

TITLE

USE OF CIRCULATING CELL-FREE METHYLATED DNA TO DETECT TISSUE DAMAGE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/224,873, filed on July 23, 2022, and U.S. Provisional Application No. 63/324,112, filed on March 27, 2022, each of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0002] This invention was made with government support under grant numbers T32 CA009686, F30 CA250307, and R01 CA231291 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

[0003] The human body is frequently exposed to agents that can have a damaging effect on tissue. Such agents may be, for instance, pathogenic such as bacteria or viruses; environmental such as sunlight; or therapeutic, such as pharmaceuticals that are associated with side effects.

[0004] Another type of therapy that can potentially lead to tissue damage are those used to treat cancer, including surgery, chemotherapy, radiotherapy, targeted therapy, and immunotherapy. Each of these interventions can have a significant systemic effect. For example, radiation therapy uses ionizing radiation to target tumor cells (Hausmann *et al.*, 2020; Xu *et al.*, 2008), but normal tissues are also impacted, leading to tissue damage and remodeling (Ruysscher *et al.*, 2019; Hubenak *et al.*, 2014). For breast cancer patients, the heart and lungs are the most common organs impacted by radiation toxicities and a linear increase in cardiovascular disease risk of 7.4% per gray mean dose to the heart was reported (Darby *et al.*, 2013; White and Joiner, 2006). In addition, radiation-induced lung injury is a severe complication reported in 5-20% of cases, presenting as radiation pneumonitis or fibrosis (Giuramno *et al.*, 2019; Arroyo-Hernández *et al.*, 2021).

[0005] The ability to distinguish different cell types participating and potentially contributing to toxicities with cell-free DNA (cfDNA) in serially drawn blood samples could significantly impact on therapeutic decision making. Although imaging modalities can be used as an indirect way to gage therapeutic efficacy, these results are often unreliable and difficult to interpret. Imaging results can be clouded by depictions of pseudoprogression making them ineffective or crude instruments to monitor for concurrent changes necessary to guide therapy decisions. In light of the risk of tissue damage from radiation therapy or from exposure to other toxic agents, a means to effectively evaluate the tissue damage and monitor the effects of therapies is essential.

SUMMARY OF INVENTION

[0006] Some of the main aspects of the present invention are summarized below. Additional aspects are described in the Detailed Description of the Invention, Examples, Drawings, and Claims sections of this disclosure. The description in each section of this disclosure is intended to be read in conjunction with the other sections. Furthermore, the various embodiments described in each section of this disclosure can be combined in various different ways, and all such combinations are intended to fall within the scope of the present invention.

[0007] The invention provides novel methods for detecting tissue damage from exposure to toxic agents.

[0008] In one aspect, the present invention relates to a method of determining if a subject has suffered tissue damage from exposure to a toxic agent. In some embodiments, the method comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the subject has suffered or suffers tissue damage from the exposure. In other embodiments, the method

comprises, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the subject has suffered or suffers tissue damage from the exposure.

[0009] In another aspect, the present invention also relates to a method of treating a subject who has suffered tissue damage from exposure to a toxic agent. In some embodiments, the method comprises administering a treatment for the tissue damage to the subject, in which the subject is determined to have suffered from tissue damage by a method comprising: (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the subject has suffered tissue damage. In other embodiments, the method comprises administering a treatment for the tissue damage to the subject, in which the subject is determined to have suffered from tissue damage by a method comprising, at two or more time points: (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the subject has suffered tissue damage.

[0010] In yet another aspect, the present invention further relates to a method of treating tissue damage in a subject. In some embodiments, the method comprising administering a treatment for the tissue damage to the subject and monitoring the tissue damage, in which the monitoring comprises: (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. A decrease in the measured quantity of the cfDNA of the determined cellular origin as compared to the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is effective, and an increase or no change in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is not effective. In other embodiments, the method comprises administering a treatment for the tissue damage to the subject and monitoring the tissue damage, in which the monitoring comprises, at two or more time points: (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and (c) measuring the quantity of the cfDNA of the determined cellular origin. A decrease in the measured quantity of the cfDNA of the determined cellular origin at later time point as compared to an earlier time point is indicative that the treatment is effective, and an increase or no change in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the treatment is not effective.

[0011] In some embodiments, the tissue damage is caused by exposure to a toxic agent. In certain embodiments, toxic agent comprises radiation.

[0012] The radiation may be for therapeutic purposes, accidental, or environmental. In some embodiments, the radiation comprises a radioactive substance. The radioactive substance

may be ingested by the subject, inhaled by the subject, or absorbed through body surface contamination by the subject.

[0013] In other embodiments, the toxic agent comprises a microorganism. The microorganism may comprise a pathogen, such as a bacterium or virus.

[0014] In some embodiments, the toxic agent is from a synthetic chemical source or from a biological source.

[0015] In some embodiments, the toxic agent comprises a pharmaceutical therapy.

[0016] In some embodiments, the toxic agent comprises a chemical or biological or radioactive substance used as a weapon.

[0017] In a further aspect, the present invention relates to method of treating a subject in need thereof. In some embodiments, the method comprises administering a treatment to the subject and monitoring whether the treatment causes tissue damage in the subject, in which the monitoring comprises: (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is causing tissue damage. In other embodiments, the method comprises administering a treatment to the subject and monitoring whether the treatment causes tissue damage in the subject, in which the monitoring comprises, at two or more time points: (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and (c) measuring the quantity of the cfDNA of the determined cellular origin, in which an increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the treatment is causing tissue damage.

[0018] In some embodiments, the methods further comprise adjusting the treatment administered to the subject when the treatment is indicated to be not effective or causing tissue damage.

[0019] In some embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who were not exposed to the toxic agent, or who were not administered the treatment.

[0020] In yet other aspects, the present invention relates to a method of treating a subject having a tumor. In some embodiments, the method comprises (A) monitoring a response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises: (i) determining whether there is an adverse reaction to the first treatment, comprising (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin, in which an increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative of an adverse reaction; (ii) determining whether there is a response to the first treatment, comprising: (a) sequencing circulating tumor DNA (ctDNA) in a biospecimen from the subject, determining clonal heterogeneity of cells of the tumor by genotyping the ctDNA, in which the presence of more than one clone of the tumor cells or the presence of a tumor cell clone that has not been previously identified in the subject is indicative of an ineffective response to the first treatment; and (B) either administering the same treatment as the first treatment when it is determined that there is no adverse reaction, that there is not an ineffective response, or a combination thereof, or administering an adjusted treatment when it is determined that there is an adverse reaction, that there is an ineffective response, or a combination thereof. In other embodiments, the method comprises (A) monitoring a response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises, at two or more time points, (i) determining whether there is an adverse reaction to the first treatment, comprising (a) sequencing cfDNA

in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and (c) measuring the quantity of the cfDNA of the determined cellular origin, in which an increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative of an adverse reaction; and (ii) determining whether there is a response to the first treatment, comprising (a) sequencing circulating tumor (ctDNA) in a biospecimen from the subject, (b) determining clonal heterogeneity of cells of the tumor by genotyping the ctDNA, in which the presence of more than one clone of the tumor cells or the presence of a tumor cell clone in a subsequent time point that has not been identified at a previous time point is indicative of an ineffective response to the first treatment; and (B) either administering the same treatment as the first treatment when it is determined that there is no adverse reaction, that there is not an ineffective response, or a combination thereof; or administering an adjusted treatment when it is determined that there is an adverse reaction, that there is an ineffective response, or a combination thereof.

[0021] In some embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who do not have a tumor. In other embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who did not receive the first treatment.

[0022] In some embodiments, the biospecimen comprises a biological fluid. In certain embodiments, the biological fluid is selected from blood, serum, plasma, cerebrospinal fluid, saliva, urine, and sputum. In preferred embodiments, the biological fluid comprises blood, serum, or plasma.

[0023] In some embodiments, the methylation pattern comprises a segment of nucleotide sequence containing at least 3 CpG dinucleotides.

[0024] In some embodiments, the known methylation patterns are set forth in Table 2.

BRIEF DESCRIPTION OF THE DRAWING FIGURES

[0025] **FIG. 1** illustrates an example of the use of predicting treatment response and therapy-related toxicities from combined genetic and epigenetic analyses of cfDNA. Predicting treatment response and therapy-related toxicities from combined genetic and epigenetic analyses of cfDNA. The minimally invasive nature of liquid biopsies allows for serial sampling to monitor changes over time, especially under selective pressures from ongoing therapy. Circulating tumor DNA (ctDNA) can be used to track clonal heterogeneity over time to assess treatment response and detect treatment-resistant clones. Normal cell-specific cfDNA methylation patterns can be used in combination with ctDNA to assess the impact of treatment to the surrounding tumor microenvironment and to monitor for therapy-related toxicities in somatic cell-types. [ctDNA = circulating tumor DNA; cme-DNA = circulating methylated cell-free DNA].

[0026] **FIG. 2** shows the overall analysis of cell-free methylated DNA in blood to identify origins of radiation-induced cellular damage, as described in the Example. Serial serum samples were collected from human breast cancer patients treated with radiation. In parallel, paired serum and tissue samples were collected from mice receiving radiation at 3Gy or 8Gy doses compared to sham control. Methylome profiling of liquid biopsy samples was performed using a bisulfite-based capture-sequencing methodology optimized for cfDNA inputs. Differential cell type-specific methylation blocks were identified from reference WGBS data compiled from healthy cell-types and tissues in human and mouse. Methylation atlases were generated emphasizing cell-types composing target organs-at-risk from radiation, including the lungs, heart, and liver. Deconvolution analysis of cfDNA using fragment-level CpG methylation patterns at these identified cell-type specific blocks was used to decode the origins of radiation-induced cellular injury.

[0027] **FIG. 3** shows sensitivity and specificity of identified mouse cell-type specific differentially methylated blocks, as described in the Example. In **Panels A-D**, the top images are a heatmap of all cell type-specific methylation blocks selected for each target cell-type. All blocks contain 3+CpG sites and have a margin of beta difference greater than or equal to 0.4 separating the target cell-type from all others included in the reference maps. All identified methylation blocks for lung endothelial (n=1,546), hepatocyte (n=616), and cardiomyocyte (n=2,917) mouse cell-types were hypomethylated. In contrast, all identified

immune cell-specific blocks (n=148) were hypermethylated relative to other solid organ cell-types in mouse. In **Panels A-D**, the right images show in-silico mix-in validation of fragment-level probabilistic deconvolution model. Target cell-type read-pairs were in-silico mixed into a background of lymphocyte or buffy coat read-pairs at various known percentages (0, 0.5, 1, 2, 5, 10, 15%) with 10 replicates per proportion. The deconvolution model was validated on these in-silico mixed samples of known cell-type proportions at the blocks selected. The average predicted %target is graphed relative to the known %mixed to assess sensitivity and specificity of the identified cell type-specific blocks and deconvolution model. Data presented as mean $\pm$ SD; n = 3 replicates per proportion. Reference WGBS samples with less than 3 replicates were split into “0.8 train” to select methylation blocks and “0.2 test” to generate in-silico mixed samples. When available, in-silico mixed samples of the same cell-type derived from different aged mice were tested. In addition, bulk tissue of the respective cell-type was tested as well.

[0028] **FIG. 4** shows sensitivity and specificity of identified human cell-type specific differentially methylated blocks, as described in the Example. In **Panels A-F**, the top images are heatmaps of all cell type-specific methylation blocks selected for each target cell-type. All blocks contain 3+CpG sites and have a margin of beta difference greater than or equal to 0.4 separating the target cell-type from all others included in the reference maps. In **Panels A-F**, the bottom images show in-silico mix-in validation of fragment-level probabilistic deconvolution model. Target cell-type read-pairs were in-silico mixed into a background of lymphocyte or buffy coat read-pairs at various known percentages (0, 0.5, 1, 2, 5, 10, 15%). The deconvolution model was validated on these in-silico mixed samples of known cell-type proportions at the blocks selected. The average predicted %target is graphed relative to the known %mixed to assess sensitivity and specificity of the identified cell type-specific blocks and deconvolution model. Data is presented as mean $\pm$ standard deviation; n = 3 replicates per proportion.

[0029] **FIG. 5** shows characterization of human and mouse cell-type specific reference methylation data, as described in the Example. **Panel A** shows a tree dendrogram depicting relationship between human reference Whole Genome Bisulfite Sequencing (WGBS) datasets included in the analysis. Methylation status at the top 30,000 variable blocks was used as input data for the unsupervised hierarchical clustering. Samples from cell-types with greater than n = 3 replicates were merged. **Panel B** shows UMAP projection of human

WGBS reference datasets, colored by tissue and cell-type. **Panel C** shows UMAP projection of mouse WGBS reference datasets. [Acronyms: HUVEV = human umbilical vein endothelial cell, PAEC = pulmonary artery endothelial cell, CAEC = coronary artery endothelial cell, PMEC = pulmonary microvascular endothelial cell, CMEC = cardiac microvascular endothelial cell, CPEC = joint cardio-pulmonary endothelial cell, LSEC = liver sinusoidal endothelial cell, NK = natural killer cell, MK = megakaryocyte.]

[0030] **FIG. 6** shows characterization of mouse cell-type specific reference methylation data, as described in the Example. **Panel A** shows a tree dendrogram depicting relationship between mouse reference WGBS datasets included in the analysis. Methylation status at the top 30,000 variable blocks was used as input data for the unsupervised hierarchical clustering. **Panel B** shows heatmaps of differentially methylated cell type-specific blocks identified from reference WGBS data compiled from healthy cell-types and tissues in mouse. Each cell in the plot marks the average methylation of one genomic region (row) at each of the 9 mouse tissues and cell-types (columns). Up to 100 blocks with the highest methylation score are shown per cell type. Differential blocks identified from cell-types comprising the target organs-at-risk from radiation (lungs, heart, and liver) were selected for generation of a radiation-specific methylation atlas, separating these solid organ cell-types from all other immune cell-types.

[0031] **FIG. 7** shows identification and biological validation of cell-type specific DNA methylation blocks in human and mouse, as described in the Example. **Panels A and B** show heatmaps of differentially methylated cell type-specific blocks identified from reference WGBS data compiled from healthy cell-types and tissues in human (**Panel A**) and mouse (**Panel B**). Each cell in the plot marks the methylation score of one genomic region (rows) at each of the 20 cell types in human and 9 in mouse (columns). Up to 100 blocks with the highest methylation score are shown per cell type. The methylation score represents the number of fully unmethylated read-pairs / total coverage or fully methylated read-pairs / total coverage for hypo- and hyper- methylated blocks, respectively. **Panel C** shows heatmap of distance scores between gene-set pathways identified from GeneSetCluster. Genes adjacent to human cell type-specific methylation blocks were identified using HOMER and pathway analysis was performed using both Ingenuity Pathway Analysis (IPA) and GREAT. Significantly enriched gene-set pathways ($p < 0.05$) from differentially methylated blocks identified in immune, cardiomyocyte, hepatocyte, and lung epithelial cell-types were

analyzed using GeneSetCluster. Cluster analysis was performed to determine the distance between all identified gene-set pathways based on the degree of overlapping genes from each individual gene-set compared to all others. Over-representation analysis was implemented in the WebgestaltR (ORAperGeneSet) plugin to interpret and functionally label identified gene-set clusters. [Acronyms: HUVEV = human umbilical vein endothelial cell, CPEC = cardio-pulmonary endothelial cell, LSEC = liver sinusoidal endothelial cell, NK = natural killer cell.]

[0032] **FIG. 8** shows biological function of mouse cell-type specific methylation blocks, as described in the Example. Heatmap of distance scores between gene-set pathways identified from GeneSetCluster. Genes adjacent to cell type-specific methylation blocks were identified using HOMER and pathway analysis was performed using both Ingenuity Pathway Analysis (IPA) and GREAT. Significantly enriched gene-set pathways ($p < 0.05$) from differentially methylated blocks identified in immune, cardiomyocyte, hepatocyte, and lung endothelial cell-types were analyzed using GeneSetCluster. Cluster analysis was performed to determine the distance between all identified gene-set pathways based on the degree of overlapping genes from each individual gene-set compared to all others. Over-representation analysis was implemented in the WebgestaltR (ORAperGeneSet) plugin to interpret and functionally label identified gene-set clusters.

[0033] **FIG. 9** shows cell type-specific DNA methylation is mostly hypomethylated and enriched at intragenic regions and developmental transcription factor (TF) binding motifs, as described in the Example. **Panel A** shows a schematic diagram depicting location of human cell-type specific hypo- and hyper- methylated blocks. Genomic annotations of cell type-specific methylation blocks were determined by analysis using HOMER. **Panels B and C** show distribution of human (**Panel B**) and mouse (**Panel C**) cell-type specific methylation blocks relative to genomic regions used in the hybridization capture probes. Captured blocks with less than 5% variance across cell types represent blocks without cell type specificity and were used as background. **Panel D** shows top 5 TF binding sites enriched among identified cell-type specific hypo- and hypermethylated blocks in human (top) and mouse (bottom), using HOMER motif analysis. The same captured blocks with less than 5% variance amongst cell-types were used as background.

[0034] **FIG. 10** shows methylation profiling of human endothelial cell-types reveals tissue-specific differences that correspond with changes in RNA expression levels and biological

functions, as described in the Example. **Panel A** shows pathways supporting the biological significance of endothelial-specific methylation blocks (all $p < 0.05$). **Panel B** shows significant functions of genes adjacent to endothelial-specific methylation blocks. Asterisked genes have nearby hypermethylated regulatory blocks. Non-asterisked genes have nearby hypomethylated regulatory blocks. **Panel C** shows gene expression at genes adjacent to tissue-specific endothelial-specific methylation blocks. Expression data was generated from paired RNA-sequencing of the same cardiopulmonary endothelial cells (CPEC) and liver sinusoidal endothelial cells (LSEC) used to generate methylation reference data. Pan-endothelial genes upregulated in both populations (ALL) are identified as common endothelial-specific methylation blocks to both LSEC and CPEC populations. **Panel D** shows top 5 transcription factor binding sites enriched among identified endothelial-specific hypomethylated blocks, using HOMER de novo and known motif analysis. The background for HOMER analysis was composed of the other 3,574 identified cell-type specific hypomethylated blocks in all cell-types besides endothelial. **Panel E** shows an example of the NOS3 locus specifically unmethylated in endothelial cells. This endothelial-specific, differentially methylated block (DMB) is 157bp long (7 CpGs), and is located within the NOS3 gene, an endothelial-specific gene (upregulated in paired RNA-sequencing data as well as in vascular endothelial cells, GTEx inset). The alignment from the UCSC genome browser (top) provides the genomic locus organization and is aligned with the average methylation across cardiomyocyte, lung epithelial, liver sinusoidal endothelial (LSEC), cardiopulmonary endothelial (CPEC), hepatocyte, and immune (PBMC) samples ($n=3$ / cell-type group). Results from RNA-sequencing generated from paired cell-types are depicted as well as peak intensity from H3K27ac and H3K4me3 published ChIP-seq data generated in endothelial cells [Acronyms: HUVEV = human umbilical vein endothelial cell, CPEC = cardiopulmonary endothelial cell, LSEC = liver sinusoidal endothelial cell.]

[0035] **FIG. 11** shows development of radiation-specific methylation atlas focusing on cell-types from target organs-at-risk (OAR), as described in the Example. **Panel A** shows representative three-dimensional conformal radiation therapy (3D-CRT) treatment planning for right-sided (i and ii) and left-sided (iii and iv) breast cancer patients, respectively. Computed tomography simulation coronal and sagittal images depicting anatomic position of target volume in relation to nearby organs. The map represents different radiation dose levels or isodose lines (95% of prescription dose, 90% isodose line, 80% isodose line, 70% isodose line, 50% isodose line). **Panel B** shows heatmaps of differentially methylated cell type-

specific blocks identified from all reference WGBS data compiled from healthy human cell-types and tissues. Each cell in the plot marks the average methylation of one genomic region (rows) at each of the 20 human cell-types (columns). Up to 100 blocks with the highest methylation score are shown per cell type. Differential blocks identified from cell-types comprising the target organs-at-risk from radiation (lungs, heart, and liver) were selected for generation of a radiation-specific methylation atlas, separating these solid organ cell-types from all other immune cell-types. [Acronyms: HUVEV = human umbilical vein endothelial cell, PAEC = pulmonary artery endothelial cell, CAEC = coronary artery endothelial cell, PMEC = pulmonary microvascular endothelial cell, CMEC = cardiac microvascular endothelial cell, CPEC = joint cardio-pulmonary endothelial cell, LSEC = liver sinusoidal endothelial cell, NK = natural killer cell, MK = megakaryocyte.]

[0036] FIG. 12 shows that dose-dependent radiation damage in mouse tissues correlates with origins of methylated cfDNA in the circulation, as described in the Example. **Panel A** shows representative hematoxylin and eosin (H&E) staining of mouse lung, heart, and liver tissues treated with 3Gy and 8Gy radiation compared to sham control. Scale bar, 200 μ m. **Panel B** shows quantitative polymerase chain reaction (qPCR) analysis of CDKN1A (p21) marker of apoptosis in mouse tissues treated with 3Gy and 8Gy radiation compared to sham control. The expression of each sample was normalized with expression of house-keeping genes ACTB (actin) and is shown relative to the expression in the sham control. Data presented as mean $\pm$ SD (n = 3). Kruskal-Wallis test was used for comparisons amongst groups; lung tissue p = 0.004, heart tissue p = 0.025, liver tissue p = 0.004. **Panels C-F** show lung endothelial, cardiomyocyte and hepatocyte methylated cfDNA in the circulation of mice treated with 3Gy and 8Gy radiation compared to sham control expressed in Genome Equivalents (Geq). CfDNA was extracted from 18 mice (n = 6 in each group) with cfDNA from 2 mice pooled in each methylome preparation. Mean $\pm$ SD; n = 3 independent methylome preparations. Kruskal-Wallis test was used for comparisons amongst groups. ns, P $\geq$ 0.05; *, P < 0.05; lung endothelial p = 0.01, cardiomyocyte p = 0.01, hepatocyte p = 0.13.

[0037] FIG. 13 shows apoptotic damage from radiation in mouse tissues, as described in the Example. qPCR analysis of markers of apoptosis (Trp53, Gadd45a, Aifm3, and Bad) in mouse lung, heart, and liver tissues treated with 3Gy and 8Gy radiation compared to sham

control. The expression of each sample was normalized with expression of house-keeping genes ACTB (actin). Data presented as mean $\pm$ SD (n = 3).

[0038] FIG. 14 shows radiation-induced effects on immune and solid organ cfDNA, as described in the Example. **Panels A-C** show the radiation-induced effects in human, and **Panels D and E** show the radiation-induced effects in mouse. **Panel A** shows predicted human immune-derived cfDNA in Geq. Human Geq are calculated by multiplying the relative fraction of cell-type specific cfDNA x initial concentration cfDNA ng/mL x the weight of the haploid human genome. Immune cfDNA was assessed at n = 222 methylation blocks found to separate immune cell types from solid organ cell-types. (g1 = Bcell, CD4Tcell, CD8Tcell, NK, MK, erythroblast, monocyte, macrophage, neutrophil; g2 = breast basal/luminal epi, lung epi, hepatocyte, kidney podocyte, pancreas islet, colon epi, cardiomyocyte, LSEC, CPEC, HUVEC, neuron, and skeletal muscle). **Panel B** shows predicted human solid organ-derived cfDNA in Geq where %solid organ is defined as 100-%immune using these same n=222 methylation blocks. **Panel C** shows fold change in human immune versus solid organ Geq at EOT and recovery relative to baseline. Data presented as mean $\pm$ SD; n = 15. For **Panels A and B**, Friedman test was performed for comparisons amongst groups. ns, P > 0.05; *, P < 0.05; immune p = 0.07, solid organ p = 0.008. **Panel D** shows predicted mouse immune-derived cfDNA in Geq. Mouse Geq are calculated by multiplying the relative fraction of cell-type specific cfDNA x initial concentration cfDNA ng/mL x the weight of the haploid mouse genome. Immune cfDNA was assessed at n = 148 methylation blocks found to separate immune cell types from solid organ cell-types. (g1 = Bcell, CD4Tcell, CD8Tcell, neutrophil; g2 = mammary epi, cardiomyocyte, hepatocyte, lung endothelial, cerebellum, hypothalamus, colon, intestine, kidney). **Panel E** shows predicted mouse solid organ-derived cfDNA in Geq. For **Panels D and E**, mean $\pm$ SD; n = 3 independent methylome preparations. Kruskal-Wallis test was used for comparisons amongst groups. ns, P > 0.05; *, P < 0.05; immune p = 0.20, solid organ p = 0.01.

[0039] FIG. 15 shows radiation-induced hepatocyte and liver endothelial cfDNAs in patient with right- versus left- sided breast cancer, as described in the Example. **Panels A and B** show hepatocyte cfDNA (in Geq/mL) in serum samples collected at different times. Fragment-level deconvolution using hepatocyte specific methylation blocks (n=200). Wilcoxon matched pairs signed rank test was used for comparison amongst groups and

results were considered significant when $*P < 0.05$; ns, $P \geq 0.05$; right-sided $p = 0.02$, left-sided $p = 0.81$. **Panel C** shows fold change in hepatocyte cfDNA after treatment (EOT) and at recovery relative to baseline. Mean $\pm$ SD; $n = 8$ right-sided, $n = 7$ left-sided. **Panels D and E** show LSEC cfDNA (in Geq/mL) in the same serum samples. Fragment-level deconvolution used LSEC specific methylation blocks ($n=89$). Wilcoxon matched pairs signed rank test was performed between groups and results were considered significant when $*P < 0.05$; ns, $P \geq 0.05$; right-sided $p = 0.02$, left-sided $p = 0.93$. **Panel F** shows fold change in LSEC cfDNA Geq at EOT and recovery relative to baseline levels. Mean $\pm$ SD; $n = 8$ right-sided, $n = 7$ left-sided.

[0040] **FIG. 16** shows that radiation-induced cardiopulmonary cfDNAs in patients correlates with the radiation dose and indicates sustained injury to cardiomyocytes, as described in the Example. **Panel A** shows lung epithelial cfDNA (in Geq/mL) in serum samples collected at different times. Fragment-level deconvolution used lung epithelial specific methylation blocks ($n = 69$). **Panel B** shows correlation of lung epithelial cfDNA with dosimetry data. EOT/Baseline represents the fraction of lung epithelial cfDNA post-radiation at end-of-treatment (EOT) relative to baseline levels. The volume of the lung receiving 20 Gy dose is represented by Lung V20 (%) and the mean dose to the total body represented by total body mean (Gy). **Panel C** shows fold change in lung epithelial cfDNA at EOT and recovery relative to baseline. **Panel D** shows CPEC cfDNA (in Geq/mL). Fragment-level deconvolution used CPEC-specific methylation blocks ($n = 132$). **Panel E** shows correlation of CPEC cfDNA with dosimetry data. The volume of the lung receiving 5 Gy dose is represented by Lung V5 (%). **Panel F** shows fold change in CPEC cfDNA at EOT and recovery relative to baseline levels. **Panel G** shows cardiomyocyte cfDNA (in Geq/mL). Fragment-level deconvolution used cardiomyocyte-specific methylation blocks ($n = 375$). **Panel H** shows correlation of cardiomyocyte cfDNA with the maximal heart dose (Gy). **Panel I** shows fold change in cardiomyocyte cfDNA at EOT and recovery relative to baseline. For **Panels A, D, and G**, Friedman test was performed comparing paired results at baseline, EOT, and recovery timepoints. The results were considered significant when $*P < 0.05$; ns, $P \geq 0.05$; lung epithelial $p = 0.98$, cardiopulmonary endothelial $p = 0.02$, cardiomyocyte $p = 0.03$. For **Panels B, E, and H**, Pearson correlation r was calculated, and linear correlation was considered significant when $*P < 0.05$. For **Panels C, F, and I**, Wilcoxon matched-pairs signed rank test was performed between groups and results were considered significant when $*P < 0.05$. Data is presented as mean $\pm$ SD; $n = 15$.

