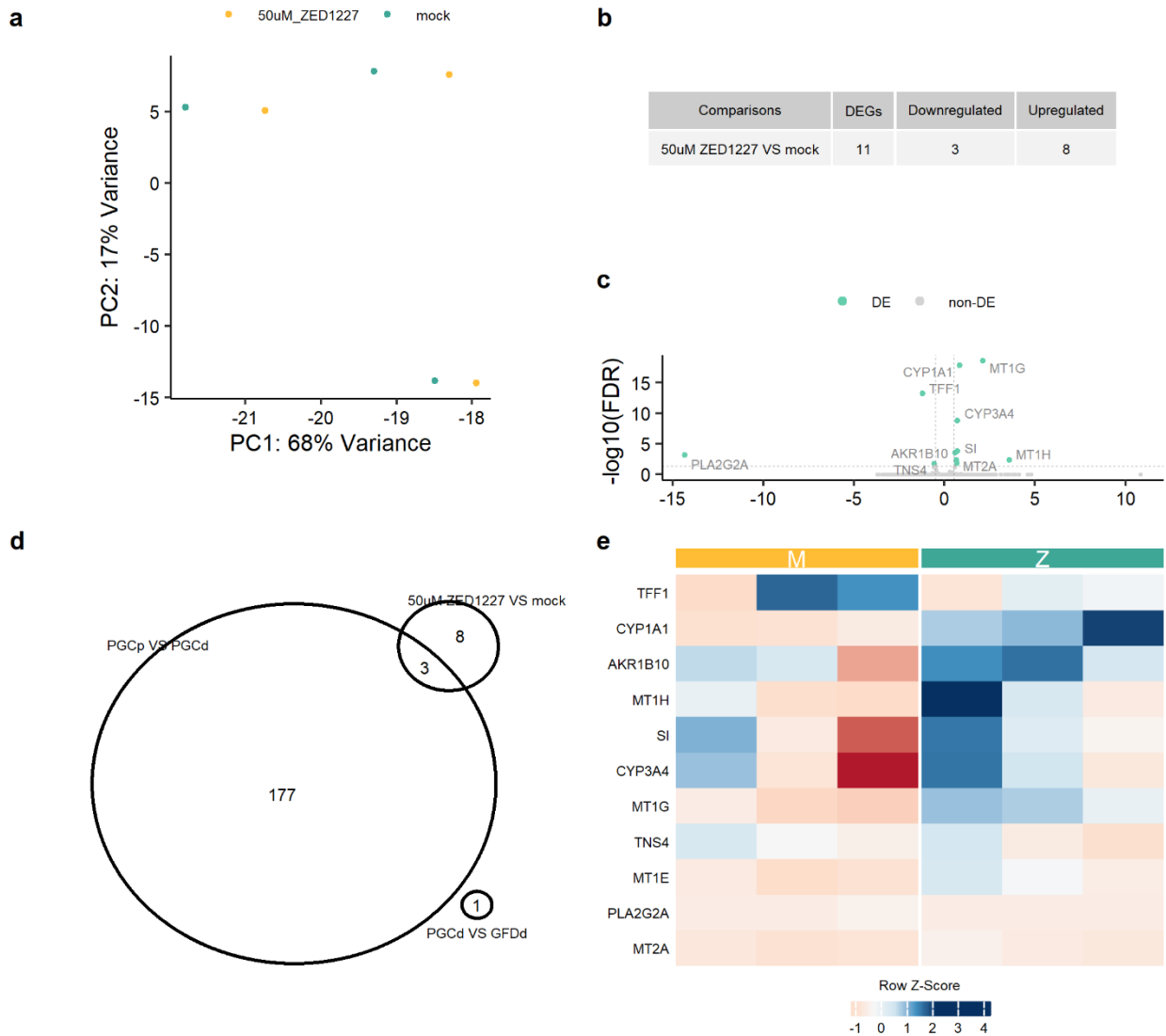
Supplementary Fig. 3: Cell type proportions according to the results of duodenal biopsies bulk transcriptomics deconvolution for cell category **a, and cell type **b**, for patients in drug and placebo groups on GFD and at PGC (GFDd, n = 34; GFDp, n = 24; PGCd, n = 34; PG Cp, n = 23). The horizontal line represents mean. **c**, Violin plots for selected genes expression. Midline denotes the median. GFDd, n = 34; GFDp, n = 24; PGCd, n = 34; PG Cp, n = 23.**

Supplementary Fig. 4



Supplementary Fig. 4: Immune Signaling Pathways, TG2 mRNA levels and TG2 activity during ZED1227 treatment. **a**, Gene set Z-score analyses for the Reactome pathway database gene set “Interferon-gamma signaling” gene set. **b**, Gene set Z-score analyses for the Reactome pathway database gene set “Interleukin-21 signaling” gene set. In **a** and **b**, GSZ scores were compared among groups using asymptotic p-value estimation, with statistical significance defined as $P < .05$. The box plot center lines represent the median, the box boundaries represent IQR and the whisker length minimum and maximum range. Values from individual patients are shown. GFDd+p ($n = 58$), PGcd ($n = 34$), and PGCp ($n = 23$). **c**, Quantitative RT-PCR of TGM2 expression in human duodenal organoids ($n = 3$) treated with human recombinant IFN γ and/or ZED1227 at specified concentrations. Gene-specific Ct values were normalized ($\Delta\Delta$ Ct) based on GAPDH house-keeping gene expression and relative to non-treated sample (0 U/mL IFN γ and 0 mM ZED1227). Data shown as mean \pm SE. **d**, Colorimetric TG activity assay in Caco2 cells treated with 100 U/mL IFN γ (I, $n = 3$), 50 μ M ZED1227 (Z, $n = 3$), their combination (I+Z, $n = 3$), or mock (M, $n = 3$) for 24 h. Data represented as mean \pm SE.

Supplementary Fig. 5



Supplementary Fig. 5: ZED1227 treatment of human intestinal organoids. **a**, Principal component analysis (PCA) plot using DESeq2-transformed counts for all organoid samples ($n = 3$), treated with $50\mu\text{M}$ ZED1227 ($n = 3$) or mock-treated ($n = 3$) for 24 hours. **b**, Table showing the number of differentially expressed genes (DEGs) ($\log_2\text{FC} \geq |0.5|$ and $\text{FDR} \leq .05$) in the $50\mu\text{M}$ ZED1227 VS mock comparison. **c**, Volcano plot representations of DEGs ($n = 11$) in $50\mu\text{M}$ ZED1227 VS mock comparison. The green dots indicate DEGs ($\text{FDR} \leq 0.05$) above the threshold ($\log_2\text{FC} \geq 0.5$ and ≤ -0.5). The dashed horizontal line represents the FDR threshold of 0.05, and the vertical dashed lines represent the $\log_2\text{FC}$ thresholds ($\geq |0.5|$). **d**, Venn diagram illustrating the number of DEGs that are shared in the PGCp VS PGCd, PGCd VS GFDD and $50\mu\text{M}$ ZED1227 VS mock comparisons. **e**, Heatmap of the 11 DEGs in $50\mu\text{M}$ ZED1227 VS mock comparison. Genes ordered according to their $\log_2\text{FC}$. Z-score of normalized expression is plotted. $50\mu\text{M}$ ZED1227 ($n = 3$), mock-treated ($n = 3$).

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