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Supplemental Information

Three-dimensional interactions between enhancers and promoters during intestinal differentiation depend upon HNF4

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SUPPLEMENTAL FIGURES

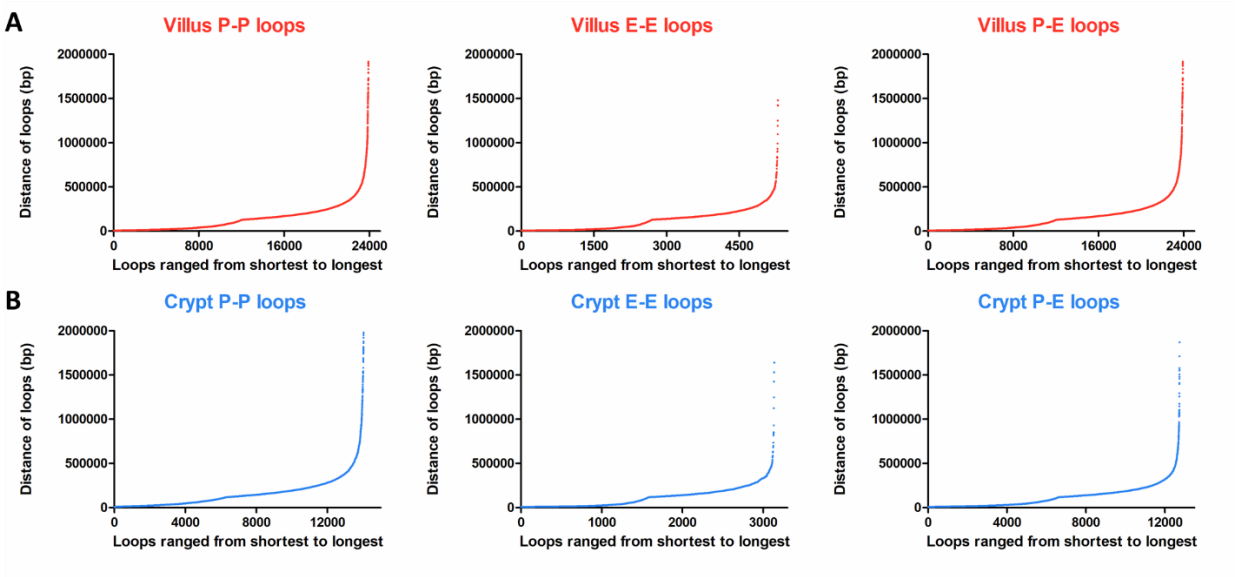


Figure S1. Distance of chromatin loops in (A) villus and (B) crypt cells as revealed by H3K4me3 HiChIP-seq. Related to Figure 1. P-P loops: promoter-promoter loops; E-E loops: enhancer-enhancer loops; P-E loops: promoter-enhancer loops (n = 2 biological replicates each condition). Loops with $q \leq 0.0001$ and counts ≥ 4 (combined 2 replicates) were used to characterize the distance of chromatin loops.