DETAILED DESCRIPTION OF THE INVENTION

[0041] The practice of the present invention can employ, unless otherwise indicated, conventional techniques of genetics, molecular biology, computational biology, genomics, epigenomics, mass spectrometry, and bioinformatics, which are within the skill of the art.

[0042] In order that the present invention can be more readily understood, certain terms are first defined. Additional definitions are set forth throughout the disclosure. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention is related.

[0043] Any headings provided herein are not limitations of the various aspects or embodiments of the invention, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0044] All references cited in this disclosure are hereby incorporated by reference in their entireties. In addition, any manufacturers' instructions or catalogues for any products cited or mentioned herein are incorporated by reference. Documents incorporated by reference into this text, or any teachings therein, can be used in the practice of the present invention. Documents incorporated by reference into this text are not admitted to be prior art.

Definitions

[0045] The phraseology or terminology in this disclosure is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0046] As used in this specification and the appended claims, the singular forms "a," "an," and "the" include plural referents, unless the context clearly dictates otherwise. The terms "a" (or "an") as well as the terms "one or more" and "at least one" can be used interchangeably.

[0047] Furthermore, "and/or" is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" is intended to include A and B, A or B, A (alone), and B (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to

include A, B, and C; A, B, or C; A or B; A or C; B or C; A and B; A and C; B and C; A (alone); B (alone); and C (alone).

[0048] Wherever embodiments are described with the language “comprising,” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are included.

[0049] Units, prefixes, and symbols are denoted in their Système International d’Unités (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range, and any individual value provided herein can serve as an endpoint for a range that includes other individual values provided herein. For example, a set of values such as 1, 2, 3, 8, 9, and 10 is also a disclosure of a range of numbers from 1-10, from 1-8, from 3-9, and so forth. Likewise, a disclosed range is a disclosure of each individual value (i.e., intermediate) encompassed by the range, including integers and fractions. For example, a stated range of 5-10 is also a disclosure of 5, 6, 7, 8, 9, and 10 individually, and of 5.2, 7.5, 8.7, and so forth.

[0050] Unless otherwise indicated, the terms “at least” or “about” preceding a series of elements is to be understood to refer to every element in the series. The term “about” preceding a numerical value includes $\pm 10\%$ of the recited value. For example, a concentration of about 1 mg/mL includes 0.9 mg/mL to 1.1 mg/mL. Likewise, a concentration range of about 1% to 10% (w/v) includes 0.9% (w/v) to 11% (w/v).

[0051] As used herein, the terms “cell-free DNA” or “cfDNA” or “circulating cell-free DNA” refers to DNA that is circulating in the peripheral blood of a subject. The DNA molecules in cfDNA may have a median size that is no greater than 1 kb (for example, about 50 bp to 500 bp, or about 80 bp to 400 bp, or about 100 bp to 1 kb), although fragments having a median size outside of this range may be present. This term is intended to encompass free DNA molecules that are circulating in the bloodstream as well as DNA molecules that are present in extra-cellular vesicles (such as exosomes) that are circulating in the bloodstream.

[0052] “Methylation site” refers to a CpG dinucleotide.

[0053] “Methylation pattern” refers to the pattern generated by the presence of methylated CpGs or non-methylated CpGs in a segment of DNA. For example, in a segment of DNA containing three CpGs, one methylation pattern is all three CpGs being methylated; a different methylation pattern is all three CpGs not being methylated; another methylation

pattern is only the first CpG being methylated; yet another methylation pattern is only the second CpG being methylated; yet a different methylation pattern is the first and second CpG being methylated, etc.

[0054] “Methylation status” refers to whether a CpG dinucleotide is methylated or not methylated.

[0055] As used herein, “hypermethylated” refers to the presence of methylated CpGs. For example, a hypermethylated genomic region means that each CpG in the genomic region is methylated.

[0056] As used herein, “hypomethylated” refers to the presence of CpGs that are not methylated. For example, a hypomethylated genomic region means that each CpG in the genomic region is not methylated.

[0057] The term “sequencing” as used herein refers to a method by which the identity of at least 10 consecutive nucleotides for example, the identity of at least 20, at least 50, at least 100 or at least 200 or more consecutive nucleotides) of a polynucleotide is obtained.

[0058] The term “next-generation sequencing” as used herein refers to the parallelized sequencing-by-synthesis or sequencing-by-ligation platforms currently employed by Illumina, Life Technologies, and Roche, etc. Next-generation sequencing methods may also include nanopore sequencing methods such as that commercialized by Oxford Nanopore Technologies, electronic-detection based methods such as Ion Torrent technology commercialized by Life Technologies, or single-molecule fluorescence-based methods such as that commercialized by Pacific Biosciences.

[0059] A “subject” or “individual” or “patient” is any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, sports animals, and laboratory animals including, e.g., humans, non-human primates, canines, felines, porcines, bovines, equines, rodents, including rats and mice, rabbits, etc.

[0060] An “effective amount” of an active agent is an amount sufficient to carry out a specifically stated purpose.

[0061] Terms such as “treating” or “treatment” or “to treat” or “alleviating” or “to alleviate” refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt progression of a diagnosed pathologic condition or disorder. In certain embodiments, a

subject is successfully “treated” for a disease or disorder if the patient shows total, partial, or transient alleviation or elimination of at least one symptom or measurable physical parameter associated with the disease or disorder.

Methods Using cfDNA to Determine Tissue Damage

[0062] The present invention relates to methods that utilize circulating cfDNA to determine tissue damage. The majority of cfDNA fragments peak around 167 bp, corresponding to the length of DNA wrapped around a nucleosome (147 bp) plus a linker fragment (20 bp). This nucleosomal footprint in cfDNA reflects degradation by nucleases as a by-product of cell death (Heitzer *et al.*, 2020).

[0063] DNA methylation typically involves covalent addition of a methyl group to the 5-carbon of cytosine (5mc) with the human and mouse genomes contain 28 and 13 million CpG sites respectively (Greenberg and Bourc’his, 2019; Michalak *et al.*, 2019). Stable, cell-type specific patterns of DNA methylation are conserved during DNA replication and thus provide the predominant mechanism for inherited cellular memory during cell growth (Kim & Costello, 2017; Dor & Cedar, 2018). DNA methylation changes associated with disease and physiological aging occur at locations throughout the epigenome that are distinct from regions critical to cell-type identity, making methylated cfDNA a robust cell-type specific readout across diverse patient populations (Michalak *et al.*, 2019; Dor & Cedar, 2018).

[0064] While recent studies have demonstrated the feasibility of Tissue-Of-Origin (TOO) analysis using cfDNA methylation, such studies traditionally averaged the methylation status across a population of fragments present at single CpG sites (Barefoot, *et al.*, 2021; Barefoot *et al.*, 2020). The present invention involves sequencing portions of cfDNA to identify patterns of differential methylation, and using these patterns of differential methylation to determine the cellular origin of the cfDNA.

[0065] The use of patterns of differential methylation to determine the cellular origin of cfDNA can be applied to methods of determining if a subject has suffered tissue damage from exposure to a toxic agent. In some embodiments, the methods comprise (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known

cell-type specific methylation pattern; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the subject has suffered or suffers tissue damage from the exposure.

[0066] In some embodiments, the methods of determining if a subject has suffered tissue damage from exposure to a toxic agent comprise, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the subject has suffered or suffers tissue damage from the exposure.

[0067] The use of patterns of differential methylation to determine the cellular origin of cfDNA can also be applied to methods of treating a subject who has suffered tissue damage from exposure to a toxic agent. In some embodiments, these methods comprise administering a treatment for the tissue damage to the subject, in which the subject was indicated as suffering tissue damage by a method comprising (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the subject has suffered tissue damage.

[0068] In some embodiments, the methods of treating a subject who has suffered tissue damage from exposure to a toxic agent comprise administering a treatment for the tissue damage to the subject, in which the subject was indicated as suffering tissue damage by a method comprising, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the subject has suffered tissue damage.

[0069] In other embodiments, the methods are for treating tissue damage in a subject. The methods comprise administering a treatment for tissue damage to the subject and monitoring the efficacy of the treatment. The monitoring comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. A decrease in the measured quantity of the cfDNA of the determined cellular origin as compared to the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is effective. An increase or no change in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is not effective.

[0070] In some embodiments, the methods for treating tissue damage comprise administering a treatment for tissue damage to the subject and monitoring the efficacy of the treatment. The monitoring comprises, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation

sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; and (c) measuring the quantity of the cfDNA of the determined cellular origin. A decrease in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the treatment is effective. An increase or no change in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the treatment is not effective.

[0071] In some embodiments, the methods may further comprise administering an adjusted treatment when the first treatment is determined to be not effective. In some embodiments, the tissue damage is caused by exposure to a toxic agent.

[0072] In some embodiments, the toxic agent comprises radiation. The radiation may be for therapeutic purposes, accidental, or environmental.

[0073] In some embodiments, the toxic agent is a radiation therapy. In certain embodiments, the radiation therapy comprises an external beam radiation therapy. Examples of external beam radiation therapy include, but are not limited to, conventional external beam radiation therapy, stereotactic radiation therapy, three-dimensional conformal radiation therapy, intensity-modulated radiation therapy, volumetric modulated arc therapy, temporally feathered radiation therapy, particle therapy, and auger therapy.

[0074] In certain embodiments, the radiation therapy comprises a brachytherapy, in which the radiation is in a sealed source. The brachytherapy may be an interstitial brachytherapy, in which the radiation source is placed directly in the target tissue of the affected site; or the brachytherapy may be a contact brachytherapy, in which the radiation source is placed in a space next to the target tissue, such as a body cavity (intracavitary brachytherapy), a body lumen (intraluminal brachytherapy), or externally (surface brachytherapy).

[0075] In certain embodiments, the radiation therapy comprises systemic radioisotope therapy, which delivers the radiation to a targeted site using, for instance, chemical properties of the isotope or attachment of the isotope to another molecule or antibody that guides the isotope to the targeted site.

[0076] In some embodiments, the toxic agent is accidental radiation, for example, work-related exposure to radiation.

[0077] In some embodiments, the toxic agent is environmental radiation. Environmental radiation include exposure to radiation resulting from, as non-limiting examples, high-altitude flights and space travel.

[0078] In some embodiments, the toxic agent comprises a radioactive substance ingested by the subject, inhaled by the subject, or absorbed through body surface contamination by the subject.

[0079] In some embodiments, the toxic agent comprises a microorganism. In certain embodiments, the toxic agent comprises a pathogen such as a bacterium or virus. Particular examples of pathogens include, but are not limited to, species of the following genus: *Bacillus*, *Brucella*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Escherichia*, *Klebsiella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Mycoplasma*, *Neisseria*, *Pseudomonas*, *Staphylococcus*, *Treponema*, *Vibrio*, and *Yersinia*.

[0080] In some embodiments, the toxic agent comprises a toxin from a synthetic chemical source or from a biological source.

[0081] In some embodiments, the toxic agent comprises a pharmaceutical therapy, such as a chemical used for therapeutic purposes.

[0082] In some embodiments, the toxic agent comprises a chemical or biological or radioactive substance used as a weapon, for example, in a terrorist attack or in a war.

[0083] In yet other embodiments, the methods of treating a subject comprise administering a treatment to the subject and monitoring whether the treatment causes tissue damage in the subject. The monitoring comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is causing tissue damage.

[0084] In other embodiments, methods of treating a subject comprise administering a treatment to the subject and monitoring whether the treatment causes tissue damage in the subject. The monitoring comprises, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at later time point as compared to an earlier time point is indicative that the treatment is causing tissue damage.

[0085] In some embodiments, the methods may further comprise administering an adjusted treatment when the first treatment is determined to cause tissue damage.

[0086] In some embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who were not exposed to the toxic agent. In other embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who were not administered the treatment.

[0087] Another aspect of the present invention is a method of determining organ-, tissue-, or cell-type damage induced by a substance administered to the subject. The method comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that an organ or tissue of the cell type, or the cell-type itself, has suffered damage. In some embodiments, the substance administered to the subject may be a pharmaceutical, such as an investigational new drug.

[0088] Yet another aspect of the present invention is a method of determining organ-, tissue-, or cell-type damage induced by a substance administered to the subject. The method comprises, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that an organ or tissue of the cell type, or the cell-type itself, has suffered damage. In some embodiments, the substance administered to the subject may be a pharmaceutical, such as an investigational new drug.

[0089] A further aspect of the present invention is a method of determining the organ-, tissue-, or cell-target of a substance administered to a subject. The method comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation pattern; (c) measuring the quantity of the cfDNA of the determined cellular origin, and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that an organ or tissue of the cell type, or the cell-type itself, is a target of the substance. In embodiments, the substance administered to the subject may be a pharmaceutical, such as an investigational new drug.

[0090] Yet, a further aspect of the present invention is a method of determining the organ-, tissue-, or cell-target of a substance administered to a subject. The method comprises, at two or more time points, (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or

more portions is the same as a known cell-type specific methylation pattern; and (c) measuring the quantity of the cfDNA of the determined cellular origin. An increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that an organ or tissue of the cell type, or the cell-type itself, is a target of the substance. In embodiments, the substance administered to the subject may be a pharmaceutical, such as an investigational new drug.

[0091] In some embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who were not exposed to the toxic agent. In other embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who were not administered the treatment.

[0092] In some embodiments, the normal quantity of cfDNA of the determined cellular origin is a quantity of cfDNA for the determined cellular origin that is expected for the determined cellular origin.

[0093] In some embodiments, the two or more time points may all be after treatment or exposure to the toxic agent. In some embodiments, at least one of the two or more time points may be before treatment or exposure to the toxic agent.

[0094] The time points may be, for instance, one or more days apart, for example, every day, every two days, every three days, every four days, every five days, every six days, every week every two weeks, every three weeks, every four weeks, every month, every two months, every three months, every four months, every five months, every six months, every seven months, every eight months, every nine months, every ten months, every 11 months, every year, or any time therebetween.

[0095] The increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin, or over a previously measured quantity of cfDNA of the determined cellular origin, may be, for example, a percent increase of about 0.1% to 100%, such as about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%; or may be a fold increase of at least about 2-fold, such as about 2-fold, or 3-fold, or 4-fold, or 5-fold, or 6-fold, or 7-fold, or 8-fold, or 9-fold, or 10-fold. In some embodiments, the increase may be any increase that is determined to be statistically significant (e.g., $p \leq 0.05$, $p \leq 0.01$, etc.) as calculated by statistical methods known in the art.

[0096] In some embodiments, the subject has cancer.

[0097] The biospecimen may be a biological fluid obtained from the subject, including, but not limited to, whole blood, plasma, serum, urine, or any other fluid sample produced by the subject such as saliva, cerebrospinal fluid, urine, or sputum. In certain embodiments, the biospecimen is whole blood, plasma, or serum.

[0098] Methods for quantifying the cfDNA are known in the art and include, but are not limited to, PCR; fluorescence-based quantification methods (e.g., Qubit); chromatography techniques such as gas chromatography, supercritical fluid chromatography, and liquid chromatography, such as partition chromatography, adsorption chromatography, ion exchange chromatography, size exclusion chromatography, thin-layer chromatography, and affinity chromatography; electrophoresis techniques, such as capillary electrophoresis, capillary zone electrophoresis, capillary isoelectric focusing, capillary electrochromatography, micellar electrokinetic capillary chromatography, isotachopheresis, transient isotachopheresis, and capillary gel electrophoresis; comparative genomic hybridization; microarrays; and bead arrays.

Methods Combining Epigenetic and Genetic Analyses

[0099] The use of patterns of differential methylation to determine the cellular origin of cfDNA can be combined with a genetic analysis of the cfDNA. Such a combination can be applied to method of treatment that involves monitoring treatment response and therapy-related adverse events. Combining changes to mutant ctDNA with altered proportions of cell-type specific cfDNA can reflect intervention-based changes. The half-life of cfDNA is between 15 minutes and 2 hours. The rapid clearance allows for serial analysis of disease evolution over time, especially under selective pressures from ongoing therapy. The methods of the invention allow for serial sampling to include a baseline comparison from which therapy-related relative changes may be assessed, taking into account patient specific co-morbidities at an individualized level.

[0100] Combining genetic and epigenetic analyses of cell-free DNA has many unique advantages when applied to precision therapeutics in cancer. Liquid biopsies have been shown to accurately characterize tumor genotypes and allow for molecular subtype classification to provide a comprehensive view of intratumor heterogeneity. High sampling frequency allows for modeling of evolutionary dynamics of tumor progression. Also,

molecular changes identified after initiation of therapy can provide insight into therapy response as well as track tumor subclones that may lead to emergence of therapy resistance. The systemic view provided by serial liquid biopsies is ideal to monitor widespread changes that may better inform clinical decision making in the face of uncertainty. For example, in the case of surgical removal of the tumor or therapeutic success, liquid biopsies can be used to monitor for minimal residual disease and recurrence. While ctDNA can be used to track molecular changes in the circulation, there is a benefit to monitoring the cancer-related changes to the host microenvironment in tandem requiring a combined genetic and epigenetic analysis. Cell-specific cfDNA methylation patterns of normal cells can be used in combination with ctDNA to assess the impact of treatment also on the surrounding tumor microenvironment. This is particularly useful to surveil for metastatic disease in distant tissue-types from the primary tumor as well as to monitor for therapy-related toxicities in somatic cell types. Further, liquid biopsies can help delineate factors that underlie clinical outcomes, providing a basis for recommending different treatments based on anticipated benefit to the patient. Liquid biopsies can identify predictive biomarkers to guide selection of treatment, recognize off-target effects and develop individualized treatment plans for patients. These applications provide a more complete picture of therapeutic response as well as tissue-specific cellular toxicity to better inform clinical care and management throughout the treatment process.

[0101] The minimally invasive nature of liquid biopsies allows for serial sampling to monitor changes over time, especially under selective pressures from ongoing therapy. ctDNA can be used to track clonal heterogeneity over time to assess treatment response and detect treatment-resistant clones. Normal cell-specific cfDNA methylation patterns can be used in combination with ctDNA to assess the impact of treatment to the surrounding tumor microenvironment and to monitor for therapy-related toxicities in somatic cell-types (FIG. 1).

[0102] The use of patterns of differential methylation to determine the cellular origin of cfDNA in combination with genetic analysis can be applied to methods of treating a subject having a tumor. In some embodiments, the methods comprise (a) monitoring the response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises, at two or more time points, performing a genetic and epigenetic analysis of cfDNA, ctDNA, or a combination thereof, and optionally comparing to normal

cfDNA, ctDNA, or a combination thereof, to determine whether to change the first treatment; and (b) administering an adjusted treatment or continuing the first treatment in accordance with the genetic and epigenetic analysis.

[0103] In other embodiments, the methods comprise (A) monitoring a response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises: (i) determining whether there is an adverse reaction to the first treatment, which comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; (c) measuring the quantity of the cfDNA of the determined cellular origin; and (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin, in which an increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative of an adverse reaction; and (ii) determining whether there is a response to the first treatment, which comprises: (a) sequencing ctDNA in a biospecimen from the subject, and (b) determining clonal heterogeneity of cells of the tumor by genotyping the ctDNA, in which the presence of more than one clone of the tumor cells or the presence of a tumor cell clone that has not been previously identified in the subject is indicative of an ineffective response to the first treatment; and (B) either administering the same treatment as the first treatment when it is determined that there is no adverse reaction, that there is not an ineffective response, or a combination thereof; or administering an adjusted treatment when it is determined that there is an adverse reaction, that there is an ineffective response, or a combination thereof.

[0104] In some embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who did not receive the first treatment. In other embodiments, the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who do not have the tumor.

[0105] In some embodiments, the normal quantity of cfDNA of the determined cellular origin is a quantity of cfDNA for the determined cellular origin that is expected for the determined cellular origin.

[0106] In yet other embodiments, the methods comprise (A) monitoring a response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises, at two or more time points, (i) determining whether there is an adverse reaction to the first treatment, which comprises (a) sequencing cfDNA in a biospecimen from the subject; (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, in which the cellular origin of the cell-free DNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and (c) measuring the quantity of the cfDNA of the determined cellular origin, wherein an increase in the measured quantity of the cfDNA of the determined cellular origin measured at a later time point as compared to an earlier time point is indicative of an adverse reaction; and (ii) determining whether there is a response to the first treatment, which comprises (a) sequencing cfDNA in a biospecimen from the subject; and (b) determining clonal heterogeneity of cells of the tumor by genotyping the cfDNA, wherein the presence of more than one clone of the tumor cells or the presence of a tumor cell clone in a subsequent time point that has not been identified at a previous time point is indicative of an ineffective response to the first treatment; and (B) either administering the same treatment as the first treatment when it is determined that there is no adverse reaction, that there is not an ineffective response, or a combination thereof; or administering an adjusted treatment when it is determined that there is an adverse reaction, that there is an ineffective response, or a combination thereof.

[0107] In some embodiments, the subject has a tumor associated with a cancer. Examples of cancer include, but are not limited to, colorectal cancer, brain cancer, ovarian cancer, prostate cancer, pancreatic cancer, breast cancer, renal cancer, nasopharyngeal carcinoma, hepatocellular carcinoma, melanoma, skin cancer, oral cancer, head and neck cancer, esophageal cancer, gastric cancer, cervical cancer, bladder cancer, lymphoma, chronic or acute leukemia (such as B, T, and myeloid derived), sarcoma, lung cancer and multidrug resistant cancer. Other examples are disease that require drug treatment with chemical compounds (small molecules) or proteins such as insulin or antibodies. Such disease can be

metabolic disease such as diabetes mellitus or infections such as bacterial or viral infections such as hepatitis or cardiovascular disease including but not limited to hypertension, coronary artery disease, cerebral vascular disease or peripheral vascular disease.

[0108] In some embodiments, cfDNA is used to compare damage to cells from the first treatment with undamaged normal cells from the same tissue.

[0109] In some embodiments, methylation patterns are assessed in the cfDNA. In certain embodiments, the methylation patterns of cfDNA from damaged cells and healthy cells are compared.

[0110] In some embodiments, the analysis includes comparing damaged cells to healthy cells, to see where the damage originated.

[0111] In some embodiments, the treatment comprises a chemotherapy, radiotherapy, targeted therapy, immunotherapy, or a combination thereof.

[0112] In some embodiments, the two or more time points may all be after the first treatment. In some embodiments, at least one of the two or more time points may be before the first treatment.

[0113] The time points may be, for instance, one or more days apart, for example, every day, every two days, every three days, every four days, every five days, every six days, every week every two weeks, every three weeks, every four weeks, every month, every two months, every three months, every four months, every five months, every six months, every seven months, every eight months, every nine months, every ten months, every 11 months, every year, or any time therebetween.

[0114] The increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin, or over a previously measured quantity of cfDNA of the determined cellular origin, may be, for example, a percent increase of about 0.1% to 100%, such as about 0.1%, 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%; or may be a fold increase of at least about 2-fold, such as about 2-fold, or 3-fold, or 4-fold, or 5-fold, or 6-fold, or 7-fold, or 8-fold, or 9-fold, or 10-fold. In some embodiments, the increase may be any increase that is determined to be statistically significant (e.g., $p \leq 0.05$, $p \leq 0.01$, etc.) as calculated by statistical methods known in the art.

[0115] The biospecimen may be a biological fluid obtained from the subject, including, but not limited to, whole blood, plasma, serum, urine, or any other fluid sample produced by the subject such as saliva, cerebrospinal fluid, urine, or sputum. In certain embodiments, the biospecimen is whole blood, plasma, or serum.

[0116] Methods for quantifying the cfDNA are known in the art and include, but are not limited to, PCR; fluorescence-based quantification methods (e.g., Qubit); chromatography techniques such as gas chromatography, supercritical fluid chromatography, and liquid chromatography, such as partition chromatography, adsorption chromatography, ion exchange chromatography, size exclusion chromatography, thin-layer chromatography, and affinity chromatography; electrophoresis techniques, such as capillary electrophoresis, capillary zone electrophoresis, capillary isoelectric focusing, capillary electrochromatography, micellar electrokinetic capillary chromatography, isotachopheresis, transient isotachopheresis, and capillary gel electrophoresis; comparative genomic hybridization; microarrays; and bead arrays.

[0117] Another aspect of the invention relates to methods of detecting and/or quantitating changes in methylated DNA in the circulation of patients undergoing treatment.

[0118] A further aspect of the invention relates to probes designed for any tissue and/or cell type in a tissue to detect changes in the abundance of tissue-specific DNA fragments in the circulation.

Analysis of cfDNA

[0119] The present invention involves analysis of cfDNA to determine the cellular origin of cfDNA. Determination of the cellular origin of cfDNA comprises identifying methylation patterns in the sequence of the cfDNA and comparing the methylation patterns in the sequence of the cfDNA to known methylation patterns associated with different cell types.

[0120] **Table 1** provides examples of cellular origins associated with different types of tissue.

Table 1. Cellular origins, and the different types of tissue with which they can be associated.

Cellular Origins	Tissue
Mature B-Cell	Blood, Bone Marrow
Naïve B-Cell	Blood, Bone Marrow

Biliary Epithelial Cell	Liver
Breast Basal Cell	Breast
Breast Luminal Cell	Breast
Bulk Endothelial Cell	Blood Vessels
Bulk Epithelial Cell	Any Epithelia
Bulk Immune Cell	Immune Organ
Cardiomyocyte	Heart
Cardiopulmonary Endothelial Cell	Heart, Lung
Colon Epithelial Cell	Colon
Dermal Epithelial Cell	Skin
Granulocyte	Blood, Bone Marrow
Hepatocyte	Liver
Keratinocyte	Skin
Kidney Epithelial Cell	Kidney
Liver Endothelial Cell	Liver
Liver Stromal Cell	Liver
Liver Resident Immune Cell	Liver
Lung Epithelial Cell	Lung
Megakaryocyte	Bone Marrow
Monocytes and Macrophage	Blood
Neuron	Neural
Natural Killer Cell	Blood
Pancreatic Cell	Pancreas
Prostate Epithelial Cell	Prostate
Skeletal Muscular Cell	Skeletal Muscle
Mature T-Cell	Blood

[0121] CfDNA can be obtained by centrifuging the biological fluid, such as whole blood, to remove all cells, and then isolating the DNA from the remaining plasma or serum. Such methods are well known (*see, e.g., Lo et al., 1998*). Circulating cfDNA and ctDNA can be double-stranded or single-stranded DNA.

[0122] Different DNA methylation detection technologies may be used in the present invention. Examples include, but are not limited to, a restriction enzyme digestion approach, which involves cleaving DNA at enzyme-specific CpG sites; an affinity-enrichment method, for instance, methylated DNA immunoprecipitation sequencing (MeDIP-seq) or methyl-CpG-binding domain sequencing (MBD-seq); bisulfite conversion methods such as whole genome bisulfite sequencing (WGBS), reduced representation bisulfite sequencing (RRBS), methylated CpG tandem amplification and sequencing (MCTA-seq), and methylation arrays; enzymatic approaches, such as enzymatic methyl-sequencing (EM-seq) or ten-eleven translocation (TET)--assisted pyridine borane sequencing (TAPS); and other methods that do not require treatment of DNA, for instance, by nanopore-sequencing from Oxford Nanopore

Technologies (ONT) and single molecule real-time (SMRT) sequencing from Pacific Biosciences (PacBio).

