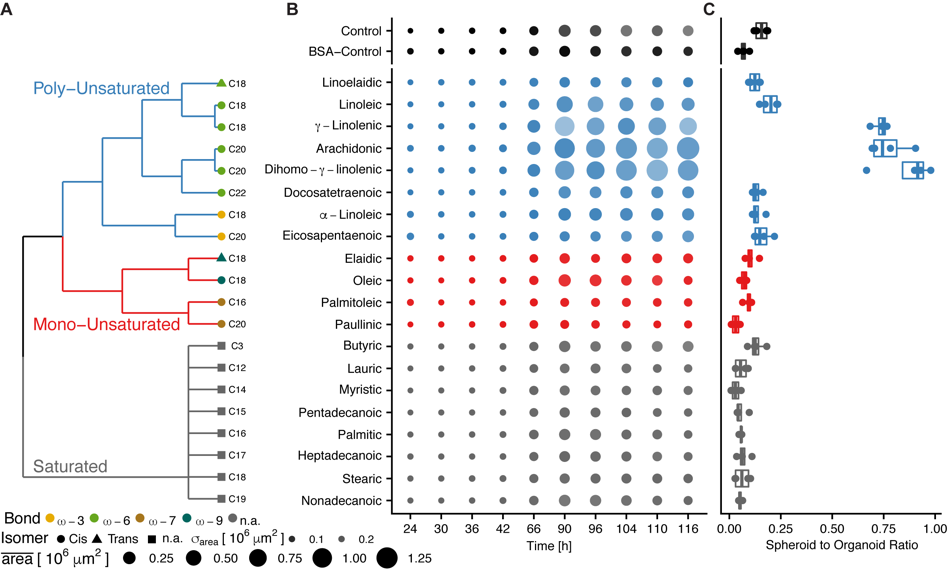
**Experience with the ENCODE- GTEx and ENTEx personalized functional genomics resource.**

ENTEx is a joint effort between ENCODE and GTEx consortia towards the goal of comparing the functional genomics data from a variety of assays between different sequenced individuals and tissues (14). The ENTEx working group has been comprehensively characterizing the personal genomes, chromatin, and transcriptome of 20-25 tissues from four individuals. Collectively, ENTEx has generated more than ~1,500 different functional genome experiments. Namely, we performed RNA-seq (long and short RNAs and RAMPAGE), chromatin characterization assays such as ATAC-seq, DNase, histone modification ChIP-Seq (H3K27ac, H3K27me3, H3K36me3, H3K4me1, H3K4me3, H3K9me3) and other marks (RNA POLII, EP300, CTCF). Tissues were also characterized in regard to their DNA methylation (WGBS and chip-array) and 3D structure (Hi-C). The data analysis committee (DAC) of which several of us were members carried out analyses of these individual data types individually and integrated fashion leading to a better understanding how these functional elements operate in and interdependent fashion.

**Experience with personal genomes and analyses of allele-specific expression and binding**

We have developed a computational tool, AlleleSeq, for the construction of personal genomes (14). The tool integrates an individual’s genomic variation data (SNVs, indels, and SVs) into the reference genome. Phase information of heterozygous variants is also incorporated, producing maternal and paternal haplotypes. Chain files generated by the program can be used to account for coordinate offsets between the individual’s parental haplotypes and the original reference genomic sequence. We have previously constructed the personal diploid genome, splice-junction libraries and personalized gene annotations for NA12878 (available as a resource at alleleseq.gersteinlab.org). Furthermore, we have implemented this on a larger scale in a recent publication (15) where we built 382 personal genomes using the variant call sets from the 1000 Genomes Project.

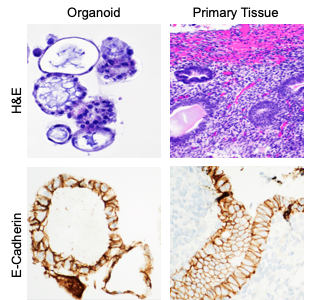


**Figure 1.** Fatty acid screen in human organoids. **A.** Fatty acids used in screen. **B.** Organoid size over time across treatments.

We have extensive experience with analyses of allele-specific expression and binding and developed a pipeline, AlleleSeq] (14), to measure and detect allele-specific events. We have spearheaded allele-specific analyses in several major consortia publications, including ENCODE and the 1000 Genomes Project (16-18). We have annotated variants associated with allele-specific expression and binding in a large pool of individuals from the 1000 Genomes Project. These results were made available as an online resource, AlleleDB (alleledb.gersteinlab.org) (15). Most recently, we constructed a high-resolution map of allelic imbalances in DNA methylation, histone marks, and transcription in 71 epigenomes from 36 distinct cell and tissue types from 13 donors (19).

**Experience with analyses of organoids and immune cells to study causal mechanisms of gene -environment interactions.**

Identifying causal mechanisms of environment – gene interactions in complex traits such as obesity has been challenging. We recently reported that a pro-obesity high fat diet increases cancer incidence in the intestine, in part through enhancing intestinal stem cell and progenitor cell function via activation of the “lipid-sensing” transcription factor PPAR-d, pushing the stem cells into a premalignant statewith increased vulnerability to undergo oncogenic transformation (14). PPAR-d is a ligand-inducible transcription factor and it is activated in response to several free fatty acids and their metabolites (15). Because a hallmark of obesity is dyslipidemia and increased free fatty acids in circulation, we hypothesized that PPAR-d is one of the mediators of increased colorectal cancer risk in obesity (16). We demonstrated that fatty acid treatment of organoids leads to activation of PPAR-d and recapitulate the stem cell enhancing effects of a pro-obesity HFD. Several PPAR-d polymorphisms are identified in colorectal cancer that may have functional significance (17). Although these variants may have clinical importance, the effect of PPAR-d or other genetic variations on gene expression and functional outcomes in obesity or in response to fatty acids is still poorly understood.



**Figure 2.** Characterization of human endometrial organoids and primary tissue using Hematoxylin&Eosin (H&E) and E-Cadherin staining.

We have significant expertise in studying causal mechanisms of how obesity impacts cellular and molecular networks in different tissues (14, 18-20). We pioneered organoid-based assays for assessing mechanisms of intestinal tumorigenesis (16, 21). Recently, we developed human patient-derived endometrial organoid models to study how obesity perturbs endometrial epithelium. (Fig 2) To explore how diverse fatty acids and metabolites influence epithelial biology in an unbiased manner, we have developed *ex vivo* fatty acid screening assay in human endometrial organoids. We have also established panel fatty acids that are stratified by length (short-chain, medium-chain, long-chain and very long chain fatty acids) and by saturation (polyunsaturated, monounsaturated and saturated). The phenotypic screens involve assessment the effect of fatty acids on organoid numbers, size and shape (i.e. cystic or branched) using microscopy.(Fig. 1) Our preliminary phenotypic screen in organoids revealed that diverse fatty acids have distinct effects on organoid growth and morphology in acute treatment for 3 days using 50 μM of fatty acids. We found that omega-6 fatty acid treatment led to increased organoid growth. We have expertise to interrogate molecular and cellular alterations that underlie these phenotypic effects by performing transcriptional, epigenetic and biochemical profiling experiments. Similarly, we have expertise in studying mechanisms of gene regulation in immune cells at steady state or in response to metabolic stress (18, 20, 22, 23).

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Go to page A

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