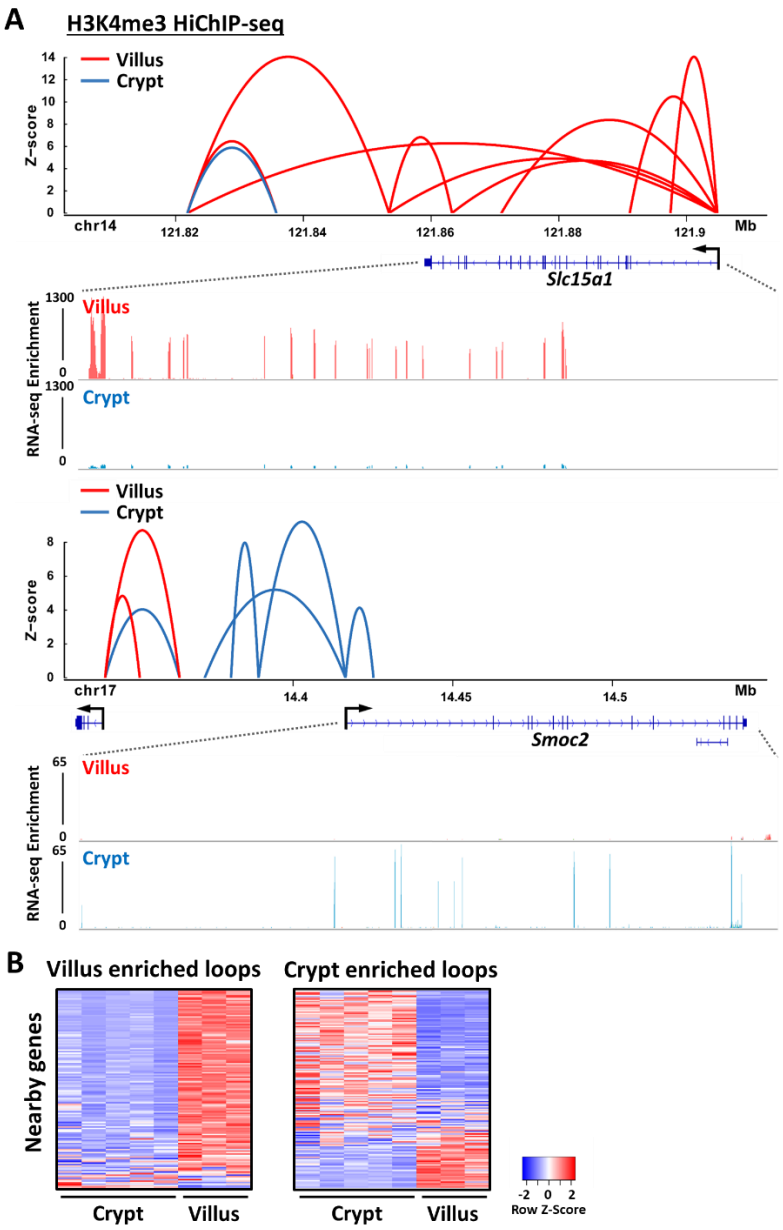
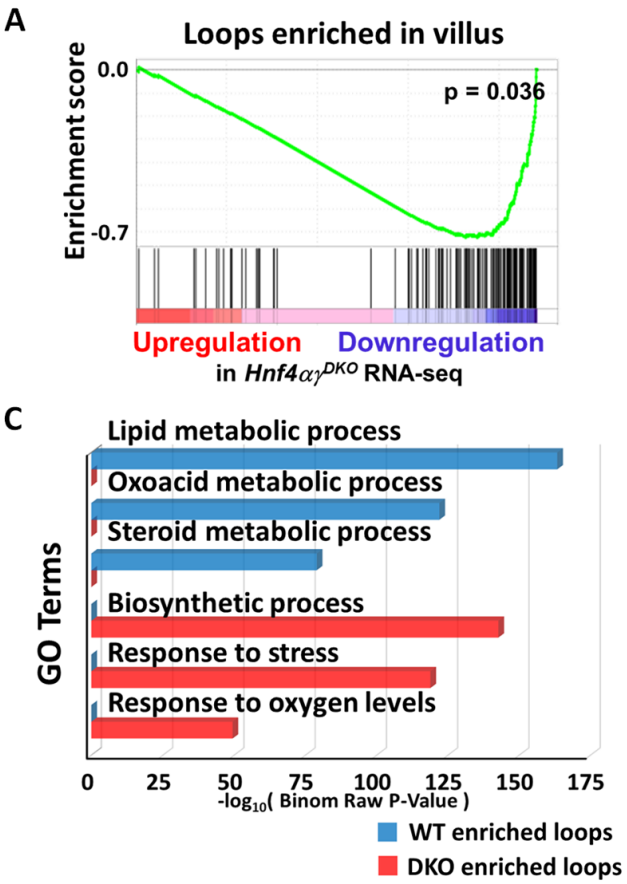


Figure S2. Differential looping events across the crypt-villus axis. Related to Figure 2. **(A)** Chromatin looping examples of a villus-specific gene and a crypt-specific gene. Loops with q ≤ 0.0001 and counts ≥ 8 (combined 2 replicates) are visualized by Sushi. **(B)** Heatmap of transcript levels of nearby genes (within 10 kb of TSSs) of villus and crypt enriched loops. RNA-seq (GSE53545, GSE70766 and GSE102171): n = 5 crypts and 3 villi; H3K4me3 HiChIP-seq: n = 2 biological replicates; TSSs: transcriptional start sites.



B

WT enriched looping regions

Motif	TFs	p value
	HNF4	1e-1666
	AP-1	1e-649
	KLF	1e-458
	GATA	1e-353
	HNF1	1e-232

DKO enriched looping regions

Motif	TFs	p value
	FRA1	1e-198
	KLF	1e-130
	GATA	1e-94
	HNF4	1e-93
	CTCF	1e-70

Figure S3. HOMER motif and gene ontology analysis of WT and *Hnf4αγ*^{DKO} enriched looping regions. Related to Figure 2. **(A)** GSEA (Kolmogorov-Smirnov test, p = 0.036) reveals that transcriptome levels of genes nearby the villus-enriched looping regions are significantly downregulated upon HNF4 loss. H3K4me3 HiChIP-seq: n = 2 biological replicates. **(B)** HOMER *de novo* motif enrichment (see full table in **Table S5**). **(C)** Functional annotation (GREAT) of nearby genes (within 10 kb of TSSs) of WT-enriched and *Hnf4αγ*^{DKO}-enriched loops. *P*-values were calculated using GREAT (see full table in **Table S6**). TSSs: transcriptional start sites.