[0123] Comparison of the methylation pattern in sequence of the cfDNA with known methylation patterns may comprise identifying the presence of a methylation pattern in the sequence of the cfDNA, or a portion thereof, that are attributed to specific cell types. In some embodiments, the presence of a methylation pattern was performed by hybridization capture sequencing of cfDNA. In other embodiments, the presence of a methylation pattern was performed using bisulfite amplicon sequencing.

[0124] The methylation pattern may comprise a segment of nucleotide sequence containing at least 1 CpG dinucleotide, or at least about 2 CpG dinucleotides, or at least about 3 CpG dinucleotides. In some embodiments, the methylation pattern may comprise a segment of nucleotide sequence containing at least about 4 CpG dinucleotides, or at least about 5 CpG dinucleotides, or at least about 6 CpG dinucleotides, or at least about 7 CpG dinucleotides, or at least about 8 CpG dinucleotides, or at least about 9 CpG dinucleotides, or at least about 10 CpG dinucleotides.

[0125] **Table 2** provides methylation status at CpG dinucleotides in genomic regions that are indicative of different cell types. The presence of a same methylation pattern between the sequence of the cfDNA and the genomic regions set forth in **Table 2** indicates the cell-type from which the cfDNA originates. **Table 2** provides contiguous methylation status across multiple adjacent CpG sites (patterns) within genomic region.

Table 2. Methylation status in genomic regions that are indicative of cell type.

Cell Type	Chromosome	Start*	End*	Methylation Status
Mature B	chr11	68139032	68139146	Hypomethylated
Mature B	chr17	80829337	80829647	Hypomethylated
Mature B	chr6	167506945	167507168	Hypomethylated
Mature B	chr19	1648937	1649129	Hypomethylated
Mature B	chr3	9694444	9695149	Hypomethylated
Mature B	chr18	77116085	77116618	Hypomethylated
Mature B	chr9	135763441	135764023	Hypomethylated
Mature B	chr12	121686411	121686789	Hypomethylated
Mature B	chr6	16306332	16306681	Hypomethylated
Mature B	chr14	96179945	96180308	Hypomethylated

Mature B	chr6	16306086	16306267	Hypomethylated
Mature B	chr16	28944140	28944468	Hypomethylated
Mature B	chr17	73316011	73316779	Hypomethylated
Mature B	chr2	112917111	112917496	Hypomethylated
Mature B	chr7	637567	637692	Hypomethylated
Mature B	chr17	79233329	79233604	Hypomethylated
Mature B	chr2	240291097	240291331	Hypomethylated
Mature B	chr17	3493609	3493935	Hypomethylated
Mature B	chr11	2415602	2415708	Hypomethylated
Mature B	chr13	111329306	111329450	Hypomethylated
Mature B	chr6	167507176	167507296	Hypomethylated
Mature B	chr10	1704918	1705006	Hypomethylated
Mature B	chr14	104158553	104158779	Hypomethylated
Mature B	chr17	80873275	80873776	Hypomethylated
Mature B	chr16	89180214	89180662	Hypomethylated
Mature B	chr11	64567087	64567240	Hypomethylated
Mature B	chr19	2324328	2324440	Hypomethylated
Mature B	chr22	50196818	50196977	Hypomethylated
Mature B	chr16	88103031	88103137	Hypomethylated
Mature B	chr19	1621010	1621318	Hypomethylated
Mature B	chr1	11395635	11395863	Hypomethylated
Mature B	chr2	464998	465071	Hypomethylated
Mature B	chr16	1495324	1495725	Hypomethylated
Mature B	chr9	101754799	101755349	Hypomethylated
Mature B	chr5	177544974	177545232	Hypomethylated
Mature B	chr6	159639094	159639294	Hypomethylated
Mature B	chr8	23083219	23083470	Hypomethylated
Mature B	chr14	104852775	104853229	Hypomethylated
Mature B	chr7	2140017	2140179	Hypomethylated
Mature B	chr19	56156225	56156453	Hypomethylated
Mature B	chr10	121201654	121201754	Hypomethylated
Mature B	chr20	57033426	57033735	Hypomethylated
Mature B	chr2	61199859	61200489	Hypomethylated
Mature B	chr15	75146404	75146784	Hypomethylated
Mature B	chr16	85289643	85290003	Hypomethylated
Mature B	chr16	88765052	88765399	Hypomethylated
Mature B	chr7	2140231	2140348	Hypomethylated
Mature B	chr16	773800	773999	Hypomethylated
Mature B	chr10	13330436	13330842	Hypomethylated
Mature B	chr15	74714681	74715017	Hypomethylated
Naïve B	chr11	68139032	68139146	Hypomethylated
Naïve B	chr17	3493609	3493935	Hypomethylated

Naïve B	chr19	1648937	1649129	Hypomethylated
Naïve B	chr17	80829337	80829647	Hypomethylated
Naïve B	chr22	20760823	20761115	Hypomethylated
Naïve B	chr17	80873275	80873776	Hypomethylated
Naïve B	chr2	240291097	240291331	Hypomethylated
Naïve B	chr10	6262461	6262727	Hypomethylated
Naïve B	chr11	64567087	64567240	Hypomethylated
Naïve B	chr14	104158553	104158779	Hypomethylated
Naïve B	chr19	2324328	2324440	Hypomethylated
Naïve B	chr16	773800	773999	Hypomethylated
Naïve B	chr8	16617187	16617280	Hypomethylated
Naïve B	chr16	88103031	88103137	Hypomethylated
Naïve B	chr5	177544974	177545232	Hypomethylated
Naïve B	chr14	96179945	96180308	Hypomethylated
Naïve B	chr4	185876236	185876334	Hypomethylated
Naïve B	chr9	135763441	135764023	Hypomethylated
Naïve B	chr11	2415602	2415708	Hypomethylated
Naïve B	chr10	121201654	121201754	Hypomethylated
Naïve B	chr7	2140017	2140179	Hypomethylated
Naïve B	chr16	2516952	2517020	Hypomethylated
Naïve B	chr16	75107515	75107841	Hypomethylated
Biliary Epithelial	chr10	7744476	7744775	Hypomethylated
Biliary Epithelial	chr19	35534546	35535085	Hypomethylated
Biliary Epithelial	chr22	39685694	39685809	Hypomethylated
Biliary Epithelial	chr16	87824936	87825139	Hypomethylated
Biliary Epithelial	chr17	48626642	48627290	Hypomethylated
Biliary Epithelial	chr1	19600102	19600251	Hypomethylated
Biliary Epithelial	chr3	128141404	128141587	Hypomethylated
Biliary Epithelial	chr5	53223677	53224181	Hypomethylated
Biliary Epithelial	chr9	137331241	137331537	Hypomethylated
Biliary Epithelial	chr11	47471307	47471401	Hypomethylated
Biliary Epithelial	chr17	79042980	79043169	Hypomethylated
Biliary Epithelial	chr22	37419795	37420227	Hypomethylated
Biliary Epithelial	chr2	74731371	74731414	Hypomethylated
Biliary Epithelial	chr5	170876495	170876741	Hypomethylated
Biliary Epithelial	chr10	135340893	135341026	Hypomethylated
Biliary Epithelial	chr20	56287198	56287355	Hypomethylated
Biliary Epithelial	chr1	9324077	9324214	Hypomethylated
Biliary Epithelial	chr2	241827906	241828206	Hypomethylated
Biliary Epithelial	chr14	101944626	101944805	Hypomethylated
Biliary Epithelial	chr21	46893085	46893254	Hypomethylated
Biliary Epithelial	chr2	241949229	241949839	Hypermethylated

Biliary Epithelial	chr17	42084940	42085300	Hypermethylated
Biliary Epithelial	chr19	50095791	50096912	Hypermethylated
Biliary Epithelial	chr10	11726866	11727466	Hypermethylated
Biliary Epithelial	chr3	13114628	13114763	Hypermethylated
Biliary Epithelial	chr10	102882978	102883606	Hypermethylated
Biliary Epithelial	chr11	2160875	2161446	Hypermethylated
Biliary Epithelial	chr19	3434917	3435465	Hypermethylated
Biliary Epithelial	chr8	11565610	11565963	Hypermethylated
Biliary Epithelial	chr8	11566699	11566916	Hypermethylated
Biliary Epithelial	chr11	10328197	10328509	Hypermethylated
Biliary Epithelial	chr8	11565188	11565531	Hypermethylated
Biliary Epithelial	chr14	38067833	38067990	Hypermethylated
Biliary Epithelial	chr16	88716968	88717851	Hypermethylated
Biliary Epithelial	chr22	37464939	37465280	Hypermethylated
Biliary Epithelial	chr7	44185404	44185968	Hypermethylated
Biliary Epithelial	chr16	127503	127698	Hypermethylated
Biliary Epithelial	chr3	197281447	197283152	Hypermethylated
Biliary Epithelial	chr18	55107463	55107887	Hypermethylated
Biliary Epithelial	chr15	53087579	53087929	Hypermethylated
Biliary Epithelial	chr11	2161456	2162090	Hypermethylated
Biliary Epithelial	chr14	38067054	38067566	Hypermethylated
Biliary Epithelial	chr7	155597344	155597625	Hypermethylated
Biliary Epithelial	chr2	128181286	128181430	Hypermethylated
Biliary Epithelial	chr3	55515070	55515736	Hypermethylated
Biliary Epithelial	chr6	152128957	152129855	Hypermethylated
Biliary Epithelial	chr5	176829535	176830185	Hypermethylated
Biliary Epithelial	chr2	128180566	128181267	Hypermethylated
Biliary Epithelial	chr11	2164937	2165828	Hypermethylated
Biliary Epithelial	chr8	27182852	27183553	Hypermethylated
Breast Basal	chr2	44497562	44497807	Hypomethylated
Breast Basal	chr17	4453891	4454402	Hypomethylated
Breast Basal	chr11	57558973	57559168	Hypomethylated
Breast Basal	chr11	392133	392695	Hypomethylated
Breast Basal	chr22	47023757	47023904	Hypomethylated
Breast Basal	chr2	240040057	240040254	Hypomethylated
Breast Basal	chr1	3321982	3322140	Hypomethylated
Breast Basal	chr18	77635864	77636069	Hypomethylated
Breast Basal	chr22	40417354	40417567	Hypomethylated
Breast Basal	chr5	137803144	137803521	Hypomethylated
Breast Basal	chr11	391681	392015	Hypomethylated
Breast Basal	chr16	27781217	27781639	Hypomethylated
Breast Basal	chr17	2278727	2279131	Hypomethylated

Breast Basal	chr17	70053558	70053834	Hypomethylated
Breast Basal	chr10	123738139	123738614	Hypomethylated
Breast Basal	chr16	81533203	81533549	Hypomethylated
Breast Basal	chr18	10588980	10589063	Hypomethylated
Breast Basal	chr18	10589150	10589279	Hypomethylated
Breast Basal	chr19	17437804	17438236	Hypomethylated
Breast Basal	chr19	46388006	46388262	Hypomethylated
Breast Basal	chr20	62045021	62045251	Hypomethylated
Breast Basal	chr1	2832006	2832144	Hypomethylated
Breast Basal	chr16	88166706	88166848	Hypomethylated
Breast Basal	chr2	233198590	233198791	Hypomethylated
Breast Basal	chr7	4804631	4805136	Hypomethylated
Breast Basal	chr8	142235391	142235926	Hypomethylated
Breast Basal	chr11	772689	773090	Hypomethylated
Breast Basal	chr19	8554999	8555062	Hypomethylated
Breast Basal	chr19	46387679	46387919	Hypomethylated
Breast Basal	chr22	29659845	29660202	Hypomethylated
Breast Basal	chr14	102093931	102094226	Hypomethylated
Breast Basal	chr17	5983894	5984066	Hypomethylated
Breast Basal	chr22	47022433	47022659	Hypomethylated
Breast Basal	chr7	2054500	2055180	Hypomethylated
Breast Basal	chr11	2170373	2170444	Hypomethylated
Breast Basal	chr5	1876857	1877139	Hypermethylated
Breast Basal	chr10	8089332	8089925	Hypermethylated
Breast Basal	chr16	56703476	56703914	Hypermethylated
Breast Basal	chr9	135463966	135464285	Hypermethylated
Breast Basal	chr10	129534178	129534481	Hypermethylated
Breast Basal	chr13	37004787	37005108	Hypermethylated
Breast Basal	chr14	95233855	95234127	Hypermethylated
Breast Basal	chr6	10381523	10382075	Hypermethylated
Breast Basal	chr9	129372570	129372906	Hypermethylated
Breast Basal	chr12	49390678	49391209	Hypermethylated
Breast Basal	chr1	22668552	22668874	Hypermethylated
Breast Basal	chr3	137489017	137489723	Hypermethylated
Breast Basal	chr5	1874836	1875551	Hypermethylated
Breast Basal	chr12	54090151	54090388	Hypermethylated
Breast Basal	chr9	129372913	129373070	Hypermethylated
Breast Luminal	chr11	65582724	65582909	Hypomethylated
Breast Luminal	chr1	3321982	3322140	Hypomethylated
Breast Luminal	chr1	2832006	2832144	Hypomethylated
Breast Luminal	chr5	148958721	148958920	Hypomethylated
Breast Luminal	chr17	2278727	2279131	Hypomethylated

Breast Luminal	chr5	176764116	176764365	Hypomethylated
Breast Luminal	chr12	115150184	115150549	Hypomethylated
Breast Luminal	chr16	90068365	90068490	Hypomethylated
Breast Luminal	chr17	47467293	47467337	Hypomethylated
Breast Luminal	chr18	10589150	10589279	Hypomethylated
Breast Luminal	chr19	48491917	48492032	Hypomethylated
Breast Luminal	chr6	169641078	169641392	Hypomethylated
Breast Luminal	chr2	121036351	121037152	Hypomethylated
Breast Luminal	chr16	30852669	30852999	Hypomethylated
Breast Luminal	chr18	10588980	10589063	Hypomethylated
Breast Luminal	chr19	7685239	7685435	Hypomethylated
Breast Luminal	chr4	757194	757416	Hypomethylated
Breast Luminal	chr1	24191684	24192034	Hypomethylated
Breast Luminal	chr6	168797654	168797892	Hypomethylated
Breast Luminal	chr9	139698878	139699275	Hypomethylated
Breast Luminal	chr1	9565292	9565531	Hypomethylated
Breast Luminal	chr13	103454025	103454177	Hypomethylated
Breast Luminal	chr3	133175047	133175550	Hypomethylated
Breast Luminal	chr1	3017607	3017703	Hypomethylated
Breast Luminal	chr1	3474045	3474246	Hypomethylated
Breast Luminal	chr5	172982435	172982535	Hypomethylated
Breast Luminal	chr14	105181998	105182486	Hypomethylated
Breast Luminal	chr14	105269038	105269404	Hypomethylated
Breast Luminal	chr3	137489017	137489723	Hypermethylated
Breast Luminal	chr10	22542284	22542463	Hypermethylated
Breast Luminal	chr17	42287754	42288090	Hypermethylated
Breast Luminal	chr5	138729659	138729816	Hypermethylated
Breast Luminal	chr5	174158786	174158926	Hypermethylated
Breast Luminal	chr6	10393138	10393779	Hypermethylated
Breast Luminal	chr9	129386162	129386326	Hypermethylated
Breast Luminal	chr5	174158548	174158782	Hypermethylated
Breast Luminal	chr9	129372460	129372565	Hypermethylated
Breast Luminal	chr10	8085311	8085801	Hypermethylated
Breast Luminal	chr5	1876857	1877139	Hypermethylated
Breast Luminal	chr5	1879621	1879706	Hypermethylated
Breast Luminal	chr9	129373594	129373647	Hypermethylated
Breast Luminal	chr5	1875800	1875940	Hypermethylated
Breast Luminal	chr5	1874836	1875551	Hypermethylated
Breast Luminal	chr9	129388506	129388993	Hypermethylated
Breast Luminal	chr5	1877986	1878242	Hypermethylated
Breast Luminal	chr6	10381523	10382075	Hypermethylated
Breast Luminal	chr9	129388068	129388495	Hypermethylated

Breast Luminal	chr9	129372570	129372906	Hypermethylated
Breast Luminal	chr9	129372913	129373070	Hypermethylated
Bulk Endothelial	chr9	139406515	139406839	Hypomethylated
Bulk Endothelial	chr6	1635704	1635851	Hypomethylated
Bulk Endothelial	chr14	69931518	69931952	Hypomethylated
Bulk Endothelial	chr17	80803660	80804189	Hypomethylated
Bulk Endothelial	chr7	4746712	4746898	Hypomethylated
Bulk Endothelial	chr12	52291132	52291323	Hypomethylated
Bulk Endothelial	chr7	150690506	150691038	Hypomethylated
Bulk Endothelial	chr6	157877066	157877221	Hypomethylated
Bulk Endothelial	chr16	2220378	2221058	Hypomethylated
Bulk Endothelial	chr7	736404	736961	Hypomethylated
Bulk Endothelial	chr6	46889597	46889761	Hypomethylated
Bulk Endothelial	chr17	1975093	1975641	Hypomethylated
Bulk Endothelial	chr12	121717850	121717948	Hypomethylated
Bulk Endothelial	chr13	29329007	29329215	Hypomethylated
Bulk Endothelial	chr19	11707038	11707247	Hypomethylated
Bulk Endothelial	chr6	167028939	167029195	Hypomethylated
Bulk Endothelial	chr7	65617005	65617363	Hypomethylated
Bulk Endothelial	chr11	86662732	86663161	Hypomethylated
Bulk Endothelial	chr14	105796697	105796899	Hypomethylated
Bulk Endothelial	chr1	3038085	3038257	Hypomethylated
Bulk Endothelial	chr5	141059753	141060199	Hypomethylated
Bulk Endothelial	chr10	13726446	13726682	Hypomethylated
Bulk Endothelial	chr11	76290451	76290615	Hypomethylated
Bulk Endothelial	chr16	1562314	1562661	Hypomethylated
Bulk Endothelial	chr2	89128482	89128683	Hypomethylated
Bulk Endothelial	chr11	126301685	126301953	Hypomethylated
Bulk Endothelial	chr7	131314332	131314441	Hypomethylated
Bulk Endothelial	chr9	138900552	138900629	Hypomethylated
Bulk Endothelial	chr6	132270337	132270745	Hypomethylated
Bulk Endothelial	chr17	73506140	73506303	Hypomethylated
Bulk Endothelial	chr17	79170799	79170897	Hypomethylated
Bulk Endothelial	chr4	1227142	1227401	Hypomethylated
Bulk Endothelial	chr4	151504816	151505028	Hypomethylated
Bulk Endothelial	chr2	128430937	128431443	Hypomethylated
Bulk Endothelial	chr5	38466902	38467249	Hypomethylated
Bulk Endothelial	chr9	35909661	35910091	Hypomethylated
Bulk Endothelial	chr10	504484	504785	Hypomethylated
Bulk Endothelial	chr7	2646484	2646629	Hypomethylated
Bulk Endothelial	chr19	18233987	18234213	Hypomethylated
Bulk Endothelial	chr22	47188803	47188952	Hypomethylated

Bulk Endothelial	chr4	1227729	1227950	Hypomethylated
Bulk Endothelial	chr17	15394429	15394554	Hypomethylated
Bulk Endothelial	chr7	131217702	131217878	Hypomethylated
Bulk Endothelial	chr7	142984797	142984913	Hypomethylated
Bulk Endothelial	chr17	73509751	73509964	Hypomethylated
Bulk Endothelial	chr9	137763917	137764041	Hypomethylated
Bulk Endothelial	chr11	72295847	72296047	Hypermethylated
Bulk Endothelial	chr11	72295460	72295843	Hypermethylated
Bulk Endothelial	chr19	8398826	8399120	Hypermethylated
Bulk Endothelial	chr8	10588820	10589153	Hypermethylated
Bulk Epithelial	chr17	37862101	37862814	Hypomethylated
Bulk Epithelial	chr11	2397153	2397487	Hypomethylated
Bulk Epithelial	chr11	27490421	27491031	Hypomethylated
Bulk Epithelial	chr7	985538	985720	Hypomethylated
Bulk Epithelial	chr10	45406802	45407004	Hypomethylated
Bulk Epithelial	chr6	168789503	168789732	Hypomethylated
Bulk Epithelial	chr16	1349427	1349853	Hypomethylated
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Bulk Epithelial	chr17	8190993	8191301	Hypomethylated
Bulk Epithelial	chr7	27163145	27163499	Hypomethylated
Bulk Epithelial	chr17	79949983	79950241	Hypomethylated
Bulk Epithelial	chr16	27237906	27238021	Hypomethylated
Bulk Epithelial	chr16	85424260	85424547	Hypomethylated
Bulk Epithelial	chr21	46678570	46678673	Hypomethylated
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Bulk Epithelial	chr14	100032685	100032895	Hypomethylated
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Bulk Epithelial	chr17	48179459	48179672	Hypomethylated
Bulk Epithelial	chr5	1183107	1183283	Hypomethylated
Bulk Epithelial	chr10	134079332	134079426	Hypomethylated
Bulk Epithelial	chr11	34622158	34622496	Hypomethylated
Bulk Epithelial	chr14	100621848	100622287	Hypomethylated
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Bulk Epithelial	chr8	29177514	29177650	Hypomethylated
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Bulk Epithelial	chr15	101991828	101991977	Hypermethylated
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Bulk Epithelial	chr4	4765147	4765382	Hypermethylated
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Bulk Epithelial	chr5	132161281	132161485	Hypermethylated
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Bulk Epithelial	chr7	155599119	155599231	Hypermethylated
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Bulk Immune	chr3	67706044	67706706	Hypomethylated
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Bulk Immune	chr8	145808729	145808892	Hypomethylated
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Bulk Immune	chr19	5139390	5139647	Hypomethylated
Bulk Immune	chr3	196367455	196367896	Hypomethylated
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Bulk Immune	chr2	233251922	233252099	Hypermethylated
Bulk Immune	chr5	176827023	176827233	Hypermethylated
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Bulk Immune	chr2	54087173	54087424	Hypermethylated
Bulk Immune	chr8	120685466	120685687	Hypermethylated
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Bulk Immune	chr7	27154911	27155334	Hypermethylated
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Bulk Immune	chr19	2540684	2541138	Hypermethylated
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Bulk Immune	chr7	27152969	27153160	Hypermethylated
Bulk Immune	chr10	101281123	101281332	Hypermethylated
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Bulk Immune	chr11	47376572	47377213	Hypermethylated
Bulk Immune	chr1	45082704	45083125	Hypermethylated
Bulk Immune	chr19	49842322	49843076	Hypermethylated
Bulk Immune	chr19	10444874	10445594	Hypermethylated
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Cardiomyocyte	chr8	124664836	124665047	Hypomethylated
Cardiomyocyte	chr10	29186554	29186755	Hypomethylated
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Cardiomyocyte	chr13	113384765	113384930	Hypomethylated
Cardiomyocyte	chr2	236877092	236877616	Hypomethylated
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Cardiomyocyte	chr12	3364736	3365604	Hypomethylated
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Cardiomyocyte	chr19	1419166	1419762	Hypomethylated
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Cardiomyocyte	chr18	19780465	19780821	Hypomethylated
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Cardiomyocyte	chr11	78534066	78534252	Hypomethylated
Cardiomyocyte	chr13	114108292	114108653	Hypomethylated
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Cardiomyocyte	chr22	26149485	26150074	Hypomethylated
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Cardiomyocyte	chr10	81059280	81059434	Hypomethylated
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Cardiomyocyte	chr2	241533173	241533990	Hypomethylated
Cardiomyocyte	chr6	6746438	6746730	Hypomethylated
Cardiomyocyte	chr6	158464164	158464475	Hypomethylated
Cardiomyocyte	chr7	820811	821315	Hypomethylated
Cardiomyocyte	chr7	4824534	4824952	Hypomethylated
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Cardiopulmonary Endothelial	chr16	2220378	2221058	Hypomethylated
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Cardiopulmonary Endothelial	chr6	167028939	167029195	Hypomethylated

Cardiopulmonary Endothelial	chr7	5549534	5549731	Hypomethylated
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Cardiopulmonary Endothelial	chr9	139406957	139407311	Hypomethylated
Cardiopulmonary Endothelial	chr7	142984797	142984913	Hypomethylated
Cardiopulmonary Endothelial	chr11	134231502	134231622	Hypomethylated
Cardiopulmonary Endothelial	chr14	105796697	105796899	Hypomethylated
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Cardiopulmonary Endothelial	chr9	139549426	139549603	Hypomethylated
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Cardiopulmonary Endothelial	chr8	102464327	102464407	Hypomethylated
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Colon Epithelial	chr11	1444733	1445067	Hypomethylated
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Dermal Endothelial	chr7	2003132	2003772	Hypomethylated
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Dermal Endothelial	chr3	128209966	128210732	Hypermethylated
Dermal Endothelial	chr11	128555051	128555481	Hypermethylated
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Dermal Endothelial	chr7	5468088	5469462	Hypermethylated
Dermal Endothelial	chr2	177022967	177023205	Hypermethylated
Dermal Endothelial	chr11	72300978	72301585	Hypermethylated
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Dermal Endothelial	chr2	177022693	177022963	Hypermethylated

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Granulocyte	chr20	1785056	1785521	Hypomethylated
Granulocyte	chr9	136919731	136919892	Hypomethylated
Granulocyte	chr13	114262862	114263522	Hypomethylated
Granulocyte	chr20	62522393	62522519	Hypomethylated
Granulocyte	chr8	131000173	131000872	Hypomethylated
Hepatocyte	chr19	2790708	2791240	Hypomethylated
Hepatocyte	chr2	118674801	118675049	Hypomethylated
Hepatocyte	chr12	133249223	133249419	Hypomethylated
Hepatocyte	chr2	128176259	128176803	Hypomethylated

Hepatocyte	chr19	16627211	16627478	Hypomethylated
Hepatocyte	chr16	27226453	27227400	Hypomethylated
Hepatocyte	chr19	59022243	59023070	Hypomethylated
Hepatocyte	chr2	119980528	119980922	Hypomethylated
Hepatocyte	chr17	41019749	41020862	Hypomethylated
Hepatocyte	chr2	44065003	44065200	Hypomethylated
Hepatocyte	chr4	155507816	155508049	Hypomethylated
Hepatocyte	chr11	47267183	47267393	Hypomethylated
Hepatocyte	chr22	50644494	50644958	Hypomethylated
Hepatocyte	chr14	70263750	70263921	Hypomethylated
Hepatocyte	chr14	103573762	103574056	Hypomethylated
Hepatocyte	chr17	48540012	48540441	Hypomethylated
Hepatocyte	chr19	11347217	11347465	Hypomethylated
Hepatocyte	chr20	60753603	60754165	Hypomethylated
Hepatocyte	chr22	38212483	38213122	Hypomethylated
Hepatocyte	chr9	130551728	130551831	Hypomethylated
Hepatocyte	chr16	1991285	1991497	Hypomethylated
Hepatocyte	chr17	80197816	80197957	Hypomethylated
Hepatocyte	chr15	64996479	64997466	Hypomethylated
Hepatocyte	chr16	12354976	12355424	Hypomethylated
Hepatocyte	chr16	31473751	31474229	Hypomethylated
Hepatocyte	chr17	80052709	80053033	Hypomethylated
Hepatocyte	chr4	185724474	185724838	Hypomethylated
Hepatocyte	chr12	57625174	57625730	Hypomethylated
Hepatocyte	chr2	44065230	44065928	Hypomethylated
Hepatocyte	chr2	127818028	127818420	Hypomethylated
Hepatocyte	chr7	1912065	1912694	Hypomethylated
Hepatocyte	chr7	158673837	158674009	Hypomethylated
Hepatocyte	chr20	62365874	62366578	Hypomethylated
Hepatocyte	chr1	11106576	11107083	Hypomethylated
Hepatocyte	chr2	44066294	44066867	Hypomethylated
Hepatocyte	chr11	679700	680254	Hypomethylated
Hepatocyte	chr17	27493494	27493786	Hypomethylated
Hepatocyte	chr22	50644200	50644478	Hypomethylated
Hepatocyte	chr12	3194286	3194554	Hypomethylated
Hepatocyte	chr16	72981972	72982166	Hypomethylated
Hepatocyte	chr19	3659410	3659740	Hypomethylated
Hepatocyte	chr20	43108645	43109079	Hypomethylated
Hepatocyte	chr14	95028058	95028332	Hypomethylated
Hepatocyte	chr9	139840215	139840491	Hypomethylated
Hepatocyte	chr12	109639260	109639506	Hypomethylated
Hepatocyte	chr17	17463355	17463878	Hypomethylated

