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Supplemental Information

Effects of Diet Choice on Stem Cell Function

Necessitate Clarity in Selection and Reporting

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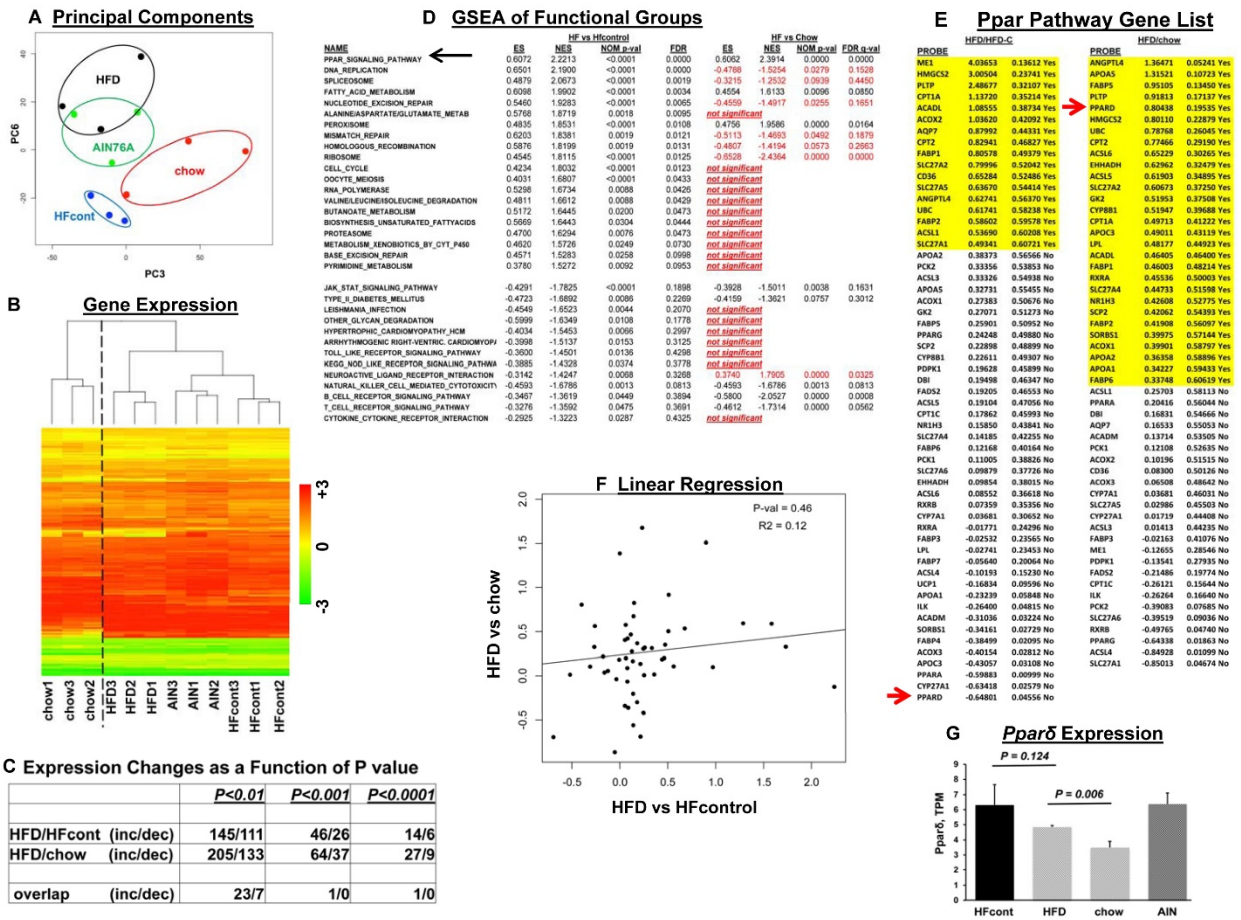
Supplemental Figure 1



C Expression Changes as a Function of P value		P<0.01	P<0.001	P<0.0001
HFD/HFcont	(inc/dec)	145/111	46/26	14/6
HFD/chow	(inc/dec)	205/133	64/37	27/9
overlap	(inc/dec)	23/7	1/0	1/0

Figure S1: Gene expression analysis of Lgr5^{hi} intestinal cells as a function of diet fed to the mice. Lgr5tm1(cre/ERT2)Cle/J mice (Jax #008875) were fed different diets *ad lib* from weaning (N=3/diet group; the chow cohort consisted of male mice, the purified control diet cohorts were mixed groups of male and female mice, and the HFD group were female mice). The diets were: the high fat purified diet (HFD) used in ((Beyaz et al., 2016), 60% calories from fat, Research Diets, 12492); a purified control (HFcontrol, 10% fat, Research Diets 12450B). This is 1 of 4 purified diets marketed by Research diets as a control for the HFD, all of which are 10% fat, 20% protein, and 70% carbohydrate, with the carbohydrate raised in different ways to balance the higher fat in HFD; another purified control diet, AIN76A (Research Diets, D10001); a chow diet (PicoLab 5058, LabDiets). Mice were sacrificed at 3 months, the small intestine dissected, Lgr5^{hi} cells from purified crypts isolated by FACS as the highest 2-3% of the green fluorescent cells, and RNAseq data generated with RNA prepared from each cell preparation (Li et al., 2019b; Peregrina et al., 2015). **A)** Principal Component Analysis as a function of diet; **B)** One-way ANOVA identified

162 genes that best distinguish the 4 groups, but without regard to diet identity. These were then used for unsupervised clustering in the heat map. **C)** Number of expressed genes up or down regulated in the Lgr5hi cells from the HFD group compared to either the HFcontrol or the chow group as a function of stringency of cut-off, and the overlap of the differentially expressed genes determined at each stringency. At $P<0.0001$, it is expected that only 1-2 false positives may be detected. **D)** The RNAseq data for the $Lgr5^{hi}$ cell populations were analyzed by GSEA (Li et al., 2019b). Tabulation of the statistically enriched functional groups in comparing the HFD to HFDcontrol mice, and for each of these its corresponding GSEA analysis when the data from HFD fed mice were compared to that from chow fed mice (black arrow, PPAR pathway). **E)** Of the 57 genes that define the Ppar Kegg pathway, those that contributed to the significant GSEA enrichment are highlighted in yellow for the HFD mice compared to either the HFcontrol or chow diet mice. In each comparison, genes are ranked from highest to lowest NES (normalized enrichment score); Red arrows indicate the Pparδ gene for each comparison. **F)** Linear regression of the ratio of gene expression for each gene of the Ppar pathway when the $Lgr5^{hi}$ cell data from mice fed HFD were compared to the data from mice fed either HFcontrol or chow diet. **G)** Pparδ expression (TPM=transcripts per million) in $Lgr5^{hi}$ cells as a function of the diet fed the mice. RNAseq data is deposited in Geo, accession number GSE151498.