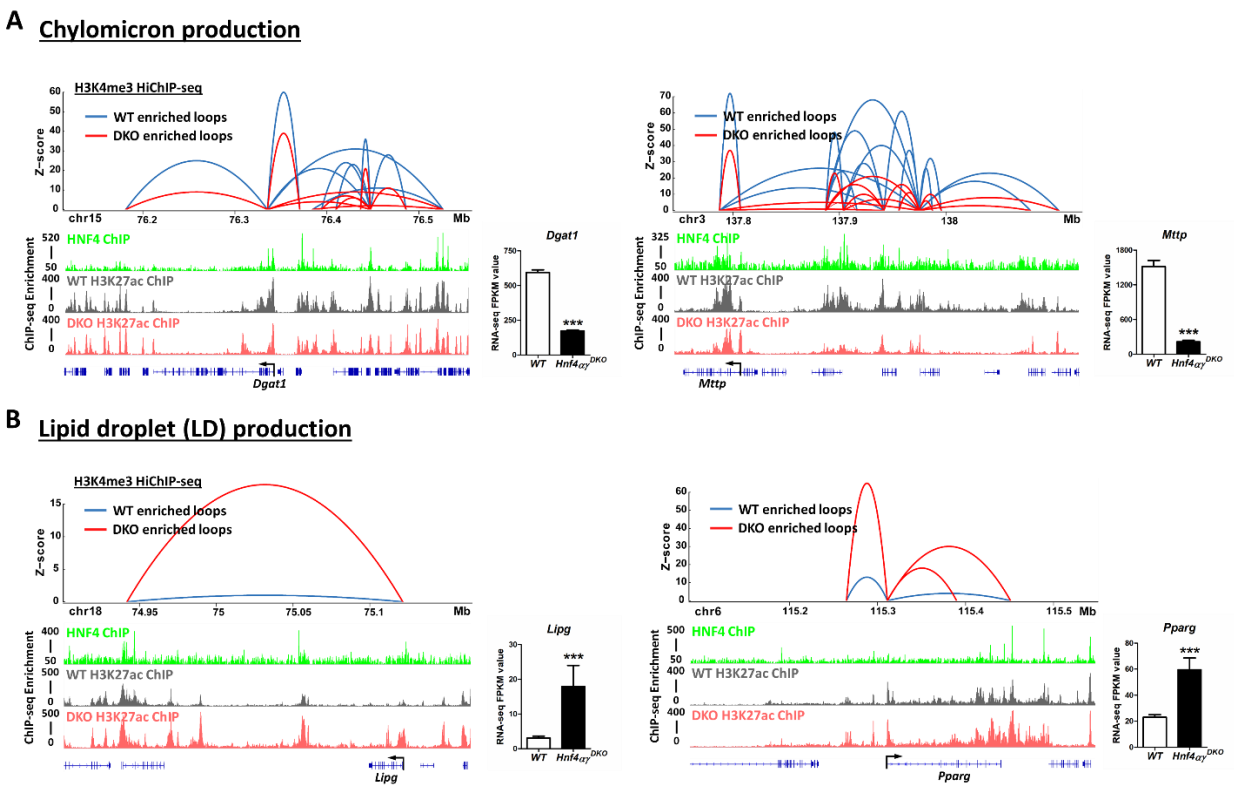


Figure S4. Additional examples of regulatory effects of HNF4 factors at genes associated with (A) chylomicron production and (B) lipid droplet production. Related to Figure 4. Differential loops (DEseq2 $p < 0.05$) are visualized by Sushi for loops with $q \leq 0.0001$ and ≥ 8 counts (over 2 combined biological replicates). H3K4me3 HiChIP-seq: n = 2 biological replicates; H3K27ac ChIP-seq (WT vs *Hnf4αγ*^{DKO}; GSE112946): n = 2 biological replicates; HNF4 ChIP-seq (WT vs *Hnf4αγ*^{DKO}; GSE112946): n = 2 biological replicates for each HNF4 paralog; RNA-seq (WT vs *Hnf4αγ*^{DKO}; GSE112946): n = 3 biological replicates, Cuffdiff FDR < 0.001***.