Hepatocyte	chr22	18324579	18324626	Hypomethylated
Hepatocyte	chr3	126060006	126060381	Hypomethylated
Hepatocyte	chr4	6755163	6755373	Hypomethylated
Hepatocyte	chr12	7280736	7281344	Hypomethylated
Keratinocyte	chr7	4802065	4802254	Hypomethylated
Keratinocyte	chr8	143463390	143463895	Hypomethylated
Keratinocyte	chr2	98349354	98349712	Hypomethylated
Keratinocyte	chr11	65306835	65307074	Hypomethylated
Keratinocyte	chr11	391681	392015	Hypomethylated
Keratinocyte	chr11	392133	392695	Hypomethylated
Keratinocyte	chr1	3321982	3322140	Hypomethylated
Keratinocyte	chr5	178565867	178566128	Hypomethylated
Keratinocyte	chr17	61558935	61559387	Hypomethylated
Keratinocyte	chr16	3129975	3130351	Hypomethylated
Keratinocyte	chr17	55037093	55037359	Hypomethylated
Keratinocyte	chr6	36936452	36936717	Hypomethylated
Keratinocyte	chr16	1017921	1018739	Hypomethylated
Keratinocyte	chr20	62705520	62705726	Hypomethylated
Keratinocyte	chr1	3322151	3322267	Hypomethylated
Keratinocyte	chr5	465181	465615	Hypomethylated
Keratinocyte	chr17	79244962	79245204	Hypomethylated
Keratinocyte	chr22	47022433	47022659	Hypomethylated
Keratinocyte	chr2	97423178	97423608	Hypomethylated
Keratinocyte	chr8	143869435	143869513	Hypomethylated
Keratinocyte	chr16	2334301	2334759	Hypomethylated
Keratinocyte	chr20	18295551	18295861	Hypomethylated
Keratinocyte	chr1	2309948	2310095	Hypomethylated
Keratinocyte	chr9	94572657	94572869	Hypomethylated
Keratinocyte	chr12	3190418	3190860	Hypomethylated
Keratinocyte	chr12	98986220	98986393	Hypomethylated
Keratinocyte	chr6	167506945	167507168	Hypomethylated
Keratinocyte	chr20	18167947	18168360	Hypomethylated
Keratinocyte	chr11	460362	460681	Hypomethylated
Keratinocyte	chr17	76027640	76027890	Hypomethylated
Keratinocyte	chr7	99227275	99227516	Hypomethylated
Keratinocyte	chr11	65582724	65582909	Hypomethylated
Keratinocyte	chr7	5648142	5648380	Hypomethylated
Keratinocyte	chr8	102076454	102076803	Hypomethylated
Keratinocyte	chr8	142235391	142235926	Hypomethylated
Keratinocyte	chr10	13771352	13771658	Hypomethylated
Keratinocyte	chr11	131707430	131707776	Hypomethylated
Keratinocyte	chr9	79631906	79632226	Hypermethylated

Keratinocyte	chr9	129386162	129386326	Hypermethylated
Keratinocyte	chr10	8094621	8094861	Hypermethylated
Keratinocyte	chr10	119292088	119292379	Hypermethylated
Keratinocyte	chr12	54384508	54385130	Hypermethylated
Keratinocyte	chr10	119292418	119292676	Hypermethylated
Keratinocyte	chr12	54359358	54359894	Hypermethylated
Keratinocyte	chr12	54338915	54339051	Hypermethylated
Keratinocyte	chr10	119294251	119294603	Hypermethylated
Keratinocyte	chr9	129372913	129373070	Hypermethylated
Keratinocyte	chr9	129388068	129388495	Hypermethylated
Keratinocyte	chr9	129388506	129388993	Hypermethylated
Keratinocyte	chr12	54338568	54338887	Hypermethylated
Kidney Epithelial	chr10	64041172	64041512	Hypomethylated
Kidney Epithelial	chr4	1625248	1625493	Hypomethylated
Kidney Epithelial	chr4	8642130	8642286	Hypomethylated
Kidney Epithelial	chr4	42363614	42363736	Hypomethylated
Kidney Epithelial	chr12	4554786	4554972	Hypomethylated
Kidney Epithelial	chr17	74908539	74908715	Hypomethylated
Kidney Epithelial	chr10	44163409	44163531	Hypomethylated
Kidney Epithelial	chr22	39685694	39685809	Hypomethylated
Kidney Epithelial	chr10	134945578	134945672	Hypomethylated
Kidney Epithelial	chr9	91321534	91321820	Hypomethylated
Kidney Epithelial	chr11	132582078	132582512	Hypomethylated
Kidney Epithelial	chr11	132912309	132912375	Hypomethylated
Kidney Epithelial	chr7	157427089	157427365	Hypomethylated
Kidney Epithelial	chr12	53517541	53517743	Hypomethylated
Kidney Epithelial	chr19	1961122	1961657	Hypomethylated
Kidney Epithelial	chr5	176225786	176225897	Hypomethylated
Kidney Epithelial	chr11	132031389	132031554	Hypomethylated
Kidney Epithelial	chr4	188097977	188098247	Hypomethylated
Kidney Epithelial	chr22	23607590	23607758	Hypomethylated
Kidney Epithelial	chr12	132661484	132661640	Hypomethylated
Kidney Epithelial	chr16	895422	895536	Hypomethylated
Kidney Epithelial	chr10	504484	504785	Hypomethylated
Kidney Epithelial	chr12	125242868	125242959	Hypomethylated
Kidney Epithelial	chr17	75695335	75695465	Hypomethylated
Kidney Epithelial	chr18	7231222	7232053	Hypomethylated
Kidney Epithelial	chr2	34902628	34903058	Hypomethylated
Kidney Epithelial	chr11	116484298	116484379	Hypomethylated
Kidney Epithelial	chr18	76151279	76151406	Hypomethylated
Kidney Epithelial	chr1	3680249	3680473	Hypomethylated
Kidney Epithelial	chr4	8642296	8642353	Hypomethylated

Kidney Epithelial	chr14	104834370	104834458	Hypomethylated
Kidney Epithelial	chr4	619473	619638	Hypomethylated
Kidney Epithelial	chr10	135120066	135120641	Hypomethylated
Kidney Epithelial	chr4	1641960	1642062	Hypomethylated
Kidney Epithelial	chr8	122961356	122961731	Hypomethylated
Kidney Epithelial	chr19	34178390	34178526	Hypomethylated
Kidney Epithelial	chr1	4193830	4193883	Hypomethylated
Kidney Epithelial	chr4	100574404	100574537	Hypomethylated
Kidney Epithelial	chr7	157258798	157258884	Hypomethylated
Kidney Epithelial	chr16	3142948	3143251	Hypomethylated
Kidney Epithelial	chr17	80192099	80192259	Hypomethylated
Kidney Epithelial	chr19	10823619	10823914	Hypomethylated
Kidney Epithelial	chr5	72677195	72677378	Hypermethylated
Kidney Epithelial	chr1	47911646	47911941	Hypermethylated
Kidney Epithelial	chr5	72597296	72597969	Hypermethylated
Kidney Epithelial	chr5	72677395	72677689	Hypermethylated
Kidney Epithelial	chr10	102586191	102586498	Hypermethylated
Kidney Epithelial	chr10	102588392	102589472	Hypermethylated
Kidney Epithelial	chr10	102586514	102588293	Hypermethylated
Liver Endothelial	chr11	128698175	128698361	Hypomethylated
Liver Endothelial	chr10	121169671	121170069	Hypomethylated
Liver Endothelial	chr17	80803660	80804189	Hypomethylated
Liver Endothelial	chr19	11707038	11707247	Hypomethylated
Liver Endothelial	chr13	29329007	29329215	Hypomethylated
Liver Endothelial	chr19	18233987	18234213	Hypomethylated
Liver Endothelial	chr1	3038085	3038257	Hypomethylated
Liver Endothelial	chr7	150690506	150691038	Hypomethylated
Liver Endothelial	chr19	4983547	4983872	Hypomethylated
Liver Endothelial	chr1	4237464	4237671	Hypomethylated
Liver Endothelial	chr16	1562314	1562661	Hypomethylated
Liver Endothelial	chr6	167028939	167029195	Hypomethylated
Liver Endothelial	chr14	69931518	69931952	Hypomethylated
Liver Endothelial	chr13	29328750	29328980	Hypomethylated
Liver Endothelial	chr17	79170799	79170897	Hypomethylated
Liver Endothelial	chr7	142984797	142984913	Hypomethylated
Liver Endothelial	chr9	35909661	35910091	Hypomethylated
Liver Endothelial	chr2	109891992	109892086	Hypomethylated
Liver Endothelial	chr6	1635704	1635851	Hypomethylated
Liver Endothelial	chr11	70266256	70266421	Hypomethylated
Liver Endothelial	chr12	52291132	52291323	Hypomethylated
Liver Endothelial	chr16	2220378	2221058	Hypomethylated
Liver Endothelial	chr16	8943021	8943199	Hypomethylated

Liver Endothelial	chr12	117113249	117113487	Hypomethylated
Liver Endothelial	chr1	101705632	101705773	Hypomethylated
Liver Endothelial	chr7	131217702	131217878	Hypomethylated
Liver Endothelial	chr9	138900552	138900629	Hypomethylated
Liver Endothelial	chr17	1975093	1975641	Hypomethylated
Liver Endothelial	chr6	159549081	159549217	Hypomethylated
Liver Endothelial	chr9	139406515	139406839	Hypomethylated
Liver Endothelial	chr12	121717850	121717948	Hypomethylated
Liver Endothelial	chr15	83781607	83781804	Hypomethylated
Liver Endothelial	chr11	72300978	72301585	Hypermethylated
Liver Endothelial	chr19	8398826	8399120	Hypermethylated
Liver Stromal	chr6	125583698	125584141	Hypomethylated
Liver Stromal	chr19	10927122	10928380	Hypomethylated
Liver Stromal	chr8	96572161	96572434	Hypomethylated
Liver Stromal	chr1	115826123	115826716	Hypomethylated
Liver Stromal	chr2	238187070	238187250	Hypomethylated
Liver Stromal	chr2	10501114	10501274	Hypomethylated
Liver Stromal	chr15	67457630	67458134	Hypomethylated
Liver Stromal	chr16	88121144	88121410	Hypomethylated
Liver Stromal	chr6	22568625	22569458	Hypomethylated
Liver Stromal	chr16	87260962	87261334	Hypomethylated
Liver Stromal	chr6	109274387	109274515	Hypomethylated
Liver Stromal	chr2	168614623	168615088	Hypomethylated
Liver Stromal	chr2	239860016	239860285	Hypomethylated
Liver Stromal	chr9	139256433	139256703	Hypomethylated
Liver Stromal	chr17	48263099	48264061	Hypomethylated
Liver Stromal	chr1	879443	879810	Hypomethylated
Liver Stromal	chr7	1162971	1163186	Hypomethylated
Liver Stromal	chr12	124514696	124514989	Hypomethylated
Liver Stromal	chr16	87261416	87261576	Hypomethylated
Liver Stromal	chr19	11276278	11277072	Hypomethylated
Liver Stromal	chr20	48600915	48601197	Hypomethylated
Liver Stromal	chr3	141162003	141163508	Hypomethylated
Liver Stromal	chr7	616047	616267	Hypomethylated
Liver Stromal	chr7	1953582	1954011	Hypomethylated
Liver Stromal	chr12	9478964	9479222	Hypomethylated
Liver Stromal	chr6	2579510	2579743	Hypomethylated
Liver Stromal	chr10	131813008	131813140	Hypomethylated
Liver Stromal	chr8	97166708	97167180	Hypermethylated
Liver Stromal	chr4	174415351	174415831	Hypermethylated
Liver Stromal	chr5	92907765	92907931	Hypermethylated
Liver Stromal	chr19	48833395	48833967	Hypermethylated

Liver Stromal	chr5	92908300	92908694	Hypermethylated
Liver Stromal	chr7	35295220	35295353	Hypermethylated
Liver Stromal	chr7	35297778	35298218	Hypermethylated
Liver Stromal	chr15	37402482	37402724	Hypermethylated
Liver Stromal	chr15	76634052	76634571	Hypermethylated
Liver Stromal	chr15	76634581	76634822	Hypermethylated
Liver Stromal	chr16	86530027	86530682	Hypermethylated
Liver Stromal	chr7	35295997	35296479	Hypermethylated
Liver Stromal	chr16	86537916	86538268	Hypermethylated
Liver Stromal	chr16	86538276	86538344	Hypermethylated
Liver Stromal	chr5	92907932	92908202	Hypermethylated
Liver Stromal	chr5	122434379	122434629	Hypermethylated
Liver Stromal	chr8	72917066	72917696	Hypermethylated
Liver Stromal	chr16	86535785	86536240	Hypermethylated
Liver Stromal	chr5	122435167	122435525	Hypermethylated
Liver Stromal	chr16	86528332	86529004	Hypermethylated
Liver Stromal	chr16	86529012	86529284	Hypermethylated
Liver Stromal	chr16	86540831	86541510	Hypermethylated
Liver Stromal	chr16	86529518	86529935	Hypermethylated
Liver Resident Immune	chr1	167485764	167486300	Hypomethylated
Liver Resident Immune	chr13	100004173	100004739	Hypomethylated
Liver Resident Immune	chr10	62671028	62672100	Hypomethylated
Liver Resident Immune	chr5	1172977	1173240	Hypomethylated
Liver Resident Immune	chr16	89408213	89408323	Hypomethylated
Liver Resident Immune	chr12	122711771	122712065	Hypomethylated
Liver Resident Immune	chr5	125798690	125800185	Hypomethylated
Liver Resident Immune	chr19	10226740	10226846	Hypomethylated
Liver Resident Immune	chr6	159457246	159457551	Hypomethylated
Liver Resident Immune	chr11	69240780	69240893	Hypomethylated
Liver Resident Immune	chr14	61799217	61801202	Hypomethylated
Liver Resident Immune	chr2	232396314	232396622	Hypomethylated
Liver Resident Immune	chr13	24825770	24826000	Hypomethylated

Liver Resident Immune	chr3	134634509	134634904	Hypomethylated
Liver Resident Immune	chr22	28501414	28501559	Hypomethylated
Liver Resident Immune	chr8	55788511	55789245	Hypomethylated
Liver Resident Immune	chr8	129089049	129089294	Hypomethylated
Liver Resident Immune	chr10	8373235	8373451	Hypomethylated
Liver Resident Immune	chr11	67254108	67254405	Hypomethylated
Liver Resident Immune	chr1	228004834	228005102	Hypomethylated
Liver Resident Immune	chr13	53507709	53507874	Hypomethylated
Liver Resident Immune	chr3	15492693	15493298	Hypomethylated
Liver Resident Immune	chr11	119897856	119898003	Hypomethylated
Liver Resident Immune	chr19	14231243	14232111	Hypomethylated
Liver Resident Immune	chr8	588952	589567	Hypomethylated
Liver Resident Immune	chr12	42627503	42629139	Hypomethylated
Liver Resident Immune	chr1	182925302	182926777	Hypomethylated
Liver Resident Immune	chr17	4079397	4079653	Hypomethylated
Liver Resident Immune	chr20	57412376	57413281	Hypomethylated
Liver Resident Immune	chr2	11775213	11775828	Hypomethylated
Liver Resident Immune	chr5	1793815	1794340	Hypomethylated
Liver Resident Immune	chr6	2414426	2415245	Hypomethylated
Liver Resident Immune	chr12	9106413	9107244	Hypomethylated
Liver Resident Immune	chr3	45984585	45986499	Hypomethylated
Liver Resident Immune	chr3	60621448	60622527	Hypomethylated
Liver Resident Immune	chr12	133413238	133413408	Hypomethylated
Liver Resident Immune	chr2	96933283	96933947	Hypomethylated

Liver Resident Immune	chr5	133452036	133452371	Hypomethylated
Liver Resident Immune	chr6	158985919	158986382	Hypomethylated
Liver Resident Immune	chr9	101754799	101755349	Hypomethylated
Liver Resident Immune	chr10	63746813	63747406	Hypomethylated
Liver Resident Immune	chr12	122712075	122712154	Hypomethylated
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Liver Resident Immune	chr8	10210347	10210483	Hypomethylated
Liver Resident Immune	chr11	61672040	61672078	Hypermethylated
Liver Resident Immune	chr2	173293597	173294219	Hypermethylated
Liver Resident Immune	chr11	64009996	64010074	Hypermethylated
Liver Resident Immune	chr9	19050493	19050678	Hypermethylated
Liver Resident Immune	chr10	85955136	85955226	Hypermethylated
Lung Epithelial	chr2	234394496	234394646	Hypomethylated
Lung Epithelial	chr6	7204780	7204927	Hypomethylated
Lung Epithelial	chr6	163731158	163731308	Hypomethylated
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Lung Epithelial	chr13	114304531	114304702	Hypomethylated
Lung Epithelial	chr22	46929575	46929762	Hypomethylated
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Lung Epithelial	chr2	235237949	235238118	Hypomethylated
Lung Epithelial	chr11	66454486	66454682	Hypomethylated
Lung Epithelial	chr12	66983978	66984318	Hypomethylated
Lung Epithelial	chr20	56296470	56296576	Hypomethylated
Lung Epithelial	chr16	85517269	85517471	Hypomethylated
Lung Epithelial	chr17	79953059	79953137	Hypomethylated
Lung Epithelial	chr1	2266262	2266414	Hypomethylated
Lung Epithelial	chr12	26261448	26262385	Hypomethylated
Lung Epithelial	chr14	104048108	104048201	Hypomethylated
Lung Epithelial	chr13	111935187	111935468	Hypomethylated
Lung Epithelial	chr7	2770561	2770802	Hypomethylated
Lung Epithelial	chr9	136728413	136728520	Hypomethylated
Lung Epithelial	chr10	1257678	1257979	Hypomethylated

Lung Epithelial	chr10	112889204	112889350	Hypomethylated
Lung Epithelial	chr17	80247859	80248026	Hypomethylated
Lung Epithelial	chr11	111171223	111172653	Hypomethylated
Lung Epithelial	chr6	46889597	46889761	Hypomethylated
Lung Epithelial	chr8	142157078	142157174	Hypomethylated
Lung Epithelial	chr17	9088165	9088467	Hypomethylated
Lung Epithelial	chr2	234393573	234394479	Hypomethylated
Lung Epithelial	chr16	677895	678174	Hypomethylated
Lung Epithelial	chr17	71548667	71548755	Hypomethylated
Lung Epithelial	chr1	2059898	2060168	Hypomethylated
Lung Epithelial	chr20	56296660	56296827	Hypomethylated
Lung Epithelial	chr2	234394372	234394479	Hypomethylated
Lung Epithelial	chr16	12354976	12355424	Hypomethylated
Lung Epithelial	chr7	33725751	33726087	Hypomethylated
Lung Epithelial	chr10	13771352	13771658	Hypomethylated
Lung Epithelial	chr22	46838804	46839245	Hypomethylated
Lung Epithelial	chr12	94093563	94093982	Hypomethylated
Lung Epithelial	chr6	155568960	155569279	Hypomethylated
Lung Epithelial	chr13	114115128	114115433	Hypomethylated
Lung Epithelial	chr4	1977200	1977236	Hypomethylated
Lung Epithelial	chr4	8248193	8248391	Hypomethylated
Lung Epithelial	chr6	46743464	46744227	Hypomethylated
Lung Epithelial	chr13	98869837	98870145	Hypomethylated
Lung Epithelial	chr16	3763054	3763348	Hypomethylated
Lung Epithelial	chr19	8554999	8555062	Hypomethylated
Lung Epithelial	chr7	5262422	5262575	Hypomethylated
Lung Epithelial	chr17	41024622	41025029	Hypomethylated
Lung Epithelial	chr21	43546441	43546655	Hypomethylated
Lung Epithelial	chr4	8436008	8436223	Hypomethylated
Lung Epithelial	chr22	19754439	19754839	Hypermethylated
Megakaryocyte	chr16	1814980	1815151	Hypomethylated
Megakaryocyte	chr16	3188474	3188511	Hypomethylated
Megakaryocyte	chr1	7911113	7911210	Hypomethylated
Megakaryocyte	chr10	467813	468083	Hypomethylated
Megakaryocyte	chr19	1872715	1873024	Hypomethylated
Megakaryocyte	chr9	71681914	71682266	Hypomethylated
Megakaryocyte	chr14	103566265	103566681	Hypomethylated
Megakaryocyte	chr16	88568536	88568989	Hypomethylated
Megakaryocyte	chr17	61498395	61498815	Hypomethylated
Megakaryocyte	chr8	145674184	145674298	Hypomethylated
Megakaryocyte	chr12	8941393	8941494	Hypomethylated
Megakaryocyte	chr5	481723	481794	Hypomethylated

Megakaryocyte	chr2	239023319	239023500	Hypomethylated
Megakaryocyte	chr5	37208967	37209202	Hypomethylated
Megakaryocyte	chr11	68522449	68522793	Hypomethylated
Megakaryocyte	chr19	56113869	56114292	Hypomethylated
Megakaryocyte	chr8	142180962	142181506	Hypomethylated
Megakaryocyte	chr14	92956120	92956450	Hypomethylated
Megakaryocyte	chr14	105167146	105167290	Hypomethylated
Megakaryocyte	chr19	16629936	16630066	Hypomethylated
Megakaryocyte	chr19	14066489	14067377	Hypomethylated
Megakaryocyte	chr5	37209212	37209381	Hypomethylated
Megakaryocyte	chr7	1785741	1786129	Hypomethylated
Megakaryocyte	chr7	137230659	137230997	Hypomethylated
Megakaryocyte	chr8	10273760	10274076	Hypomethylated
Megakaryocyte	chr7	631951	632605	Hypomethylated
Megakaryocyte	chr7	632621	632817	Hypomethylated
Megakaryocyte	chr11	63977307	63977540	Hypomethylated
Megakaryocyte	chr1	1871823	1871974	Hypomethylated
Megakaryocyte	chr19	5593710	5594329	Hypomethylated
Megakaryocyte	chr22	39909921	39910015	Hypomethylated
Megakaryocyte	chr11	68898452	68898667	Hypomethylated
Megakaryocyte	chr19	12799867	12800212	Hypomethylated
Megakaryocyte	chr19	49254245	49254406	Hypomethylated
Megakaryocyte	chr7	149129744	149130209	Hypomethylated
Megakaryocyte	chr15	74714681	74715017	Hypomethylated
Megakaryocyte	chr19	50376109	50376389	Hypomethylated
Megakaryocyte	chr8	145814448	145814812	Hypomethylated
Megakaryocyte	chr14	102963357	102963929	Hypomethylated
Megakaryocyte	chr16	88557690	88558056	Hypomethylated
Megakaryocyte	chr2	179640101	179641010	Hypomethylated
Megakaryocyte	chr3	194968078	194968601	Hypomethylated
Megakaryocyte	chr5	1924041	1924100	Hypomethylated
Megakaryocyte	chr5	53223677	53224181	Hypomethylated
Megakaryocyte	chr8	141312889	141313313	Hypomethylated
Megakaryocyte	chr16	1545317	1545676	Hypomethylated
Megakaryocyte	chr3	194835984	194836405	Hypomethylated
Megakaryocyte	chr8	53323520	53323719	Hypomethylated
Megakaryocyte	chr16	85551464	85551806	Hypomethylated
Megakaryocyte	chr16	86755593	86756024	Hypomethylated
Monocytes and Macrophage	chr3	196351807	196352171	Hypomethylated
Monocytes and Macrophage	chr12	6659484	6659682	Hypomethylated

Monocytes and Macrophage	chr13	32888845	32889052	Hypomethylated
Monocytes and Macrophage	chr3	195897812	195898123	Hypomethylated
Monocytes and Macrophage	chr19	8568502	8568649	Hypomethylated
Monocytes and Macrophage	chr16	85577692	85577882	Hypomethylated
Monocytes and Macrophage	chr12	132469818	132470033	Hypomethylated
Monocytes and Macrophage	chr17	80581522	80581992	Hypomethylated
Monocytes and Macrophage	chr3	128370161	128370565	Hypomethylated
Monocytes and Macrophage	chr4	3531428	3531642	Hypomethylated
Monocytes and Macrophage	chr9	95799785	95800025	Hypomethylated
Monocytes and Macrophage	chr18	74824341	74824414	Hypomethylated
Monocytes and Macrophage	chr12	56731566	56731735	Hypomethylated
Monocytes and Macrophage	chr16	56892384	56892556	Hypomethylated
Monocytes and Macrophage	chr16	85873312	85873440	Hypomethylated
Monocytes and Macrophage	chr2	134866101	134866288	Hypomethylated
Monocytes and Macrophage	chr8	602054	602159	Hypomethylated
Monocytes and Macrophage	chr5	79422700	79423348	Hypomethylated
Monocytes and Macrophage	chr16	3597075	3597441	Hypomethylated
Monocytes and Macrophage	chr6	26385502	26385842	Hypomethylated
Monocytes and Macrophage	chr14	69091384	69091490	Hypomethylated
Monocytes and Macrophage	chr2	240225004	240225176	Hypomethylated
Monocytes and Macrophage	chr21	45773615	45773734	Hypomethylated
Monocytes and Macrophage	chr11	47350123	47350267	Hypomethylated
Monocytes and Macrophage	chr19	2073026	2073176	Hypomethylated
Monocytes and Macrophage	chr22	37309307	37309488	Hypomethylated

Monocytes and Macrophage	chr2	28697740	28697866	Hypomethylated
Neuron	chr6	163558238	163558675	Hypomethylated
Neuron	chr1	2006859	2007311	Hypomethylated
Neuron	chr16	81731208	81731484	Hypomethylated
Neuron	chr9	138775499	138775772	Hypomethylated
Neuron	chr17	30815173	30815470	Hypomethylated
Neuron	chr8	143463390	143463895	Hypomethylated
Neuron	chr1	14113094	14113343	Hypomethylated
Neuron	chr1	110082688	110083518	Hypomethylated
Neuron	chr17	76675816	76676403	Hypomethylated
Neuron	chr18	42324837	42325188	Hypomethylated
Neuron	chr3	47932918	47934038	Hypomethylated
Neuron	chr11	117232011	117232455	Hypomethylated
Neuron	chr17	76818594	76819359	Hypomethylated
Neuron	chr10	131694516	131694773	Hypomethylated
Neuron	chr7	636539	636950	Hypomethylated
Neuron	chr11	6425899	6426501	Hypomethylated
Neuron	chr1	2236562	2236748	Hypomethylated
Neuron	chr1	6363384	6363883	Hypomethylated
Neuron	chr1	111145683	111147040	Hypomethylated
Neuron	chr2	1828347	1828664	Hypomethylated
Neuron	chr9	138627849	138628084	Hypomethylated
Neuron	chr10	3283414	3283766	Hypomethylated
Neuron	chr1	2238334	2238847	Hypomethylated
Neuron	chr19	4770747	4770983	Hypomethylated
Neuron	chr7	150816696	150817291	Hypomethylated
Neuron	chr17	77083111	77083194	Hypomethylated
Neuron	chr2	43019573	43020395	Hypermethylated
Neuron	chr3	49941216	49941579	Hypermethylated
Neuron	chr19	1467071	1467140	Hypermethylated
Neuron	chr6	166267918	166268069	Hypermethylated
Neuron	chr11	31841762	31842091	Hypermethylated
Neuron	chr17	8924080	8924226	Hypermethylated
Neuron	chr21	34755391	34755801	Hypermethylated
Neuron	chr19	38886319	38886635	Hypermethylated
Neuron	chr19	46142860	46142980	Hypermethylated
Neuron	chr9	38672385	38672729	Hypermethylated
Neuron	chr10	103534498	103534585	Hypermethylated
Neuron	chr11	64066758	64067406	Hypermethylated
Neuron	chr12	130529868	130530228	Hypermethylated
Neuron	chr19	1455008	1456059	Hypermethylated
Neuron	chr17	72352876	72353282	Hypermethylated

Neuron	chr19	46379859	46380198	Hypermethylated
Neuron	chr4	8200669	8201295	Hypermethylated
Neuron	chr12	49366026	49366367	Hypermethylated
Neuron	chr7	140346986	140347127	Hypermethylated
Neuron	chr7	131514718	131515081	Hypermethylated
Neuron	chr11	62693619	62693987	Hypermethylated
Neuron	chr8	145697840	145698117	Hypermethylated
Neuron	chr2	97151733	97152183	Hypermethylated
Neuron	chr14	70038530	70038749	Hypermethylated
Natural Killer	chr6	159457324	159457551	Hypomethylated
Natural Killer	chr6	10733158	10733571	Hypomethylated
Natural Killer	chr17	1104805	1105072	Hypomethylated
Natural Killer	chr5	10377133	10377320	Hypomethylated
Natural Killer	chr2	426239	426502	Hypomethylated
Natural Killer	chr6	158985966	158986382	Hypomethylated
Natural Killer	chr16	2892965	2893055	Hypomethylated
Natural Killer	chr10	72358669	72358804	Hypomethylated
Natural Killer	chr10	72362893	72363273	Hypomethylated
Natural Killer	chr19	4657680	4658152	Hypomethylated
Natural Killer	chr13	114874439	114874735	Hypomethylated
Natural Killer	chr3	39322103	39322776	Hypomethylated
Natural Killer	chr21	47845235	47846073	Hypomethylated
Natural Killer	chr22	46685653	46686131	Hypomethylated
Natural Killer	chr1	11395635	11395863	Hypomethylated
Natural Killer	chr11	120827449	120827536	Hypomethylated
Natural Killer	chr19	10226728	10226846	Hypomethylated
Natural Killer	chr13	77565285	77565577	Hypomethylated
Natural Killer	chr10	72357918	72358611	Hypomethylated
Natural Killer	chr16	89336192	89336628	Hypomethylated
Natural Killer	chr21	47830003	47830198	Hypomethylated
Natural Killer	chr16	84553497	84553694	Hypomethylated
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Pancreatic	chr17	80395185	80395451	Hypomethylated
Pancreatic	chr7	97843854	97844802	Hypomethylated
Pancreatic	chr16	3706036	3706908	Hypomethylated
Pancreatic	chr1	22303212	22303543	Hypomethylated
Pancreatic	chr9	139394558	139395025	Hypomethylated
Pancreatic	chr19	56658298	56658723	Hypomethylated
Pancreatic	chr6	35762792	35763566	Hypomethylated
Pancreatic	chr11	794340	794623	Hypomethylated
Pancreatic	chr4	186742139	186742364	Hypomethylated
Pancreatic	chr16	75252683	75252951	Hypomethylated

Pancreatic	chr17	78337515	78338076	Hypomethylated
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Pancreatic	chr9	135944497	135946085	Hypomethylated
Pancreatic	chr8	145685382	145685798	Hypomethylated
Pancreatic	chr16	630101	630351	Hypomethylated
Pancreatic	chr17	77923890	77924179	Hypomethylated
Pancreatic	chr8	103942869	103943309	Hypomethylated
Pancreatic	chr17	705656	706287	Hypomethylated
Pancreatic	chr19	39691205	39691581	Hypomethylated
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Pancreatic	chr9	139393948	139394113	Hypomethylated
Pancreatic	chr16	75255120	75255591	Hypomethylated
Pancreatic	chr10	130546138	130546266	Hypomethylated
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Pancreatic	chr5	1702312	1702471	Hypomethylated
Pancreatic	chr5	10757768	10758645	Hypomethylated
Pancreatic	chr7	97846514	97847025	Hypomethylated
Pancreatic	chr11	1321822	1322132	Hypomethylated
Pancreatic	chr12	110353054	110353218	Hypomethylated
Pancreatic	chr12	126676605	126676791	Hypomethylated
Pancreatic	chr17	37328203	37328335	Hypomethylated
Pancreatic	chr19	49254245	49254406	Hypomethylated
Pancreatic	chr7	97857387	97857615	Hypomethylated
Pancreatic	chr1	22854310	22854475	Hypomethylated
Pancreatic	chr7	150034206	150034367	Hypomethylated
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Pancreatic	chr6	35764704	35765094	Hypomethylated
Pancreatic	chr20	17633665	17633859	Hypomethylated
Pancreatic	chr4	1372943	1373417	Hypomethylated
Pancreatic	chr7	156797845	156798469	Hypermethylated
Pancreatic	chr13	28496765	28497184	Hypermethylated
Pancreatic	chr13	28497826	28498280	Hypermethylated
Pancreatic	chr11	119227020	119227708	Hypermethylated
Pancreatic	chr14	105714520	105715440	Hypermethylated
Pancreatic	chr7	157476754	157477033	Hypermethylated
Pancreatic	chr15	53089968	53090394	Hypermethylated
Pancreatic	chr19	47960704	47960978	Hypermethylated
Pancreatic	chr12	65218052	65218948	Hypermethylated
Pancreatic	chr13	28491265	28491526	Hypermethylated
Prostate Epithelial	chr20	18295551	18295861	Hypomethylated
Prostate Epithelial	chr7	2054500	2055180	Hypomethylated
Prostate Epithelial	chr17	40823869	40824215	Hypomethylated

Prostate Epithelial	chr17	9088165	9088467	Hypomethylated
Prostate Epithelial	chr22	40415244	40415401	Hypomethylated
Prostate Epithelial	chr1	27220656	27221119	Hypomethylated
Prostate Epithelial	chr7	5549534	5549731	Hypomethylated
Prostate Epithelial	chr16	81533203	81533549	Hypomethylated
Prostate Epithelial	chr11	70022863	70023099	Hypomethylated
Prostate Epithelial	chr7	1491787	1491952	Hypomethylated
Prostate Epithelial	chr8	143386225	143386548	Hypomethylated
Prostate Epithelial	chr8	142235391	142235926	Hypomethylated
Prostate Epithelial	chr19	8554999	8555062	Hypomethylated
Prostate Epithelial	chr10	116080333	116080495	Hypomethylated
Prostate Epithelial	chr19	14636919	14637391	Hypomethylated
Prostate Epithelial	chr9	140708598	140709066	Hypomethylated
Prostate Epithelial	chr16	23158676	23159171	Hypomethylated
Prostate Epithelial	chr19	30298144	30298342	Hypomethylated
Prostate Epithelial	chr6	6894045	6894183	Hypomethylated
Prostate Epithelial	chr18	77231461	77231655	Hypomethylated
Prostate Epithelial	chr20	60953594	60953740	Hypomethylated
Prostate Epithelial	chr1	17307773	17308093	Hypomethylated
Prostate Epithelial	chr1	19208516	19208778	Hypomethylated
Prostate Epithelial	chr2	174147832	174148599	Hypomethylated
Prostate Epithelial	chr8	8748227	8749041	Hypomethylated
Prostate Epithelial	chr11	129802069	129803188	Hypomethylated
Prostate Epithelial	chr3	124859057	124859348	Hypomethylated
Prostate Epithelial	chr3	197121225	197121420	Hypomethylated
Prostate Epithelial	chr19	48796188	48796381	Hypomethylated
Prostate Epithelial	chr4	1225408	1225653	Hypomethylated
Prostate Epithelial	chr19	1425504	1425707	Hypomethylated
Prostate Epithelial	chr1	3615514	3615746	Hypomethylated
Prostate Epithelial	chr10	5567123	5567503	Hypomethylated
Prostate Epithelial	chr10	81915434	81915647	Hypomethylated
Prostate Epithelial	chr11	120007968	120008155	Hypomethylated
Prostate Epithelial	chr20	18167947	18168360	Hypomethylated
Prostate Epithelial	chr7	551344	551759	Hypomethylated
Prostate Epithelial	chr1	38493014	38493557	Hypomethylated
Prostate Epithelial	chr6	33740395	33740572	Hypomethylated
Prostate Epithelial	chr7	26480584	26480922	Hypomethylated
Prostate Epithelial	chr10	133796508	133796631	Hypomethylated
Prostate Epithelial	chr22	43463194	43463331	Hypomethylated
Prostate Epithelial	chr22	47023029	47023196	Hypomethylated
Prostate Epithelial	chr1	2359816	2359937	Hypomethylated
Prostate Epithelial	chr21	46232702	46232873	Hypomethylated

Prostate Epithelial	chr22	43164347	43164751	Hypomethylated
Prostate Epithelial	chr4	1317409	1317679	Hypomethylated
Prostate Epithelial	chr10	30317521	30317689	Hypomethylated
Prostate Epithelial	chr16	73099735	73099893	Hypermethylated
Prostate Epithelial	chr6	10420567	10421060	Hypermethylated
Skeletal Muscular	chr7	148497704	148497985	Hypomethylated
Skeletal Muscular	chr18	46289370	46289545	Hypomethylated
Skeletal Muscular	chr10	99330013	99330204	Hypomethylated
Skeletal Muscular	chr11	129613860	129614016	Hypomethylated
Skeletal Muscular	chr1	9324223	9324364	Hypomethylated
Skeletal Muscular	chr18	55922618	55922960	Hypomethylated
Skeletal Muscular	chr3	14582440	14582904	Hypomethylated
Skeletal Muscular	chr17	78724929	78725376	Hypomethylated
Skeletal Muscular	chr17	3769455	3770035	Hypomethylated
Skeletal Muscular	chr10	23353063	23354028	Hypomethylated
Skeletal Muscular	chr18	55923895	55924181	Hypomethylated
Skeletal Muscular	chr1	1490850	1491199	Hypomethylated
Skeletal Muscular	chr2	201342380	201342659	Hypomethylated
Skeletal Muscular	chr7	611233	612221	Hypomethylated
Skeletal Muscular	chr3	52869808	52871468	Hypomethylated
Skeletal Muscular	chr17	65040730	65041120	Hypomethylated
Skeletal Muscular	chr7	1351324	1351753	Hypomethylated
Skeletal Muscular	chr17	76672617	76673183	Hypomethylated
Skeletal Muscular	chr1	54856305	54856608	Hypomethylated
Skeletal Muscular	chr4	25097386	25097706	Hypomethylated
Skeletal Muscular	chr4	100572972	100573578	Hypomethylated
Skeletal Muscular	chr7	613015	613422	Hypomethylated
Skeletal Muscular	chr14	20903959	20904321	Hypomethylated
Skeletal Muscular	chr17	79898313	79898988	Hypomethylated
Skeletal Muscular	chr5	138730990	138731670	Hypomethylated
Skeletal Muscular	chr17	11461316	11461624	Hypomethylated
Skeletal Muscular	chr7	1351335	1351753	Hypomethylated
Skeletal Muscular	chr7	56149083	56150012	Hypomethylated
Skeletal Muscular	chr14	104636600	104636829	Hypomethylated
Skeletal Muscular	chr7	612876	613698	Hypomethylated
Skeletal Muscular	chr10	134894695	134894939	Hypomethylated
Skeletal Muscular	chr17	11461335	11461638	Hypomethylated
Skeletal Muscular	chr4	126572121	126572399	Hypomethylated
Skeletal Muscular	chr16	89258456	89259792	Hypermethylated
Mature T	chr13	24825869	24826000	Hypomethylated
Mature T	chr22	37544974	37545667	Hypomethylated
Mature T	chr1	17054071	17054128	Hypomethylated

Mature T	chr10	13330036	13330429	Hypomethylated
Mature T	chr10	1156420	1156511	Hypomethylated
Mature T	chr2	85667088	85667542	Hypomethylated
Mature T	chr13	111841565	111842079	Hypomethylated
Mature T	chr14	99715693	99716236	Hypomethylated
Mature T	chr7	625073	625320	Hypomethylated
Mature T	chr11	60775001	60775404	Hypermethylated
Mature T	chr3	42113514	42114020	Hypermethylated

* The start and end points of the genomic region is with reference to the Homo sapiens full genome as provided by University of California Santa Cruz, version hg19 (Genome Reference Consortium GRCh37, February 2009).

Analysis of ctDNA

[0126] Aspects of the present invention involve analysis of ctDNA to determine clonal heterogeneity of tumor cells. The determination of the heterogeneity of cells of the tumor cells comprises genotyping the ctDNA in order to obtain a genotype profile of the ctDNA. The genotype profile of the ctDNA can be compared with the genotype profile of ctDNA previously obtained from the subject and is well established in the genotyping of cancers for signature mutations or for previously unknown mutations. These mutations may be a point mutation, , methylation changes, tumor-specific rearrangements (e.g., inversions, translocations, insertions and deletions), or cancer-derived viral sequences.

[0127] Examples of methods that can be used in genotyping include, but are not limited to, sequencing such as whole-genome sequencing or whole-exome sequencing; PCR; the Sanger-based ctDNA detection method (Newman *et al.*, 2014); BEAMing (beads, emulsion, amplification, and magnetics) developed by Diehl *et al.* (2008); and cancer personalized profiling by deep sequencing (CAPP-seq) (Newman *et al.*, 2014).

EXAMPLES

[0128] A study was conducted to establish sequencing-based, cell-type specific DNA methylation reference maps of human and mouse tissues to enable the assignment of DNA released from dying cells into the circulation back to its cellular origin. The study showed that cell-free, methylated DNA in blood samples revealed tissue-specific, cellular damage from radiation treatment.

Methods

[0129] Human serum sample collection. Breast cancer patients undergoing adjuvant radiation-therapy participated in the study. For serum isolation, peripheral blood (~8-12 ml) was collected and allowed to clot at room temperature for 30 minutes before centrifugation at 1500 x g for 20 min at 4 °C to separate the serum fraction. The serum was aliquoted in 0.5 mL fractions and stored at -80 °C until use. Serial serum samples were collected from 15 breast cancer patients at Baseline (before radiation treatment), End-of-Treatment (EOT; 30 minutes after the last radiation treatment), and Recovery (one month after cessation of radiation treatment), thus allowing for a within-patient internal control and baseline. A schematic of the time series for sample collection can be found in **FIG. 2**. Patients received either three-dimensional conformal RT (3D-CRT) or a combination of proton beam therapy (PBT) and 3D-CRT. Patient characteristics and treatment details including radiation dosimetry are summarized in **Table 3** and in Barefoot *et al.*, 2022, Supplemental Table 8.

[0130] Mouse serum and tissue collection. C57Bl6 mice (n=18) were irradiated to the upper thorax at varying dose (sham control, 3Gy, 8Gy) for 3 consecutive treatments. Serum and tissues were collected 24 hours after the last radiation dose. For serum isolation, blood was collected via cardiac puncture (~1 mL) and allowed to clot at room temperature for 30 minutes before centrifugation at 1500 x g for 20 min at 4 °C to separate the serum fraction. Heart, lung, and liver tissues were harvested and sectioned to be both flash frozen and formalin fixed for subsequent analysis.

[0131] Cell isolation. Reference methylomes were generated for mouse immune cell-types and human endothelial cell-types to augment publicly available datasets. Peripheral blood and bone marrow were isolated and spleens from healthy C57Bl6 mice were dissociated to single cells and FACS sorted using cell-type specific antibodies. Buffy coat (n=4), bone marrow (n=3), CD19+ B cell (n=1), CD4 T cell (n=1), CD8 T cell (n=1) and Gr1+ Neutrophil (n=1) methylomes were generated using the following antibodies: FITC anti-mouse CD45, Alexa Fluor 647 anti-mouse CD3, Brilliant Violet 711 anti-mouse CD4, Brilliant Violet 421 anti-mouse CD8a, PE anti-mouse CD19, PE/Cy7 anti-mouse Ly-6G/Ly-6C (Gr-1) (all BioLegend 1:20). Cryopreserved passage 1 human liver sinusoidal endothelial cells (LSEC) were purchased. Purity was determined by immunofluorescence with antibodies specific to vWF/Factor VIII and CD31 (PECAM). Cryopreserved passage 2 human coronary artery, cardiac microvascular, pulmonary artery, and pulmonary

microvascular endothelial cells were isolated from single donor healthy human tissues purchased. All endothelial cell populations were CD31 positive and Dil-Ac-LDL uptake positive. Paired RNA-seq data was generated from the same cell-populations used for DNA methylome profiling to validate the identity of purchased cell populations through analysis of cell-type expression markers.

[0132] *RNA isolation, RNA-sequencing, and RT-qPCR analysis.* RNA was isolated from tissues or sorted cells using the RNeasy Kit following homogenization step using the MagNA Lyser according to the manufacturer's protocol and quantified by Qubit RNA BR assay. Total RNA samples were validated using an Agilent RNA 6000 nano assay on the 2100 Bioanalyzer TapeStation. The resulting RNA Integrity number (RIN) of samples selected for downstream qPCR or RNAseq analysis was at least 7. Reverse transcription was done using iScript cDNA Synthesis Kit according to the manufacturer's protocol. Real-time quantitative RT-PCR was performed with iQ SYBR Green Supermix. Primers used for RT-qPCR were purchased from Integrated DNA Technologies. Fold change was calculated as a percentage normalized to housekeeping gene human actin (ACTB) using the delta-Ct method. All RT-qPCR assays were done in triplicate. RNA-sequencing libraries were prepared using TruSeq Total RNA library Prep Kit at Novogene Corporation Inc., and 150bp paired-end sequencing was performed on an Illumina Hiseq 4000 with a depth of 50 million paired reads per sample. A reference index was generated using GTF annotation from GENCODEv28. Raw FASTQ files were aligned to GRCh38 or GRCm38 with HISAT2. Derived counts per million and P-value were used to create a rank ordered list, which was then used for subsequent analysis and confirmation of the identity of isolated cell-types for methylome analysis. Expression levels at known cell type markers from single cell expression databases were used to validate the identity of isolated cell-type populations for methylome analysis (Khan *et al.*, 2018).

[0133] *Isolation of circulating cfDNA.* Circulating cfDNA was extracted from 3-4 mL human serum and 0.5 mL mouse serum, using the QIAamp Circulating Nucleic Acid kit according to the manufacturer's instructions. CfDNA was quantified via Qubit fluorometer using both the dsDNA High Sensitivity Assay Kit. As a quality control, fragment size distribution of isolated cfDNA was verified based on analysis using a 2100 Bioanalyzer TapeStation. Additional purification using Beckman Coulter beads was implemented to remove high-molecular weight DNA reflective of cell-lysis and leukocyte contamination as

previously described (Maggi *et al.*, 2018). Size distribution of cfDNA fragments were re-verified using 2100 Bioanalyzer TapeStation analysis following purification.

[0134] *Isolation and fragmentation of genomic DNA.* Genomic DNA from tissues was extracted with DNeasy Blood and Tissue Kit following the manufacturer's instructions and quantified via Qubit fluorometer dsDNA BR Assay Kit. Genomic DNA was fragmented via sonification using a Covaris E220 instrument to the recommended 150-200 base pairs before library preparation. Lambda phage DNA was also fragmented and included as a spike-in to all DNA samples at 0.5%w/w, serving as an internal unmethylated control. Bisulfite conversion efficiency was calculated through assessing the number of unconverted C's on unmethylated lambda phage DNA. The SeqCap Epi capture pool contains probes to capture the lambda genomic region from base 4500 to 6500. The conversion rate was calculated as follows: conversion rate = $1 - (\text{sum}(\text{C_count}) / \text{sum}(\text{CT_count}))$ across the lambda genomic region captured.

[0135] *Bisulfite capture-sequencing library preparation.* Bisulfite capture-sequencing libraries were generated from either cfDNA or reference DNA inputs according to the same protocol. As a first step, WGBS libraries were generated using the Zymo Research Pico Methyl-Seq Library Prep Kit (D5455) with the following modifications. Bisulfite-conversion was carried out using the Zymo EZ DNA Methylation Gold kit instead of the EZ DNA Methylation-Lightning Kit. For mouse samples, cfDNA from two mice in the same group was pooled as input to library preparation. An additional 2 PCR cycles were added to the recommended cycle number based on total input cfDNA amounts. WGBS libraries were eluted in 15 μL 10 mM Tris-HCl buffer, pH 8. Library quality control was performed with an Agilent 2100 Bioanalyzer and quantity determined via KAPA Library Quantification Kit.

[0136] Cell-free WGBS libraries were pooled to meet the required 1 μg DNA input necessary for targeted enrichment. However, no more than four WGBS libraries were pooled in a single hybridization reaction and the 1 μg input DNA was divided evenly between the libraries to be multiplexed. Hybridization capture was carried out according to the SeqCap Epi Enrichment System protocol using SeqCap Epi CpGiant probe pools for human samples and SeqCap Epi Developer probes for mouse samples with xGen Universal Blocker-TS Mix as the blocking reagent. Washing and recovering of the captured library, as well as PCR amplification and final purification, were carried out as recommended by the manufacturer. The capture library products were assessed by Agilent Bioanalyzer DNA 1000 assays.

Bisulfite capture-sequencing libraries with inclusion of 15-20% spike-in PhiX Control v3 library were clustered on an Illumina Novaseq 6000 S4 flow cell followed by 150-bp paired-end sequencing.

[0137] *Bisulfite sequencing data alignment and preprocessing.* Paired-end FASTQ files were trimmed using Trim Galore (<https://github.com/FelixKrueger/TrimGalore>) with parameters “--paired -q 20 --clip_R1 10 --clip_R2 10 --three_prime_clip_R1 10 --three_prime_clip_R2 10” (<https://github.com/FelixKrueger/Bismark>). Trimmed paired-end FASTQ reads were mapped to the human genome (GRCh37/hg build) using Bismark (V 0.22.3) with parameters “--non-directional”, then converted to BAM files using Santools (V 1.12). BAM files were sorted and indexed using Santools (V1.12). Reads were stripped from non-CpG nucleotides and converted to BETA and PAT files using webstools (V 0.1.0), a tool suite for working with WGBS data while preserving read-specific intrinsic dependencies (https://github.com/nloyfer/wgbs_tools) (Loyfer *et al.*, 2022; Loyfer & Kaplan).

[0138] *Reference DNA methylation data from healthy tissues and cells.* Controlled access to reference WGBS data from normal human tissues and cell-types was requested from public consortia participating in the International Human Epigenome Consortium (IHEC) and upon approval downloaded from the European Genome-Phenome Archive (EGA), Japanese Genotype-phenotype Archive (JGA), and database of Genotypes and Phenotypes (dbGAP) data repositories (**Table 4**; *see also* Barefoot *et al.*, 2022, Supplemental Table 1). Reference mouse WGBS data from normal tissues and cell-types was downloaded from select GEO and SRA datasets (**Table 5**). Downloaded FASTQs were processed and realigned in a similar manner as the locally generated bisulfite-sequencing libraries described above. However, parameters were adjusted to account for each respective WGBS library type at both trimming and alignment steps as previously described in the Bismark User Guide (<http://felixkrueger.github.io/Bismark/Docs/>). WGBS libraries were deduplicated using deduplicate_bismark (V 0.22.3). Special consideration of bisulfite conversion efficiency was given to samples prepared by the μ WGBS protocol and reads with a bisulfite conversion rate below 90% or with fewer than three cytosines outside a CpG context were removed.

[0139] *Segmentation and clustering analysis.* The genome was segmented into blocks of homogenous methylation as previously described in Loyfer *et al.* 2022 using wgbstools (with parameters segment --max_bp 5000) (Loyfer *et al.*, 2022; Loyfer & Kaplan). In brief, a

multi-channel Dynamic Programming segmentation algorithm was used to divide the genome into continuous genomic regions (blocks) showing homogenous methylation levels across multiple CpGs, for each sample. The segmentation algorithm was applied to 278 human reference WGBS methylomes and retained 351,395 blocks covered by the hybridization capture panel used in the analysis of cfDNA in human serum (captures 80Mb, ~20% of CpGs). Likewise, segmentation of 103 mouse WGBS datasets from healthy cell types and tissues identified 1,344,889 blocks covered by the mouse hybridization capture panel (captures 210 Mb, ~75% of CpGs). The hierarchical relationship between reference tissue and cell type WGBS datasets was visualized through creation of a tree dendrogram. The top 30,000 most variant methylation blocks containing at least three CpG sites and coverage across 90% of samples were selected. The average methylation for each block and sample was computed using `wgbstools (--beta_to_table)`. Trees were assembled using the unweighted pair-group method with arithmetic mean (UPGMA), using `scipy` (V 1.7.1) and L1 distance, and then visualized in R with the `ggtree` package (V 2.4.1). The similarity between samples was assessed by the degree of variation in distance between samples of the same cell-type (average 23,056) compared to samples between different cell-types (average 273,018). Dimensional reduction was also performed on the selected blocks using the UMAP package (V 0.2.8.2.0). Default UMAP parameters were used (15 neighbors, 2 components, Euclidean metric, and a minimum distance of 0.1).

[0140] *Identification of cell-type specific methylation blocks.* The original 278 human WGBS samples were reduced to a final set of 104 samples to identify differentially methylated cell-type specific blocks. Samples from bulk tissues and those that did not have sufficient coverage (missing values in >50% of methylation blocks) were excluded. Outlier replicates, or those clustering with fibroblasts or stromal cell types were excluded, due to possible contamination. Only immune cell methylomes that were reprocessed from raw sequencing data to PAT files were used to identify DMBs. The final 104 human reference samples were organized into groupings of 20 cell-types (see **Table 4** and Barefoot *et al.*, 2022, Supplemental Table 1). Similarly, the starting 103 mouse WGBS samples were reduced to a final set of 44 samples that were organized into a final grouping of 9 cell-types and tissues (see **Table 5** and Barefoot *et al.*, 2022, Supplemental Table 2). Tissue and cell-type specific methylation blocks were identified from the final reduced reference WGBS data using custom scripts. A one-vs-all comparison was performed to identify differentially methylated blocks unique for each group. This was done separately for human and mouse.

First, blocks covering a minimum of three CpG sites, with length less than 2Kb and at least 10 observations, were identified. Then, the average methylation per block/sample was calculated, as the ratio of methylated CpG observations across all sequenced reads from that block. Differential blocks were sorted by the margin of separation, termed “delta beta”, defined as the minimal difference between the average methylation in any sample from the target group versus all other samples. Blocks with a delta-beta ≥ 0.4 in human and ≥ 0.35 in mouse were then selected. This resulted in a variable number of cell-type specific blocks available for each tissue and cell-type. Each DNA fragment was characterized as U (mostly unmethylated), M (mostly methylated) or X (mixed) based on the fraction of methylated CpG sites as previously described (Loyfer *et al.*, 2022). Thresholds of $\leq 33\%$ methylated CpGs for U reads and $\geq 66\%$ methylated CpGs for M were used. A methylation score was calculated for each identified cell-type specific block based on the proportion of U/X/M reads among all reads. The U proportion was used to define hypomethylated blocks and the M proportion was used to define hyper methylated blocks. Selected human and mouse blocks for cell-types of interest can be found in Barefoot *et al.*, 2022, Supplemental Tables 3 and 4. Heatmaps were generated using the pretty heatmap function in the RStudio Package for the R Bioconductor.

[0141] *Likelihood-based probabilistic model for fragment-level deconvolution.* The cell type origins of cfDNA were determined using a probabilistic fragment-level deconvolution algorithm. Using this model, the likelihood of each cfDNA molecule was calculated using a 4th order Markov Model, considering the joint methylation status of up to 5 adjacent CpG sites. Within individual tissue and cell-type specific blocks, this model is used to predict whether each molecule is classified as belonging to the tissue of interest or alternatively is classified as background. The posterior probability of each cfDNA molecule is calculated based on the log-likelihood that the origins of the specific read-pair came from the target cell-type times the prior knowledge of the probability that any read should originate from the target cell-type. The model was trained on reference bisulfite-sequencing data from normal cells and tissues to learn the distribution of each marker in the target tissue/cell-type of interest compared to background. Then the model was applied to test cfDNA methylomes for binary classification of the origins of each cfDNA molecule. The proportion of molecules assigned to the tissue of interest across all cell-type specific blocks was then summed and used to determine the relative abundance of cfDNA derived from that tissue origins in each respective sample. The resulting proportions were adjusted to have a sum of 1 through

imposing a normalization constraint. Relative tissue-of-origin percentages were converted to genome equivalents and reported as an absolute measure (Geq/mL) considering the initial cfDNA concentrations [*i.e.*, fraction cell-type specific cfDNA x initial concentration cfDNA ng/mL x 3.3×10^{-12} grams per human haploid genome equivalent (or 3.0×10^{-12} grams per mouse haploid genome equivalent)].

[0142] *In-silico simulations WGBS deconvolution.* In silico mix-in simulations were performed to validate the fragment-level deconvolution algorithm at identified cell-type specific blocks included in the radiation-specific methylation atlas (**FIGS. 3 and 4**). Reference data with greater than three replicates per cell-type was split into independent training and testing sets, leaving at least one replicate out for testing. Since the mouse cardiomyocyte reference WGBS data had less than three replicates, fragments were merged across replicates for this cell-type and split into training (80%) and testing (20%) sets. For each cell-type profiled, known proportions of target fragments were mixed into a background of leukocyte fragments across identified cell-type specific methylation blocks (leukocyte fragments obtained from n=4 buffy coat samples in mouse and n=10 buffy coat samples in human). Ten replicates for each admixture ratio assessed (0.001, 0.005, 0.01, 0.02, 0.05, 0.1, 0.15) were performed, and the average predicted proportion and standard deviation across all replicates was presented. Model accuracy was assessed through correct classification of the actual percent target mixed and relative degree of incremental change with increasing amount of target reads admixed was used to assess accuracy in estimating proportional changes across groups (mouse) and timepoints from serial samples (human). The cell-type specific blocks included in the radiation-specific methylation atlas were constructed using training set fragments only. Merging, splitting, and mixing of reads were preformed using wgbstools (Loyfer & Kaplan).

[0143] *Longitudinal analysis of serial serum samples.* Longitudinal analysis was performed on serial serum samples collected from breast cancer patients. Changing cell-type proportions of cfDNA at the end of treatment (EOT) and at Recovery were evaluated relative to baseline levels before the start of therapy (Baseline). Fold change (FC) from baseline was used to represent the percent cell-type cfDNA at EOT and Recovery relative to Baseline within the same individual. An exploratory correlation analysis was performed to evaluate linear relationship of changing cell-type proportions from EOT relative to Baseline, using Pearson's Correlation Coefficient.

[0144] *Functional annotation and pathway analysis.* Identified cell-type specific methylation blocks were provided as input for analysis in HOMER (<http://homer.ucsd.edu/homer/>). Each block was associated with its closest nearby gene and provided a genomic annotation. By default, TSS (transcription start site) was defined from -1kb to +100 bp, TTS (transcription termination site) was defined from -100 bp to +1kb, and CpG islands were defined as a genomic segment with GC content $\geq 50\%$, genomic length > 200 bp and the ratio of observed/expected CpG number > 0.6 . Prediction of known and de-novo transcription factor binding motifs were also assessed by HOMER. The top 5 motifs based on p value were selected from each analysis. Pathway analysis of identified tissue and cell-type specific methylation blocks was performed using Ingenuity Pathway Analysis (IPA) and Genomic Regions Enrichment of Annotations Tool (GREAT) (McLean *et al.*, 2010). GeneSetCluster was used to cluster identified gene-set pathways based on shared genes (Ewing *et al.*, 2020). The WebgestaltR (ORAperGeneSet) plugin was used to interpret and functionally label identified gene-set clusters by reducing all identified significant gene-set pathways to the topmost representative one. Integration of methylome and transcriptome data generated from tissue-specific endothelial cells was performed using an expanded set of cell-type specific blocks (--bg.quant 0.2) compared to the more restricted set of blocks used for deconvolution analysis in the circulation (--bg.quant 0.1). The extended endothelial-specific methylation blocks can be found in Barefoot *et al.*, 2022, Supplemental Table 10.

[0145] *Cluster analysis and visualization techniques.* The hierarchical relationship between reference tissue and cell-type WGBS datasets was visualized through creation of a tree dendrogram. The top 30,000 most variant methylation blocks containing at least three CpG sites and coverage across 90% of samples were selected. The average methylation for each block and sample was computed using wgbstools (--beta_to_table). Trees were assembled using the unweighted pair-group method with arithmetic mean (UPGMA) and visualized in R with the ggtree package. Dimensional reduction was also performed on the selected blocks using the UMAP algorithm. Default UMAP parameters were used (15 neighbors, 2 components, Euclidean metric, and a minimum distance of 0.1). Heatmaps were generated using the pretty heatmap function in the RStudio Package for the R bioconductor (RStudioTeam, 2015). Statistical analyses for group comparisons and correlations were performed using Prism and R. Sequencing reads were visualized using the Integrative Genomics Viewer (IGV) using the bisulfite CG mode for alignment coloring (Robinson *et al.*, 2011). The BEDTools suite and AWK programming were used to overlay the

sequencing data across samples to compare across sample groups and replicates. Python was used to operate WGBS tools and also to create visualization plots.

Results

[0146] *DNA methylation is highly cell-type specific and reflects cell lineage specification.*

Access to reference human and mouse WGBS datasets was obtained from publicly available databases and identified cell-type specific differential DNA methylation patterns, preferentially from primary cells isolated from healthy human and mouse tissues. Additionally, cell-type specific methylomes were generated for purified mouse immune cell-types (CD19+ B cell, Gr1+ Neutrophil, CD4+ T cell, and CD8+ T cell) and human tissue-specific endothelial cell-types (coronary artery, pulmonary artery, cardiac microvascular, pulmonary microvascular, and liver sinusoidal endothelial). Due to limited cell-type specific data available for mouse, reference data from mouse bulk tissues were included if none was available from purified cell-types within those tissues. This resulted in curation of methylation data from 10 different cell-types and 18 tissues for mouse and over 30 distinct cell-types for human (**Tables 4 and 5**; see also Barefoot *et al.*, 2022, Supplemental Table 10).

[0147] To better understand the epigenomic landscape of these healthy human and mouse cell-types in tissues, the methylomes were characterized by first segmenting the data into homogeneously methylated blocks where DNA methylation status at adjacent CpG sites is highly co-regulated due to the processivity of methylation enzymes (Loyfer *et al.*, 2022). Exploring the epigenetic variation amongst cell-types at the block-level increased robustness of down-stream analysis, proving more resistant to noise introduced as a by-product of the bisulfite sequencing. The segmentation was applied to 275 publicly available human WGBS datasets from purified cell-types to identify 351,395 blocks that are contained in the probes used for hybridization capture sequencing to enrich for cfDNA in human serum (**Table 4**). Segmentation of 83 WGBS datasets from normal cell-types and tissues in mouse identified 1,344,889 blocks that are contained in the mouse hybridization capture probes (**Table 5**). On average, each block was greater than 300 bp with 4-8 CpG sites per block. Unsupervised hierarchical clustering analysis of the top 30,000 most variable methylation blocks in human and mouse, respectively shows the relationship between samples as a dendrogram and UMAP projection (**FIGS. 5 and 6**). The tightly correlated relationship between methylomes of the same cell-type observed from the cluster analysis reinforces the concept that methylation status is conserved at regions critical to cell-type identify. The within cell-type variation is

noticeably reduced compared to the between cell-type variation. This stability allows methylated DNA to serve as a robust biomarker in the face of patient heterogeneity, capable of being generalized across diverse patient populations. For the most part, cell-types composing distinct lineages remain closely related, including immune, epithelial, muscle, neuron, endothelial, and stromal cell-types. Examples are tissue-specific endothelial and tissue-resident immune cells that cluster with endothelial or immune cells respectively, independent of the germ layer origin of their tissues of residence. Also, some cell types cluster separately from their bulk tissue counterparts. For instance, cardiomyocytes cluster separately from heart tissue in the mouse dendrogram, indicating heterogenous composition and distinct embryonic origins of different cell-types that contribute to organs (**FIG. 6, Panel A**). Surprisingly, a large epigenetic distance between immune cells of hematopoietic origins and solid organ cells from other lineages was observed (**FIG. 5, Panels A and B**). This is important for the tissue-of-origin analysis of cfDNA in the circulation, to distinguish solid organ from the hematopoietic origins of the DNA. Quite unexpectedly, a large number of epigenetic signatures capable of distinguishing amongst immune cells was also found, with cell-types of lymphoid and myeloid lineages forming distinct clusters. Within the immune cell cohort, increased separation of terminally differentiated cells compared to precursors was observed, with naïve B and T cells clustering separately from their more mature central and effector memory counterparts (**FIG. 5, Panel B**). Collectively, these findings support that DNA methylation is highly cell-type specific and reflects cell lineage specification.

[0148] *Differential DNA methylation distinguishes amongst cell-types in healthy human and mouse tissues.* Based on the above unsupervised clustering analysis, the inclusion/exclusion criteria were further refined to select a final set of reference methylomes used to identify differentially methylated cell-type specific blocks. Low coverage WGBS samples were excluded from bulk tissues. Also, samples that did not cluster with other replicates were excluded from the same cell-type and instead clustered with fibroblast and other stromal cell-types. This resulted in a reduction of the starting 278 human WGBS samples to a final set of 104 samples that were organized into a grouping of 20 cell-types. Similarly, the starting 103 mouse WGBS samples were reduced to a final set of 44 samples that were organized into a final grouping of 9 cell-types and tissues. Subsets of some related cell-types were considered together to form the final groups (i.e., monocytes grouped together with macrophages and colon grouped together with small intestine). This final combination of groups was found to best represent the cell-specific epigenetic variation as a whole without overlap, using this

publicly available data. Cell-type specific differentially methylated blocks (DMBs) that contained a minimum of 3 CpG sites were identified. The co-methylation status of neighboring CpG sites in these blocks were able to distinguish amongst all cell-types included in the final groups. 4,502 human and 7,344 mouse DMBs (*see Barefoot et al., 2022, Supplemental Tables 3 and 4*) with a lower margin of separation for mouse (0.35) versus human (0.40) due to more limited data were identified. A complete summary of human and mouse cell-type specific methylation blocks identified is in **Tables 6 and 7**. A variable number of blocks was required to achieve the same specificity for each cell-type based on the depth of coverage, purity, and degree of separation from other tissues and cell-types included in the atlas. Similar to others, we found enhanced separation of reference datasets using methylomes of purified cell-types as opposed to more heterogenous mixtures from bulk tissues (Moss *et al.*, 2018). This is evident from the low number of DMBs identified from mouse bulk tissues as compared to mouse purified cell-types (average of 310 DMBs/tissue versus 1,488 DMBs/cell-type). Although over 85% of cell-type specific DMBs are hypomethylated, the blocks were depicted as a heatmap using a methylation score that is agnostic to the directionality of the methylation status and emphasizes the degree of separation of both hypo- and hyper-methylated blocks in the target group relative to all other groups. The methylation score calculates the number of fully unmethylated or methylated read-pairs divided by total coverage for hypo- and hyper- methylated blocks, respectively. The heatmaps in **FIG. 7** depicts up to 100 blocks for each cell-type group with the highest methylation score.

[0149] *Differential DNA methylation is closely linked to regulation of cell-type specific functions.* The role of cell-type specific methylation in shaping cellular identity and function was investigated. Genes adjacent to cell-type specific methylation blocks were identified using HOMER and performed pathway analysis of annotated genes using both Ingenuity Pathway Analysis (IPA) and GREAT. GeneSetCluster was used to group significantly enriched pathways based on shared genes and WebgestaltR functionally labeled each cluster by its top defining biological process (**FIG. 7, Panel C**; and **FIG. 8**). Gene-set pathways largely clustered within independent cell-type groups, reinforcing that cell-specific differential methylation occurs adjacent to unique genes integral to cell-type specific functions. Collectively, cell-type specific methylation was preferentially located adjacent to genes with biological functions involving cell development, movement, proliferation, differentiation, and morphology. In addition, transcriptional machinery genes including

transcription factors and co-regulators were significantly associated with cell-type specific DNA methylation, specifically those involving assembly of RNA polymerase III complex and pre-mRNA catabolic process (*see Table 11*). However, despite these commonalities, important biological differences were also observed in the gene sets identified based on specific processes unique to the cell-types profiled. For example, the biological function of genes associated with immune cell-type specific methylation reflects processes of leukocyte cell-cell adhesion, immune response-regulating signaling, and hematopoietic system development (**FIG. 7, Panel C**). In contrast, fatty acid metabolic process, lipid metabolism, and acute phase response signaling were identified for hepatocytes. These findings suggest that cell-type specific methylation is involved in regulation of these cellular processes. Significantly enriched biological pathways and functions for genes associated with differential methylation in each cell-type examined are provided in **Table 11**.

[0150] Cell-type specific DNA methylation is majority hypomethylated and enriched at intragenic regions containing developmental TF binding motifs. The majority of identified human and mouse cell-type specific blocks were hypomethylated, consistent with the proposed mechanisms of methylation resetting during embryonic development that leads to highly regulated cell-type specific differences (Greenberg & Bourc'his, 2019; Dor & Cedar, 2018). It was found that, in human samples, 86% of cell-type specific DMBs hypomethylated and only 14% hypermethylated. Strikingly in the mouse samples, 98% of cell-type specific DMBs were hypomethylated and only 2% were hypermethylated. The schematic in **FIG. 9, Panel A** depicts the location of identified human cell-type specific hypo- and hyper- methylated blocks. Interestingly, regardless of directionality the majority of cell-type specific blocks were located within intragenic regions. To see if this distribution was enriched, the genomic loci of cell-type specific blocks were compared to blocks that did not vary amongst cell-types (**FIG. 9, Panels B and C; Table 8**). It was found that for both human and mouse, there was a significant enrichment of cell-type specific blocks within intragenic regions relative to other captured regions ($p<0.05$). Furthermore, the intragenic distribution of cell-type specific blocks showed a significant increase of locations within exons and decrease in promoter-TSS segments ($p<0.05$). There was also a significant relationship between directionality and intragenic distribution, with a larger proportion of cell-type specific blocks being hypermethylated in exons and hypomethylated in introns ($p<0.05$). The similar distribution of cell-type specific methylation blocks in human and mouse suggests a conserved biological function of these genomic regions across species.

[0151] To further explore what common purpose these identified regions may have in human and mouse development, motif analysis was performed using HOMER to see if there were commonly enriched transcription factor binding sites (TFBS). MADS motifs bound by MEF2 transcription factors were significantly enriched in both human and mouse cell-type specific hypomethylated blocks (**FIG. 9, Panel D, left**). The MEF2 transcription factors are established developmental regulators with roles in the differentiation of many cell-types from distinct lineages. In comparison, Homeobox motifs bound by several different HOX TFs were enriched in the human cell-type specific hypermethylated blocks (**FIG. 9, Panel D, right**). Specifically, HOXB13 was the top TF associated with binding at sites within the human hypermethylated DMBs. Recently, HOXB13 has been found to control cell state through binding to super-enhancer regions, suggesting a novel regulatory function for cell-type specific hypermethylation. In addition to the common TFBS enriched by all cell-type specific blocks, endothelial-specific TFs were found to be enriched in the endothelial-cell hypomethylated blocks, including EWS, ERG, Fli1, ETV2/4, and SOX6 (*see FIG. 10, Panel D*). As a whole, this data reveals unknown functions of these cell-type specific blocks that represent cell-specific biology.

[0152] Methylation profiling of tissue-specific endothelial cell-types reveals epigenetic heterogeneity associated with differential gene expression. Radiation-induced endothelial damage is a major complicating factor of radiotherapy that is thought to be a leading cause for development of late-onset cardiovascular disease (Tapio, 2016; Wagner & Dimmeler, 2019). The microvasculature is particularly sensitive to radiation, with dysfunction of these cells potentially contributing to damage in a variety of tissues (Wijerathne et al., 2021; Park et al., 2012). Thus, tissue-specific endothelial methylomes and paired transcriptomes were generated in order to profile damage from distinct populations of microvascular and large vessel endothelial cell-types including coronary artery, pulmonary artery, cardiac microvascular, pulmonary microvascular, and liver sinusoidal endothelial. Also made use were publicly available umbilical vein endothelial methylomes from the Blueprint Epigenome Consortium to complement our data (**Table 4**; *see also Barefoot et al., 2022, Supplemental Table 1*). Previous studies support modeling the heart and lung as an integrated system in the development of radiation damage since the heart and lungs are linked by the cardiopulmonary circulation (Barazzuol et al., 2020). Therefore, cardiac and pulmonary endothelial cell-types were merged together to generate a joint cardiopulmonary endothelial signal and identified the specific methylation blocks for cardiopulmonary (CPEC,

n = 132), liver sinusoidal endothelial (LSEC, n = 89), and umbilical vein endothelial (HUVEC, n = 116) cell-types. Pathway analysis of genes associated with these methylation blocks confirmed endothelial cell identity, revealing genes involved in regulation of vasculogenesis, angiogenesis, and vascular development (**FIG. 10, Panel B**). In addition, unique pathways were identified capturing the tissue-specific epigenetic diversity of these different endothelial cell populations. For example, Hepatic Fibrosis Signaling was found to be LSEC-specific, Cardiac Hypertrophy Signaling identified as CPEC-specific, and Thioredoxin Pathway activity was specific to HUVEC (**FIG. 10, Panel A**). The identity of starting material used to generate these human endothelial methylomes was validated through paired RNA-sequencing analysis. Integrative analysis of DNA methylation and paired RNA expression allowed for better understanding of the relationship between cell-type specific DNA methylation and corresponding changes in gene expression. Methylation status at several identified blocks was found to correspond with RNA expression of known endothelial-specific genes, confirming the identity of the LSEC and CPEC populations isolated (**FIG. 10, Panel C and E**; Barefoot *et al.*, 2022, Supplemental Table 10). For example, hypomethylation was associated with increased expression at several pan-endothelial genes, including NOTCH1, ACVRL1, FLT1, MMRN2, NOS3 and SOX7. Likewise, hypomethylation at CPEC- and LSEC-specific genes led to differential expression when comparing the two populations, reflecting tissue-specific differences. CPEC- and LSEC-specific expression of selected genes have been reported in previous studies examining vascular heterogeneity at the transcriptome level (Feng *et al.*, 2019; Sabbagh *et al.*, 2018; Nolan *et al.*, 2013; Cleuren *et al.*, 2019). However, linking these expression patterns with cell-type specific methylation is a novel feature. While the majority of endothelial-specific methylation blocks were hypomethylated, select hypermethylated blocks were identified as well, including CCM2L in CPEC that corresponded with decreased gene expression compared with LSEC. As a relatively abundant cell-type in the circulation, the ability to non-invasively detect distinct damage to different types of endothelial cell populations could prove useful to monitor tissue-specific damages.

[0153] *Development of a radiation-specific methylation atlas focusing on cell-types from target organs-at-risk (OAR).* After ensuring specificity of identified cell-type specific methylation blocks by comparison to all other cell-types with available WGBS data, the assessment of cfDNA origins in the circulation was limited to select cell-types originating from target organs-at-risk for radiation damage. Restriction to a focused radiation-specific

methylation atlas helped to maintain sensitivity of radiation-induced damage to cell-types of interest based on prior knowledge of organs targeted and damaged due to existing clinical correlates. Representative treatment planning for breast cancer patients receiving adjuvant radiation provides an estimate organ volume impacted and radiation dose level for target organs-at-risk from radiation damage, including the heart and lungs (**FIG. 11, Panel A**). In addition to organs that are in close proximity with the target treatment area, the liver is another organ that may receive a substantial dose from radiation, especially in right-sided breast cancer patients. Differential blocks identified from cell-types comprising these target organs-at-risk from radiation (lungs, heart, and liver) were selected for generation of a radiation-specific methylation atlas, separating these solid organ cell-types of interest from all other immune cell-types (**FIG. 11, Panel B; FIG. 6, Panel B**). The human and mouse blocks specific to these cell-types can be found in Barefoot *et al.*, 2022, Supplemental Tables 3 and 4. Due to the large degree of separation of the epigenetic signature of hematopoietic cells from other solid organ cell lineages, all hematopoietic cell-types were merged into one joint “immune” super-group. This approach also accounts for the majority hematopoietic origins of cfDNA at baseline and helps reveal signals coming from solid organ cell-types of interest. Focus was on these same target organs in both human and mouse, resulting in a final curation of six groups for human (immune, lung epithelial, cardiopulmonary endothelial, cardiomyocyte, hepatocyte, and liver sinusoidal endothelial) and four groups for mouse (immune, lung endothelial, cardiomyocyte, hepatocyte) based on the reference cell type data available.

[0154] *Cell-free Methylated DNA in blood identifies origins of radiation-induced cellular damage in tissues.* Serial serum samples were collected from breast cancer patients undergoing standard radiation therapy. In addition, paired serum and tissue samples were collected from mice receiving radiation. Unbiased methylome-wide hybridization capture sequencing of DNA from human or mouse serum samples was performed. Deconvolution analysis was used to trace the origins of cfDNA fragments allowing for minimally invasive monitoring of radiation-induced cellular toxicities from blood samples (**FIG. 2**). In comparison to previous studies using single CpG sites, the sequencing-based approach allows for fragment-level cfDNA analysis using CpG methylation patterns (Scott *et al.*, 2020; Li *et al.*, 2018). For this, the co-methylation status was modeled of adjacent CpG sites on the same molecule implemented by a novel probabilistic deconvolution method. The model was applied using cell-type specific blocks from the human and mouse radiation-specific

methylation atlases described above. The prediction accuracy of the fragment-level deconvolution was validated through in silico mix-in simulations for each tissue and cell-type of interest (**FIGS. 3 and 4**).

[0155] *Dose-dependent indicators of radiation damage in mice.* To explore the relationship between radiation-induced damage in tissues to changing proportions of cfDNA origins in the circulation, mice were used to model exposure from different radiation doses. Mice received upper thorax radiation at 3Gy or 8Gy doses relative to sham control, forming three groups for comparison (**FIG. 2**). Tissues and serum were harvested 24 hours after the last fraction of treatment and tissues in line with the path of the radiation-beam (heart, lung, and liver) were targeted for subsequent analyses. Through histological analysis, dysregulated tissue architecture corresponding to higher dose radiation was observed (**FIG. 12, Panel A**). These changes were most apparent in tissue sections of the lungs showing noticeable alveolar collapse with increased radiation dose. Liver tissues showed increased fibrosis with increased radiation doses and only minor changes were apparent in cardiac tissues matching with its higher resilience to radiation. Tissue effects were also assessed through qPCR analysis of established indicators of radiation effects, including expression of CDKN1A (p21), that exhibited a dose-dependent increase in expression in response to radiation in all tissues (**FIG. 12, Panel B; FIG. 13**) (Hyduke *et al.*, 2013).

[0156] To assess indicators of heart, lung, and liver damage in serum samples, data from capture sequencing of methylated cfDNA was analyzed (**FIG. 2**). For the analysis, the above-described mouse cardiomyocyte (n=2,917), lung endothelial (n=1,546), hepatocyte (n=616) and immune (n=148) cell-type specific methylation blocks derived from the radiation atlas for target organs-at-risk was used. Combining signals from 3Gy and 8Gy treated mice, a significant increase was found in percent lung endothelial, cardiomyocyte and hepatocyte cfDNA in the radiation-treated group relative to sham control that correlated with apoptotic cell death in the corresponding tissues. In addition, a significant dose-dependent increase was observed in percent lung endothelial, cardiomyocyte and combined solid organ cfDNA across all three treatment groups that correlated with radiation-induced cell death in the corresponding tissues ($p < 0.05$, Kruskal-Wallis Test) (**FIG. 12, Panels C and D; FIG. 14, Panel E**). However, there was no dose-dependent increase in hepatocyte or immune cfDNA (**FIG. 12, Panel E; FIG. 14, Panel D**). As proof of principle, this supports that methylated DNA in blood can indicate the source of radiation-induced cellular damage in tissues.

[0157] *Radiation treatment of patients with breast cancer.* To evaluate whether changes in cfDNA patterns could indicate damages to tissues in patients after radiation, serum samples were collected from breast cancer patients at three timepoints during their standard-of-care radiation therapy after surgery (**FIG. 2**). A baseline sample was taken for each patient before onset of radiation-therapy and after a total of 20-30 treatments a second End-Of-Treatment (EOT) sample was taken 30 minutes after the last treatment. Finally, a recovery sample was taken one month after completion of radiation-therapy. Demographic information and clinical characteristics of patients enrolled in this study are in **Table 3** and in Barefoot *et al.*, 2022, Supplemental Table 8. For analysis of cfDNA focus was on cell-types composing heart, lung, and liver tissues.

[0158] *Radiation-induced liver damage.* While liver damage is not a common radiation-induced toxicity experienced by breast cancer patients, a substantial dose may still be administered to the liver, especially with right-sided tumors (**FIG. 11, Panel A**). The top hepatocyte (n=200) and liver sinusoidal endothelial (n= 89) methylation blocks were used to assess the sequence data for the presence of liver-derived cfDNA. Surprisingly, in patients receiving radiation treatment of right-sided breast cancer, an increase in hepatocyte plus liver sinusoidal endothelial methylated DNA in the circulation indicated significant radiation-induced cellular damage in the liver ($p<0.05$, Wilcoxon matched-pairs signed rank test) (**FIG. 15, Panels A-F**). Elevated levels of either hepatocyte and/or liver sinusoidal endothelial cfDNA were detected in seven of the eight breast cancer patients with right-sided tumors. In contrast, there was not significant increase in hepatocyte or liver sinusoidal endothelial cfDNA in patients with left-sided breast cancer.

[0159] *Radiation-induced heart and lung damage.* Due to close proximity with the target treatment area, the heart and lungs are common organs-at-risk for breast cancer patients undergoing radiotherapy. To assess radiation-induced lung damage, cfDNAs from serum were examined for the presence of lung epithelial methylated DNA blocks (n=69). Interestingly, no significant increase in lung epithelial cfDNA across all patients was observed ($p\geq 0.05$, Friedman Test) (**FIG. 16, Panel A**). However, a few patients showed increased lung epithelial cfDNA indicating lung damage that correlated with increasing dose and volume of the lungs targeted (**FIG. 16, Panel B**). Specifically, longitudinal changes in lung epithelial cfDNA after radiation were found to correlate with the volume of the ipsilateral lung receiving 20Gy dose (Lung V20) (Pearson's $r = 0.67$, $p < 0.05$) and the total

body mean dose (Pearson's $r = 0.90$, $p < 0.05$). In addition to lung injury, cardiovascular disease is one of the most serious complications from radiation exposure that is associated with increasing morbidity and mortality (White & Joiner, 2006; Brownlee *et al.*, 2018). Through deconvolution using cardiopulmonary endothelial (CPEC, $n=132$) and cardiomyocyte-specific ($n=375$) DNA methylation blocks, increased CPEC and cardiomyocyte cfDNA was found in the serum samples indicating significant cardiovascular cell damage across all breast cancer patients ($p < 0.05$, Friedman Test) (**FIG. 16, Panels D and G**). Surprisingly, cardiomyocyte-specific methylated DNA in the circulation correlated with the maximum radiation dose to the heart (Pearson's $r = 0.63$, $p < 0.05$), but not the mean dose to the heart (Pearson's $r = -0.09$, $p \geq 0.05$) (**FIG. 16, Panel H**). This suggests that cardiomyocyte susceptibility to radiation-induced damage requires a sufficiently high dose, reinforcing the resilience of this cell-type to radiation damage compared to corresponding epithelial and endothelial cell-types from the heart and lungs.

[0160] *Distinct endothelial and epithelial damages from radiation.* Distinct epithelial and endothelial cell-type responses to radiation across the different tissues profiled were observed. Different responses to radiation were observed when comparing hepatocyte to lung epithelial damages (**FIG. 15, Panels A-C** versus **FIG. 16, Panels A-C**), demonstrating the ability of methylated DNA to distinguish between tissue-specific epithelial cell-types from serum samples. Likewise, analysis for tissue-specific endothelial populations reveals differences in cardiopulmonary microvascular and liver sinusoidal endothelial responses to radiation (**FIG. 15, Panels D-F** vs **FIG. 16, Panels D-F**). In general, there was greater magnitude of damage to the endothelium compared to the epithelium in different organs. The endothelium forms a layer of cells lining blood as well as lymphatic vessels. As a result, turnover from this cell-type likely may contribute to the high amplitude of signal detected from serum (Moss *et al.*, 2018). This could, however, also be a result of the different sensitivities of endothelial versus epithelial cell-types to radiation-induced damage. There was a five-fold higher signal from CPEC cfDNA compared to lung epithelial cfDNA. Likewise, there was a two-fold increase in LSEC cfDNA compared to hepatocyte in right-sided cases. Also, in comparison to epithelial- and endothelial-derived cfDNA, sustained injury and delayed recovery is indicated by elevated cardiomyocyte cfDNA (**FIG. 16, Panels C, F, and I**). This may reflect important differences in cell turnover rates leading to differential processes of regeneration and repair in these cell-types. Notably, one month after completion of radiation therapy, epithelial damage signatures detected from cfDNA had

returned to baseline levels although increased turnover of endothelial cells and cardiomyocytes indicate lingering tissue remodeling. Taken as a whole, these findings demonstrate applicability of this approach to uncover distinct cellular damages in different tissues during the course of treatment with a minimally invasive approach.

[0161] *Comparison of results in humans and mice.* Comparing the cfDNA origins after radiation, similar radiation-related changes were observed in both human and mouse serum samples. In both human and mouse, there was a significant increase in lung endothelial and cardiomyocyte cfDNAs after radiation. Likewise, there was an overall increase in cfDNA derived from any solid-organ tissue post-radiation in both breast cancer patients and mice receiving radiation (**FIG. 14**). The total concentration of cfDNA was elevated in some breast cancer patients at EOT as well, suggesting an overall increase in cfDNA shortly after radiation treatment (**Table 9**). Changes in mouse cfDNA concentration with increasing radiation dose were not significant (**Table 13**) as similarly reported in previous studies.^{78,79}

[0162] This study demonstrated the ability of tissue-of-origin analysis of cell-free methylated DNA to monitor systemic responses to radiotherapy. The assignment of DNA fragments extracted from serum samples from patients undergoing treatment as well as from experimental animals to specific cell types required in-depth analysis of tissue- and cell-type methylation patterns. It was surprising that there was a significant association of the cell-type specific DNA methylation blocks with cell-type specific gene expression, transcription factor binding motifs and signaling pathway regulation. This study resulted in the development of a methylation atlas containing cell-type specific methylation patterns from target organs-at-risk from radiation damage, including the heart, lungs, and liver. It was found that methylated DNA in blood samples is an indicator of radiation damage that may be useful to predict patients who are more likely to develop severe adverse effects.

Table 3. Characteristics of breast cancer patients enrolled in the study.

Demographics				
Age	Range 38-76	Average 60	STDEV 13.6	
Race	AA 26.67%	EA 66.67%	Other 6.67%	
Tumor Characteristics				
Stage	stage 0 25.00%	stage 1 26.70%	stage 2 33.00%	Stage 3 20.00%
Histology	DCIS 20.00%	IDC 53.33%	ILC 26.67%	
Laterality of breast cancer	Right 53.33%	Left 46.67%		
Hormone Receptors	ER+/PR+/HER2+ ER+/PR+/HER2- Triple Negative 6.67% 86.67% 6.67%			
Cancer Treatment				
Radiotherapy	3D-CRT 100.00%	PBT 6.67%	SBRT 6.67%	
Prior Radiation	Yes 20.00%	No 80.00%		
LR Node Irradiation	Yes 26.67%	No 73.00%		
Mean Lung Gy	Range 0.6-16	Mean 6.8	STDEV 4.71	
Mean Heart Gy	Range 0.1-1.65	Mean 0.64	STDEV 0.42	
Total Body Mean Gy	Range 1.05-14	Mean 4.6	STDEV 3.2	
Fx	Range 5.0-30	Mean 22	STDEV 7.2	
TO dose (cGY)	Range 3000-6000	Mean 4988	STDEV 876.2	
Adjuvant hormone therapy	Yes 40.00%	No 60.00%		
Adjuvant Chemotherapy	Yes 26.67%	No 73.00%		
Medical History / Comorbidities				
Diabetes	Yes 26.67%	No 73.33%		

Table 4. Human reference methylation data from healthy tissues and cell-types.

Consortium/ Source	Tissue/ Cell-type	Samples	DatasetID	Included in Atlas
Blueprint	Macrophage	6	EGAD00001002732; EGAD00001000923; EGAD00001001192; EGAD00001002501	X
Blueprint	Bcell	6	EGAD00001002732; EGAD00001000710; EGAD00001001304	X
Blueprint	Monocyte	6	EGAD00001002732; EGAD00001000673; EGAD00001000941; EGAD00001001206	X
Blueprint	Megakaryocyte	3	EGAD00001002732; EGAD00001000932; EGAD00001002311	X
Blueprint	CD4Tcell	6	EGAD00001002732; EGAD00001001157; EGAD00001001516	X
Blueprint	CD8Tcell	6	EGAD00001002732; EGAD00001000921; EGAD00001001189	X
Blueprint	NKcell	6	EGAD00001002732; EGAD00001001128; EGAD00001002403	X
Blueprint	Eosinophil	6	EGAD00001002732; EGAD00001001507; EGAD00001002309	
Blueprint	Erythroblast	2	EGAD00001000909; EGAD00001001133; EGAD00001002423	X
Blueprint	Neutrophil	6	EGAD00001002732; EGAD00001000673; EGAD00001000935; EGAD00001001201; EGAD00001002508	X
Blueprint	Endothelial cell of umbilical vein (large vessel endothelial)	2	EGAD00001002294	X
KNIH	Adipocyte	2	EGAD00001002755; EGAD00001002756	
KNIH	Podocyte	4	EGAD00001002758; EGAD00001002759;	X

			EGAD00001003469; EGAD00001003470	
AMED-CREST / DEEP	Hepatocyte	10	JGAD000026; EGAD00001002527	X
CEEHRC / KNIH	Skeletal muscle	21	EGAD00001001289; EGAD00001003871; EGAD00001003872; EGAD00001003873; EGAD00001003875; EGAD00001003876; EGAD00001003877	X
ENCODE	Smooth muscle	1	ENCSR076OHG	
NIH Roadmap / CEEHRC	Breast luminal epithelium	5	EGAD00001005060; EGAD00001005335; EGAD00001006220	X
NIH Roadmap / CEEHRC	Breast basal epithelium	4	EGAD00001005060; EGAD00001005335;	X
KNIH	Pancreatic islet cell	5	EGAD00001002750; EGAD00001002751; EGAD00001002752; EGAD00001002753; EGAD00001002754	X
KNIH	Neuron	2	EGAD00001003474; EGAD00001003477	X
CEEHRC	Thyroid	8	EGAD00001001228	
Stueve et al. 2017 (PMID:28854564)	Lung epithelium	12	PRJNA375086	X
AMED-CREST	Endometrial epithelium	2	JGAD000073	
ENCODE	Esophagus squamous epithelium	2	ENCSR515MHO; ENCSR853BXB	
AMED-CREST	Colon epithelial cells	12	JGAD000078	X
Gilsbach et al. 2018 (PMID:29374152)	Cardiomyocyte	5	PRJNA353755	X
Pidsley et al. 2017 (PMID:27717381)	Prostate epithelium	4	PRJNA342657	
CUHK CNARG	Ureter Urothelial	4	Data generated by "Chen THT et al in Clin Biochem. 2017;50:496–501".	
CUHK CNARG	Bladder Epithelial	1	Data generated by "Chen THT et al in Clin Biochem. 2017;50:496–501".	

NIH Roadmap / ENCODE	Gastric epithelium / stomach	2	ENCSR999RWT; ENCSR669BAL	
Jamil et al. 2020 (PMID:33096636) / in-house	Liver sinusoidal endothelial	6	PRJNA596240	X
in-house	Cardiopulmonary endothelial	6		X

Table 5. Mouse reference methylation data from healthy tissues and cell-types.

Consortium/Source	Tissue/Cell-type	Samples	DatasetID	Included in Atlas
Duncan et al. 2018 (PMID:29326230) / in-house	Bcell	4	PRJNA391196	X
Delacher et al. 2017 (PMID:28783152) / in-house	CD4Tcell	5	PRJEB14591	X
in-house	CD8Tcell	1		X
in-house	Neutrophil	1		X
in-house	Buffy coat	4		X
ENCODE / Hon et al 2013 (PMID:23995138)	Bone marrow	5	ENCBS218HTH; ENCBS430NOM; GSM1051150; ENCFF802SFU; ENCFF703DEV; ENCFF306ZPW; ENCFF340YVI	X
Delacher et al. 2017 (PMID:28783152)	Tissue-resident Treg	9	PRJEB14591	
Hon et al 2013 (PMID:23995138)	Spleen	1	GSM1051163	
Hon et al 2013 (PMID:23995138)	Thymus	1	GSM1051165	
Gilsbach et al. 2014 (PMID:25335909)	Cardiomyocyte	3	PRJNA229470	X
ENCODE / Hon et al 2013 (PMID:23995138)	Heart	8	ENCSR633CON; ENCSR835OJU; ENCSR258MDR; ENCSR397YEG; ENCSR641SDF; ENCSR265OMO; ENCSR149GUT; ENCSR050EXR; GSM1051154	
Gravina et al. 2016	Hepatocyte	3 (13)	PRJNA310298	X
ENCODE / Hon et al 2013 (PMID:23995138)	Liver	8	ENCSR550CYA; ENCSR660CKG; ENCSR788XSZ; ENCSR334GBD; ENCSR129SBE; ENCSR324NAF; ENCSR033PGF; GSM1051157	
Sabbagh et al. 2018 (PMID:30188322)	Endothelial	8	GSE111839	

Schlereth et al. 2017 (PMID:29749927)	Lung endothelial	3	PRJNA344551	X
ENCODE / Hon et al 2013 (PMID:23995138)	Lung	5	ENCSR191UKH; ENCSR409HKJ; ENCSR535YCH; ENCSR027ICI; GSM1051158	
ENCODE / Hon et al 2013 (PMID:23995138)	Cerebellum	5	GSM1051151; ENCBS358CVB; ENCBS588RGY	X
Lagger et al. 2017 (PMID:28498846)	Hypothalamus	3	PRJNA329552	X
Hon et al 2013 (PMID:23995138)	Olfactory Bulb	1	GSM1051159	
ENCODE / Hon et al 2013 (PMID:23995138)	Intestine	5	ENCSR089FFK; ENCSR217TMK; ENCSR353IFP; ENCSR842QTB; GSM1051155	X
Hon et al 2013 (PMID:23995138)	Colon	1	GSM1051152	
ENCODE / Hon et al 2013 (PMID:23995138)	Stomach	5	ENCSR953WFU; ENCSR013VIR; ENCSR286OOJ; ENCSR545WRA; GSM1051164	
ENCODE / Hon et al 2013 (PMID:23995138)	Kidney	5	ENCSR425NDU; ENCSR128HOP; ENCSR841TRV; ENCSR906HLA; GSM1051156	X
Hon et al 2013 (PMID:23995138)	Pancreas	1	GSM1051160	
Gravina et al. 2016	Fibroblast	2	PRJNA310298	
dos Santos et al. 2015 (PMID:25959817)	Mammary epithelial	5	GSE67386	X
Hon et al 2013 (PMID:23995138)	Placenta	1	GSM1051161	
Hon et al 2013 (PMID:23995138)	Uterus	1	GSM1051166	
Hon et al 2013 (PMID:23995138)	Skin	1	GSM1051162	

Table 6. Summary of identified human cell-type specific methylation blocks (AMF > |0.4|, minimum 3CpG sites).

Cell-type	#Hypomethylated	#Hypermethylated
Bcell	53	0
Cardiopulmonary Endothelial (CPEC)	128	4
Breast Basal Epithelial	705	155
Breast Luminal Epithelial	287	43
Cardiomyocyte	649	15
CD4Tcell	52	2
CD8Tcell	73	2
Colon Epithelial	519	58
Large Vessel Endothelial (LVEC)	104	12
Erythroblast	12	1
Hepatocyte	482	79
Kidney Podocyte	60	13
Liver Sinusoidal Endothelial (LSEC)	88	1
Lung Epithelial	68	1
Monocyte / Macrophage	97	0
Neuron	106	197
Neutrophil	51	0
Nkcell	52	0
Skeletal Muscle	211	3
Pancreas Islet	92	22
Bulk Immune	117	105

Table 7. Summary of identified mouse cell-type specific methylation blocks (AMF > |0.4|, minimum 3CpG sites).

Cell-type	#Hypomethylated	#Hypermethylated
Bulk Immune	0	148
Cardiomyocyte	2917	0
Hepatocyte	616	0
Lung Endothelial	1546	0
Cerebellum	1229	0
Mammary Epithelial	874	0
Intestine	6	0
Hypothalamus	4	0
Kidney	4	0

Table 8. Genomic annotation of identified human and mouse cell-type specific hypomethylated and hypermethylated blocks relative to all captured blocks.

Human_Hypomethylated blocks (n=3889)	
intergenic	915
intragenic	2942
promoter-TSS	274
TTS	142
exon	631
intron	1895

Mouse_Hypomethylated blocks (n=7964)	
intergenic	2700
intragenic	5264
promoter-TSS	119
TTS	136
exon	716
intron	4293

Human_Hypomethylated blocks (n=608)	
intergenic	204
Intragenic	404
promoter-TSS	80
TTS	24
exon	117
intron	183

Mouse_Hypomethylated blocks (n=148)	
intergenic	17
Intragenic	131
promoter-TSS	16
TTS	5
exon	40
intron	70

Human Background Captured Blocks (n=317963)	
intergenic	98202
intragenic	219761
promoter	39216
TTS	9943
exon	39260
intron	131342

Mouse Background Captured Blocks (n=1214889)	
intergenic	608763
intragenic	606126
promoter	42339
TTS	16699
exon	55317
intron	491771

Table 9. Human cDNA sample concentrations and predicted precursors from deconvolution analysis at identified cell-type specific blocks for target cell-types

Sample	ng/mL	%Lung Epithelial	%Hepatocyte	%Cardiomyocyte	%Cardiopulmonary Endothelial (CPEC)	%Liver Sinusoidal Endothelial (LSEC)	%Immune
101.1	142.0	1.675904524	0.287332084	0.029971414	1.057076778	0	96.9497152
101.2	100.4	0.348632095	0.105411607	0.0783332303	1.203961225	0.09027696	98.1733858
101.3	116.4	0.0942617	0.336054406	0.220777008	0.312584367	0.03922258	98.9970999
102.1	68.4	0.159638429	0.415568659	0.465308433	0.374905104	0.07881589	98.5057635
102.2	372.0	0.011095914	0.446790142	0.628657336	1.430265589	0.07488822	97.4083028
102.3	96.8	0.051613574	0.47360535	0.984144776	0	0	98.4906363
103.1	19.1	0.256707102	0.563417383	0.28425303	0.24044444	0.18948499	98.4656931
103.2	88.0	0.16686356	0.426237369	0.390857292	0.347458494	0.08661508	98.5819682
103.3	33.6	0.045541196	0.710362406	0.217893557	0.382335646	0.05237159	98.5914956
104.1	74.7	0.04140701	0.358170659	0.281567649	0.420281174	0	98.8985735
104.2	88.8	0.263052706	0.624499172	0.259036654	1.283134955	0.15643783	97.4138387
104.3	90.1	0.032511234	0.674608147	0.304792838	0.22554669	0.04420491	98.7183362
105.1	15.6	0.057912071	0.403966669	0.250549456	0.583406255	0.36333617	98.3408294
105.2	26.9	0.033082363	0.333533186	0.460896505	1.211293535	0.18785466	97.7733397
105.3	21.5	0.107636608	0.293577302	0.487267321	1.051777845	0.15455843	97.9051825
106.1	39.7	0.069472616	0.250285823	0.774141116	1.90445977	0.29539538	96.7062453
106.2	182.7	0.951953399	0.294805455	0.981969082	0.457496611	0.04868031	97.2650951
106.3	24.5	1.80978571	0.465130627	0.812483429	1.729335411	0.93515001	94.2481128
107.1	44.3	0.497611236	1.053494562	0.542070916	1.447219552	0.02240723	96.4371965
107.2	632.0	0	1.542284221	0.157889618	1.071853544	0.21960132	97.0083713
107.3	320.0	0.211757934	1.416242727	0.505639938	1.376137521	0.71798595	95.7722359
108.1	29.1	0	0.688671158	0.382439275	0.603764532	0.16302918	98.1620959
108.2	148.8	0.735053495	0.783024014	0.479429223	0.568956322	0.01666383	97.4168731
108.3	32.3	0.408248008	0.779447652	0.605693045	0.64876015	0.14714937	97.4107018

Table 9 (cont.)

Sample	ng/mL	%Lung Epithelial	%Hepatocyte	%Cardiomyocyte	%Cardiopulmonary Endothelial (CPEC)	%Liver Sinusoidal Endothelial (LSEC)	%Immune
109.1	38.4	0	0.474231281	0.248280445	0.426939593	0.3631607	98.487388
109.2	722.7	0.013676029	0.65980246	0.161370039	1.718976989	0.52657702	96.9195975
109.3	45.9	0.272650817	0.361996258	0.103163038	0.803899375	0.43873763	97.9995529
110.1	30.9	0.4742599	1.403818565	1.191351612	0.392211011	0.16481115	96.3735478
110.2	17.6	0.242255832	0.794249572	0.882501569	0.680428153	0.08111527	97.3194496
110.3	26.3	0.372535977	0.825663635	1.085022006	0.99582761	0.23200288	96.4889479
111.1	30.9	0.080724628	1.502603313	0.55841863	0.934586383	0.16762099	96.7560461
111.2	92.8	0.167643817	6.60401336	1.377649585	1.211801446	0.31783789	90.3210539
111.3	26.7	0.091107031	6.34023715	1.181319466	0.091158051	0.13545335	92.1607264
112.1	59.7	0.021991448	0.551215254	0.005929387	0.134739401	0	99.2861245
112.2	64.8	0.04623058	0.747756312	0.358843279	1.814499911	0.12394989	96.90872
112.3	36.5	1.2638928	0.632178819	0.016952957	0.492033467	0	97.594942
113.1	111.5	0.767210315	0.405267296	0.418200983	0.979478906	0	97.4298335
113.2	174.9	0.014953727	0.357760609	1.38443133	0.078292337	0.08876883	98.0767932
113.3	61.1	0	0.895662241	1.435923237	0	0	97.6684145
114.1	304.0	0.65783443	0.377453852	0.382912335	0.275136473	0.14017883	98.1664841
114.2	46.9	0.02814705	0.327169124	0.60028721	1.103292289	0.27702703	97.6640773
114.3	68.5	0.458553005	0.969397206	0.496765788	0.386149925	0.19633411	97.4928
115.1	47.2	1.3088228	2.28363898	1.013656941	0.755232344	0.26146117	94.3771878
115.2	72.8	1.609908701	2.064005032	0.995458717	0.684814019	0.23204816	94.4137654
115.3	594.7	0.259518205	0.730465023	0.596255318	0.488497441	0.23923304	97.686031

Table 10. Mouse cfDNA sample concentrations and predicted precents from deconvolution analysis at identified cell-type specific blocks for target cell-types.

Sample	ng/mL	%cardiomyocyte	%hepatocyte	%Lung Endothelial	%Immune
shamA	25.12	0.221790643	0.12116778	0.310984938	99.34605664
shamB	21.04	0	0.26485898	0.007434279	99.72770674
shamC	29.92	0	0	0	100
3GyA	23.92	2.406149764	1.586386567	0.918922438	95.08854123
3GyB	18.32	0.14614797	0.107333799	0.188790206	99.55772802
3GyC	18.32	1.34771749	2.179727824	0.452849459	96.01970523
8GyA	47.52	2.772568704	1.20751631	3.597659949	92.42225504
8GyB	48.16	1.35983355	1.946827849	0.589794119	96.10354448
8GyC	23.2	8.351210369	1.467364049	1.660177126	88.52124846

Table 11. Enriched biological pathways and functions for genes associated with differential methylation in each cell-type examined.

HUMAN LUNG EPITHELIAL		
Biological Pathways and Functions	-log(p-value)	Molecules
Binding of pulmonary fibroblasts	4.72	HS6ST1, SDC1
Abnormal morphology of larynx	4.64	BMPER, FSTL1, TBX1
Abnormality of cartilage tissue	3.43	ADRA2A, BMPER, CCN4, FSTL1, TBX1
Cough	3.18	ADORA2B, ADRA2A, ATP4B
Quantity of type II pneumocytes	3.13	EPAS1, FSTL1
Hyperpolarization of membrane	3.08	BMPER, FSTL1
Lower respiratory tract disorder	3.04	ADORA2B, ADRA2A, ATP4B, BMPER, FSTL1, HS6ST1, USP40
Pulmonary Hypertension	2.90	ADORA2B, ADRA2A, EPAS1, RPTOR
Migration of Langerhans cells	2.89	RPTOR, SIRPA
Respiratory failure	2.86	ADRA2A, EPAS1, TBX1, WNT7B
Abnormal morphology of tracheal ring	2.77	FSTL1, PRKCZ
α -Adrenergic Signaling	2.58	ADRA2A, GNA12, PRKCZ
AMPK Signaling	2.48	ADRA2A, GNA12, PPM1H, RPTOR
Expression of RNA	2.46	BHLHE41, EHMT1, EPAS1, GNA12, GRIP1, HMG20A, LIMS1, MAD1L1, MCF2L, PRKCZ, RREB1, SIRPA, STAU2, TBX1, TCF25, VAV2, WNT7B
Migration of lung cell lines	2.45	ARHGEF7, MCF2L
Circadian Rhythm Signaling	2.33	BHLHE41, CSNK1D, PRKCZ, RPTOR
Xenobiotic Metabolism CAR Signaling Pathway	1.91	GRIP1, HS6ST1, PRKCZ
3-phosphoinositide Degradation	1.90	PALD1, PPM1H, SIRPA
Xenobiotic Metabolism PXR Signaling Pathway	1.89	GRIP1, HS6ST1, PRKCZ

Role of WNT/GSK-3 β Signaling in the Pathogenesis of Influenza	1.77	CSNK1D, WNT7B
Integrin Signaling	1.77	ARHGEF7, LIMS1, TNK2
RHO GDI Signaling	1.76	ARHGEF7, GNA12, GRIP1
lung morphogenesis	1.59	CELSR1, RDH10, WNT7B
lung development	1.38	CELSR1, EPAS1, HS6ST1, RDH10, WNT7B
Pulmonary Fibrosis Idiopathic Signaling Pathway	1.30	CCN4, GNA12, WNT7B
HUMAN CARDIOMYOCYTE		
Biological Pathways and Functions	-log(p-value)	Molecules
myofibril assembly	35.02	ACTC1, ACTG1, CASQ2, LDB3, LMOD2, LMOD3, MEF2A, MYBPC3, MYH6, MYL2, MYLK3, MYOM2, MYOZ1, MYOZ3, MYPN, TPM1, TTN
sarcomere organization	31.96	ACTG1, CASQ2, LDB3, LMOD2, MYBPC3, MYH6, MYLK3, MYOM2, MYPN, TPM1, TTN
actomyosin structure organization	30.16	ACTC1, ACTG1, CASQ2, FRMD5, LDB3, LMOD2, LMOD3, MEF2A, MYBPC3, MYH6, MYL2, MYLK3, MYOM2, MYOZ1, MYOZ3, MYPN, PHACTR1, SORBS1, TPM1, TTN
cellular component assembly involved in morphogenesis	28.36	ACTC1, ACTG1, CASQ2, GPC1, LDB3, LMOD2, LMOD3, MEF2A, MYBPC3, MYH6, MYL2, MYLK3, MYOM2, MYOZ1, MYOZ3, MYPN, TENM4, TPM1, TTN
striated muscle cell development	28.22	ACTC1, ACTG1, CASQ2, FHL2, LDB3, LMOD2, LMOD3, LRRC10, MEF2A, MYBPC3, MYH6, MYL2, MYLK3, MYOM2, MYOZ1, MYOZ3, MYPN, NPPA, PDLIM5, SORBS2, TBX3, TPM1, TTN
actin filament-based process	27.40	ABLIM2, ACTC1, ACTG1, ARHGAP26, BAIAP2, BAIAP2L1, CACNA1C, CAP2, CAPN10, CASQ2, COBL, CORO6, DES, DNAJB6, DTNBP1, EVL, FGF12, FNBP1L, FRMD5, FRY, GAB1, INF2, INPP5K, KCND3, KCNQ1, LDB3, LMOD2, LMOD3, MEF2A, MICAL3, MYBPC3, MYH6, MYH7, MYL2, MYLK3, MYO5A, MYOM2, MYOZ1, MYOZ3, MYPN, NEDD4L, NISCH, NPHP4, PARVA, PARVB, PDGFA, PDLIM3, PDPK1, PHACTR1, PKP2, PPARGC1B, SCN5A, SNTA1, SORBS1, SORBS2, SPTBN5, SYNE2, TNNC1, TPM1, TTN, WASL, XIRP1
muscle cell development	26.60	ACTC1, ACTG1, ATP2A2, CASQ2, ENG, FHL2, LDB3, LMOD2, LMOD3, LRRC10, MEF2A, MYBPC3, MYH6, MYL2, MYLK3, MYOM2,

		MYOZ1, MYOZ3, MYPN, NPPA, PDLIM5, SORBS2, TBX3, TPM1, TRIM54, TTN
Morphology of heart	21.57	ACACB, ACTC1, ADAM19, ADCY5, ADCY6, ADRB1, AGO2, ANK3, ATP2A2, BNIP3, CABIN1, CACNA1C, CASP9, CASQ2, CASZ1, CCND1, CKMT2, CUX1, DCHS1, DES, DPF3, F3, FBXO32, FGF12, FHL2, FITM1, GATA6, HAND1, HCN4, HDAC4, HSPB7, IGF1R, JARID2, JPH2, KCNQ1, KIDINS220, LDB3, LIMS2, LMOD2, LRRC10, MEF2A, mir-208, mir-486, MYBPC3, MYH7, MYL2, MYL9, MYLK3, MYOCD, MYPN, MYZAP, NEDD4L, NPPA, PDLIM5, PDPK1, PKP2, PLEC, PPARGC1B, PRDM16, RARB, RPL3L, RPS6KA2, RXRA, SAV1, SCN5A, SGTA, SLC25A21, SMARCD3, SMYD1, SNTA1, SPEG, SYNE2, TBX5, TNNC1, TNS1, TPM1, TRAF3, TRIM63, TTN, UBE4B, UCN, XIRP1, ZMIZ1
cardiac muscle tissue development	19.82	ACTC1, BMPR1A, ENG, FHL2, FOXC1, GATA6, HAND1, LRRC10, MEF2A, MYBPC3, MYH6, MYH7, MYL2, MYLK3, MYOCD, MYPN, NDRG4, NPPA, PDLIM5, PKP2, RARB, RXRA, SORBS2, TBX3, TBX5, TENM4, TNNC1, TPM1, TTN, UBE4B
Cardiogenesis	17.87	ACTC1, ADAM19, APC, ATP2A2, CACNA1C, CASQ2, CASZ1, CCND1, COL5A1, CTBP2, CUX1, DCHS1, FGF12, GAB1, GATA6, GYS1, HAND1, HDAC4, IFT140, JARID2, JPH2, KCNQ1, KLF7, LDB3, LIMS2, LMOD2, LRRC10, mir-208, MTHFD1, MYBPC3, MYH7, MYL2, MYLK3, MYO18B, MYOCD, MYOM2, MYPN, NPPA, PDPK1, PKP2, PLEC, PPARGC1B, PTCH1, RPS6KA2, RXRA, SAV1, SCN5A, SMARCD3, SMYD1, SPEG, SYNPO2L, TBX5, TENM4, TNNC1, TPM1, TRIM63, TTN, VGLL4, XIRP1, ZMIZ1
Dilated Cardiomyopathy Signaling Pathway	13.70	ACTC1, ADCY5, ADCY6, ADCY9, ADRB1, ATP2A2, CACNA1C, CAMK2B, CASP9, DES, GAB1, ITPR1, MYBPC3, MYH7, MYH7B, MYL2, MYL9, MYO18B, PRKAR1B, SCN5A, TNNC1, TPM1, TTN
Morphology of muscle cells	13.39	ACTC1, ADCY6, ADRB1, ATP2A2, CABIN1, CASQ2, COL13A1, CUX1, DES, FBXO32, FHL2, GATA6, HSPB7, ILF3, JARID2, JPH2, LDB3, LIMS2, LMO7, LMOD2, LRRC10, MEF2A, mir-208, MYBPC3, MYLK3, MYOCD, NPPA, PARVA, PDLIM5, PDPK1, PLEC, PSAP, RPS6KA2, SGCA, SMARCD3, SNTA1, SPEG, TBX5, TRIM63, TTN, UBE4B, UCN, XIRP1

Morphology of cardiac muscle	12.31	ACTC1, ADCY6, ADRB1, ATP2A2, CABIN1, CASP9, CASQ2, CUX1, DES, FBXO32, FHL2, GATA6, HSPB7, JPH2, LIMS2, LRRC10, mir-208, MYBPC3, MYL9, MYLK3, MYOCD, NPPA, PDPK1, PKP2, PLEC, PPARGC1B, RPS6KA2, RXRA, SMARCD3, TBX5, TRIM63, TTN, UBE4B, UCN, XIRP1
Morphology of cardiomyocytes	10.00	ACTC1, ADCY6, ADRB1, ATP2A2, CABIN1, CASQ2, CUX1, DES, FBXO32, FHL2, GATA6, HSPB7, JPH2, LIMS2, LRRC10, mir-208, MYBPC3, MYLK3, MYOCD, NPPA, PDPK1, PLEC, RPS6KA2, TBX5, TRIM63, TTN, UBE4B, UCN
Calcium Signaling	7.20	ACTC1, ATP2A2, CABIN1, CACNA1C, CAMK2B, CASQ2, CREBBP, HDAC4, ITPR1, LETM1, MEF2A, MYH7, MYH7B, MYL2, MYL9, MYO18B, PRKAR1B, TNNC1, TPM1
White Adipose Tissue Browning Pathway	5.52	ADCY5, ADCY6, ADCY9, CACNA1C, CREBBP, CTBP2, ITPR1, NPPA, NRF1, PRDM16, PRKAR1B, RARB, RXRA
ILK Signaling	4.39	ACTC1, CCND1, CREBBP, FBLIM1, LIMS2, MYH7, MYH7B, MYL2, MYL9, MYO18B, PARVA, PARVB, PDPK1, PPP2R5C
Factors Promoting Cardiogenesis in Vertebrates	4.33	APC, CAMK2B, CCND1, CREBBP, LRP5, MYH7, MYL2, MYOCD, NPPA, PLCL2, SCN5A, TBX5
Role of NFAT in Cardiac Hypertrophy	3.31	ADCY5, ADCY6, ADCY9, CABIN1, CACNA1C, CAMK2B, HAND1, HDAC4, IGF1R, ITPR1, MEF2A, PLCL2, PRKAR1B
Assembly of RNA Polymerase III Complex	2.73	GTF3A, GTF3C1, GTF3C5
Cardiac Hypertrophy Signaling	2.69	ADCY5, ADCY6, ADCY9, ADRB1, CACNA1C, CREBBP, HAND1, IGF1R, MEF2A, MYL2, MYL9, PLCL2, PRKAR1B
Cardiac β -adrenergic Signaling	2.05	ADCY5, ADCY6, ADCY9, ADRB1, ATP2A2, CACNA1C, PPP1R7, PPP2R5C, PRKAR1B
Thrombin Signaling	2.02	ADCY5, ADCY6, ADCY9, CAMK2B, GATA6, ITPR1, MYL2, MYL9, PDPK1, PLCL2
Cardiomyocyte Differentiation via BMP Receptors	2.00	MYH7, MYL2, NPPA
Apelin Cardiomyocyte Signaling Pathway	1.34	ATP2A2, ITPR1, MYL2, MYL9, PLCL2
HUMAN HEPATOCYTE		
Biological Pathways and Functions	-log(p-value)	Molecules

FXR/RXR Activation	21.80	A1BG, ABCG5, ABCG8, AHSG, APOB, APOC2, APOC4, APOF, CLU, CYP8B1, FASN, FBP1, FGA, FOXA1, HPR, HPX, IL1RN, LIPC, ORM1, ORM2, PON1, RBP4, RXRA, SLC10A1, SLC27A5, SLC51B, VTN
LXR/RXR Activation	19.60	A1BG, ABCG5, ABCG8, AHSG, APOA5, APOB, APOC2, APOC4, APOF, CLU, FASN, FGA, HPR, HPX, IL1RN, LBP, NCOR2, ORM1, ORM2, PON1, RBP4, RXRA, SCD, UGT1A3, VTN
organic anion transport	16.63	ABCG8, ACACB, ACSL1, AGXT, AKR1C4, APOA5, APOC2, CYB5R2, DRD4, GOT2, MPC1, PLA2G12B, PLA2G2C, PLA2G2F, RXRA, SERINC2, SERINC3, SLC10A1, SLC13A5, SLC16A1, SLC16A11, SLC16A13, SLC16A3, SLC17A1, SLC17A3, SLC19A1, SLC22A1, SLC22A3, SLC22A9, SLC25A25, SLC25A4, SLC25A42, SLC27A5, SLC35A3, SLC38A10, SLC38A7, SLC38A8, SLC43A1, SLC51B, SLC5A12, SLC6A1, SLC7A10, SLC7A5, SLC7A9, THRSF
Liver lesion	12.91	A1BG, A2M, ABCG5, ABCG8, ACACB, ACSL1, ADM, AGBL1, AGTR1, AHSG, AKR1C4, ALDH2, ALDH8A1, ALPL, ANGPTL7, ANPEP, AP1M1, APOA5, APOB, APOF, ARC, ARHGAP44, ARMC5, ARNTL, ATP2B2, BDH1, C1R, C1S, C5, CABLES2, CAMK2G, CAMTA2, CAPN5, CBFA2T3, CCDC57, CDK9, CDKN1B, CENPP, CHRD, CHRNA4, CLASP1, CLU, COL18A1, CPN2, CTSB, CUX2, CYP2E1, CYP8B1, DAB2IP, DCPS, DHCR24, DNAJB12, DOCK6, DOCK9, DUSP1, DUSP3, ELFN1, EPPK1, ERICH1, EXOC3L4, F12, F2, FAAP100, FAM20C, FASN, FBP1, FGA, FGF1, FOXD2-AS1, GNPNTA1, GOT1, GOT2, GPER1, GPRC5C, GRHPR, GYS2, H6PD, HAAO, HAGH, HIF1AN, HP, HPN, HPX, HR, IGF2R, IGFALS, IHH, IL1RN, INHBE, INPP5A, INS-IGF2, INTS6, IRS1, ITGB4, ITIH1, LBP, LPAL2, LRP5, MAD1L1, MASP1, MASP2, MAT1A, MEGF6, MGMT, MGRN1, MICAL3, MINK1, mir-122, MLXIP, MYO1C, MYO7A, NAGS, NAT8, NCMAP, NCOR2, NFIC, NINL, NLRP6, NPC1L1, NRDE2, NTN1, OPLAH, ORM1, OSGIN1, PAH, PC, PCK1, PCSK6, PEMT, PIEZO1, PLEC, PMEPA1, PMFBP1, PML, POLE, PRLR, PROC, PROZ, PTPRF, RBM33, RBP4, RFC1, RIN3, RNF220, RPS6KA2, RXRA, SARM1, SCD, SEBOX, SELENOP, SERINC2, SH3BP2, SH3BP4, SH3PXD2A, SIGIRR, SLC10A1, SLC16A13, SLC22A1, SLC22A9, SLC25A4, SLC25A42,

		SLC25A47, SLC27A5, SLC29A1, SLC43A1, SLC51B, SLC6A1, SLC7A10, SLC7A5, SLC7A9, SMAD3, SNED1, SNTG1, SORBS2, SOX10, SOX11, SPTBN1, SQSTM1, SS18L1, STK24, SUN1, TBC1D14, TEDC1, TEX2, TFR2, TIMP2, TM6SF2, TMEM33, TMEM82, TNK2, TNS3, TOP1MT, TRAF3, TRAPPC4, TRIP10, UBE2V2, UGT1A1, UGT1A3, UGT1A4, UROC1, VAV2, VPS37B, VPS4A, VTN, WDR62, WNT5A, ZC3H10, ZC3H7B, ZFPM1, ZFYVE28, ZMYM4, ZNF358, ZNF444
Synthesis of lipid	12.23	ABCG5, ABCG8, ACACB, ACSL1, ADM, AGTR1, AKR1C4, ALDH8A1, ANPEP, APOA5, APOB, APOC2, ARMC5, ARNTL, ATAD3A, C5, CAMK2G, CYP2E1, CYP8B1, DGAT1, DGKD, DHCR24, ETNPPL, F2, FASN, FGF1, FOXA1, GATA4, GPD1, GPER1, GYS2, H6PD, HSD17B1, IL1RN, LIPC, NPC1L1, NTN1, PC, PCK1, PEMT, PIP5K1C, PLA2G2F, PLPP3, PON1, PRLR, PTK2B, RXRA, SCD, SERINC2, SIGIRR, SLC22A1, SLC27A5, SLC9A3R2, SMAD3, SRD5A1, ST6GALNAC6, THRSP, VTN
pre-mRNA catabolic process	11.50	ZNF259
Quantity of steroid	11.30	ABCG5, ABCG8, ACACB, ADM, AGTR1, APOA5, APOB, ARNTL, CDKN1B, CLU, CRY2, CYP8B1, DGAT1, DHCR24, DUSP1, FASN, GATA4, GPER1, H6PD, HIF1AN, HP, HPN, HSD17B1, IHH, IL1RN, IRS1, LIPC, LRP5, mir-122, NPC1L1, PEMT, PON1, PRLR, RXRA, SCD, SMAD3, SRD5A1, TM6SF2, TRAF3, UGT1A1, VAV2
Liver cancer	10.27	A1BG, ABCG5, ABCG8, ACACB, ACSL1, AGBL1, AHSG, AKR1C4, ALDH2, ALDH8A1, ALPL, ANPEP, AP1M1, APOA5, APOB, APOF,

		ARC, ARHGAP44, ARMC5, ARNTL, ATP2B2, BDH1, C1R, C1S, C5, CABLES2, CAMK2G, CAMTA2, CBFA2T3, CCDC57, CDK9, CDKN1B, CENPP, CHRD, CHRNA4, CLASP1, CLU, COL18A1, CTSB, CUX2, CYP8B1, DAB2IP, DHCR24, DNAJB12, DOCK9, DUSP3, EPPK1, ERICH1, F12, F2, FAAP100, FAM20C, FASN, FBP1, FGA, FOXD2-AS1, GNPAT1, GOT1, GPER1, GPRC5C, GRHPR, GYS2, H6PD, HAAO, HAGH, HP, HPX, HR, IGF2R, IHH, INHBE, INPP5A, INS-IGF2, INTS6, IRS1, ITGB4, ITIH1, LBP, LPAL2, LRP5, MAD1L1, MASP1, MASP2, MEGF6, MGMT, MGRN1, MICAL3, MINK1, mir-122, MLXIP, MYO1C, MYO7A, NAT8, NCMAP, NCOR2, NFIC, NLRP6, NRDE2, NTN1, OPLAH, ORM1, OSGIN1, PAH, PC, PCK1, PCSK6, PIEZO1, PLEC, PMEPA1, PMFBP1, PML, POLE, PROZ, PTPRF, RBM33, RBP4, RFC1, RIN3, RNF220, RPS6KA2, RXRA, SARM1, SCD, SEBOX, SELENOP, SERINC2, SH3BP2, SH3PXD2A, SLC10A1, SLC16A13, SLC22A1, SLC22A9, SLC25A42, SLC25A47, SLC27A5, SLC29A1, SLC6A1, SLC7A10, SLC7A5, SLC7A9, SMAD3, SNED1, SNTG1, SOX10, SOX11, SPTBN1, SQSTM1, STK24, SUN1, TBC1D14, TEDC1, TEX2, TFR2, TIMP2, TM6SF2, TMEM33, TMEM82, TNK2, TNS3, TOP1MT, TRAPPC4, TRIP10, UBE2V2, UGT1A1, UGT1A3, UGT1A4, UROC1, VAV2, VPS37B, VPS4A, VTN, WDR62, ZC3H10, ZC3H7B, ZFPM1, ZFYVE28, ZMYM4, ZNF358
Concentration of cholesterol	9.67	ABCG5, ABCG8, ACACB, ADM, APOA5, APOB, ARNTL, CDKN1B, CYP8B1, DGAT1, DHCR24, DUSP1, FASN, GPER1, HIF1AN, HP, HPN, IL1RN, IRS1, LIPC, LRP5, mir-122, NPC1L1, PEMT, PON1, RXRA, SCD, TM6SF2, UGT1A1

Hepatobiliary neoplasm	9.33	A1BG, A2M, ABCG5, ABCG8, ACACB, ACSL1, AGBL1, AHSG, AKR1C4, ALDH2, ALDH8A1, ALPL, ANGPTL7, ANPEP, AP1M1, APOA5, APOB, APOF, ARC, ARHGAP44, ARMC5, ARNTL, ATP2B2, BDH1, C1R, C1S, C5, CABLES2, CAMK2G, CAMTA2, CBFA2T3, CCDC57, CDK9, CDKN1B, CENPP, CHRD, CHRNA4, CLASP1, CLU, COL18A1, CPN2, CTSB, CUX2, CYP8B1, DAB2IP, DCPS, DHCR24, DNAJB12, DOCK6, DOCK9, DUSP1, DUSP3, ELFN1, EPPK1, ERICH1, EXOC3L4, F12, F2, FAAP100, FAM20C, FASN, FBP1, FGA, FOXD2-AS1, GNPAT1, GNRH2, GOT1, GPER1, GPRC5C, GRHPR, GYS2, H6PD, HAAO, HAGH, HIF1AN, HP, HPN, HPX, HR, IGF2R, IGFALS, IHH, INHBE, INPP5A, INS-IGF2, INTS6, IRS1, ITGB4, ITIH1, KATNAL1, LBP, LPAL2, LRP5, MAD1L1, MASP1, MASP2, MEGF6, MGMT, MGRN1, MICAL3, MINK1, mir-122, MLXIP, MYO1C, MYO7A, NAGS, NAT8, NCMAP, NCOR2, NFIC, NINL, NLRP6, NRDE2, NTN1, OPLAH, ORM1, OSGIN1, PAH, PC, PCK1, PCSK6, PIEZO1, PLEC, PMEPA1, PMFBP1, PML, POLE, PRLR, PROZ, PTPRF, RBM33, RBP4, RFC1, RIN3, RNF220, RPS6KA2, RXRA, SARM1, SCD, SEBOX, SELENOP, SERINC2, SH3BP2, SH3PXD2A, SLC10A1, SLC16A13, SLC22A1, SLC22A9, SLC25A42, SLC25A47, SLC27A5, SLC29A1, SLC43A1, SLC6A1, SLC7A10, SLC7A5, SLC7A9, SMAD3, SNED1, SNTG1, SORBS2, SOX10, SOX11, SPTBN1, SQSTM1, SS18L1, STK24, SUN1, TBC1D14, TEDC1, TEX2, TFR2, TIMP2, TM6SF2, TMEM33, TMEM82, TNK2, TNS3, TOP1MT, TRAPPC4, TRIP10, UBE2V2, UGT1A1, UGT1A3, UGT1A4, UROC1, VAV2, VPS37B, VPS4A, VTN, WDR62, WNT5A, ZC3H10, ZC3H7B, ZFAND2A, ZFHX3, ZFPM1, ZFYVE28, ZMYM4, ZNF358, ZNF444
Acute Phase Response Signaling	7.09	A2M, AHSG, C1R, C1S, C5, F2, FGA, HP, HPX, HRG, IL1RN, ITIH3, LBP, ORM1, ORM2, RBP4
Clathrin-mediated Endocytosis Signaling	6.86	AP1M1, APOB, APOC2, APOC4, APOF, CLU, F2, FGF1, FGF3, ITGB4, ORM1, ORM2, PIP5K1C, PON1, RBP4, SH3BP4
LPS/IL-1 Mediated Inhibition of RXR Function	5.92	ABCG5, ABCG8, ACSF2, ACSL1, ALAS1, ALDH2, ALDH8A1, APOC2, APOC4, CYP2E1, IL1RN, LBP, LIPC, MGMT, RXRA, SLC10A1, SLC27A5

Morphology of liver	5.73	AGL, ARHGAP1, CDKN1B, CYP2E1, DUSP1, FGA, FGF1, GATA4, GPER1, HIF1AN, IGF2R, IRS1, MAT1A, mir-122, PCK1, PEMT, PROC, RXRA, SLC13A5, SLC22A1, SMAD3, SPTBN1, WNT5A, ZFPM1
Extrinsic Prothrombin Activation Pathway	5.35	F12, F2, F7, FGA, PROC
Growth of epithelial tissue	5.06	ADM, AGTR1, C5, CAMK2G, CBFA2T3, CDKN1B, CLU, COL18A1, CTSC, CYBA, DAB2IP, EPPK1, F12, F2, FGF1, FGF3, HIF1AN, HPN, IHH, IL1RN, ITGB4, NFIC, NLRP6, NTN1, PC, PML, PRLR, PROC, RXRA, SEPTIN9, SLC7A5, SLC9A3R2, SMAD3, SOX11, SPTBN1, TIMP2, TOP1MT, VAV2, WNT5A
TR/RXR Activation	4.99	APOA5, FASN, FGA, HP, NCOR2, PCK1, RXRA, SLC16A3, THRSP
Coagulation System	4.68	A2M, F12, F2, F7, FGA, PROC
liver development	4.41	CEBPB, CEBPG, FPGS, GNPAT1, IGF2R, IHH, MPST, ONECUT2, PROC, SEBOX, SMAD3, SRD5A1, UGT1A1, UGT1A8
Complement System	3.47	C1R, C1S, C5, MASP1, MASP2
Fatty Acid Activation	2.76	ACSF2, ACSL1, SLC27A5
Bile Acid Biosynthesis, Neutral Pathway	2.52	AKR1C4, CYP8B1, SLC27A5
Iron homeostasis signaling pathway	2.07	ATP6V0D1, HP, HPX, SLC25A37, SMAD3, STEAP3, TFR2
Hepatic Cholestasis	1.84	ABCG5, ABCG8, CYP8B1, IL1RN, LBP, RXRA, SLC10A1, TJP2
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1.81	A2M, AGTR1, COL18A1, CYP2E1, FGF1, LBP, SMAD3, TIMP2
<u>HUMAN ENDOTHELIAL</u>		
Biological Pathways and Functions	-log(p-value)	Molecules
Development of vasculature	13.09	ABCA4, ANGPT2, APLNR, ARAP3, ATXN1, CCL24, CCN1, CCN2, CD9, CTNBP1, CTTN, DLC1, ECSCR, EFNB2, EGFL7, EPHA2, EPHB2, ESM1, FAT1, FGF18, FLI1, FLT1, FOXC1, GBX2, HOXD3, HSPG2, HYAL1, IGF1R, JCAD, LAMA4, MAP2K5, MMP14, MMRN2, NFATC1, NOS3, NOTCH1, PDE2A, PIK3R1, PLPP3, PRKCE, RAPGEF1, RASA3, SEMA6A, SMAD3, SOX7, TCF7L2, TGM2, TMEM204, VWF, WLS, ZEB1

Angiogenesis	13.08	ABCA4, ANGPT2, APLNR, ARAP3, ATXN1, CCL24, CCN1, CCN2, CD9, CTNNBIP1, ECSCR, EFNB2, EGFL7, EPHA2, EPHB2, ESM1, FAT1, FGF18, FLI1, FLT1, FOXC1, GBX2, HOXD3, HSPG2, HYAL1, IGF1R, JCAD, LAMA4, MAP2K5, MMP14, NFATC1, NOS3, NOTCH1, PDE2A, PIK3R1, PLPP3, PRKCE, RAPGEF1, RASA3, SEMA6A, SMAD3, SOX7, TCF7L2, TGM2, TMEM204, VWF, WLS, ZEB1
Morphology of cardiovascular system	10.10	ANGPT2, ANK3, APLNR, ATXN1, CACNA1D, CAPZB, CCN2, CTTN, EFNB2, EPHA2, FGF18, FLT1, GBX2, GRK5, HDAC4, HOXD3, HSPG2, IGF1R, LAMA4, LDLRAD3, MAP2K5, MIR5093, MME, MMP14, MMRN2, MTERF4, NCOR2, NFATC1, NOS3, NOTCH1, PARD3, PIK3R1, PLPP3, PRDM16, PRKCE, RHEB, RPS6KA2, SMAD3, TGM2, VWF, ZEB1
Vasculogenesis	9.97	ABCA4, ANGPT2, APLNR, ATXN1, CCL24, CCN1, CCN2, CD9, CTNNBIP1, ECSCR, EFNB2, EGFL7, EPHA2, EPHB2, FAT1, FLI1, FLT1, FOXC1, GBX2, HSPG2, IGF1R, MAP2K5, MMP14, NFATC1, NOS3, NOTCH1, PDE2A, PLPP3, PRKCE, RASA3, SEMA6A, SMAD3, SOX7, TCF7L2, TGM2, VWF, WLS, ZEB1
Cell movement of endothelial cells	8.20	ANGPT2, CCL24, CCN1, CCN2, CD9, DLC1, ECSCR, EFNB2, EGFL7, FGF18, FLT1, FOXC1, HSPG2, MAP2K5, MMP14, NOS3, NOTCH1, PDE2A, PLPP3, PRKCE, SMAD3, VWF
Transcription of RNA	7.39	ABLIM2, AEBP2, ANKRD33, ATXN1, CAMKK2, CCN1, CCN2, CCNDBP1, CD9, CRCP, CTBP1, CTNNBIP1, FLI1, FOXC1, FOXL1, GBX2, GLI2, GRHL2, HDAC4, HOXD3, IBTK, IGF1R, INO80, ITGA6, KANK2, MAD1L1, MAMSTR, MAP2K5, MME, MTA1, MXD3, MYEF2, MYT1L, NACC2, NCOR2, NFATC1, NOTCH1, PDE2A, PIAS4, PIK3R1, PRDM16, PRKCE, RAPGEF1, RAX2, SMAD3, SOX2, SOX7, TCF7L2, TFCP2L1, TRAF7, TRIM44, ZEB1, ZHX2
Transcription of DNA	6.27	ABLIM2, AEBP2, ANKRD33, ATXN1, CAMKK2, CCN1, CRCP, CTBP1, CTNNBIP1, FLI1, FOXC1, FOXL1, GBX2, GLI2, GRHL2, HDAC4, HOXD3, IGF1R, INO80, ITGA6, KANK2, MAMSTR, MAP2K5, MXD3, MYEF2, MYT1L, NACC2, NCOR2, NFATC1, NOTCH1, PDE2A, PIAS4, PIK3R1, PRDM16, RAX2, SMAD3, SOX2, SOX7, TCF7L2, TFCP2L1, TRAF7, TRIM44, ZEB1, ZHX2
Migration of vascular endothelial cells	5.49	ANGPT2, CCN1, CD9, DLC1, ECSCR, EFNB2, FLT1, FOXC1, MMP14, PDE2A, PLPP3, SMAD3

Development of vascular endothelial cells	4.79	CCN1, CD9, FLT1, MMP14, NOTCH1, RASA3
Apelin Endothelial Signaling Pathway	4.43	ADCY4, APLNR, GNG7, HDAC4, NOS3, PIK3R1, PRKCE, SMAD3
IGF-1 Signaling	4.41	CCN1, CCN2, GRB10, IGF1R, NEDD4, PIK3R1, PRKAR1B
Nitric Oxide Signaling in the Cardiovascular System	4.04	CACNA1D, FLT1, NOS3, PDE2A, PIK3R1, PRKAR1B, PRKCE
Pulmonary Fibrosis Idiopathic Signaling Pathway	3.80	AEBP2, CCN2, EFNB2, FGF18, GLI2, MMP14, NOTCH1, PIK3R1, RPS6KA2, SMAD3, TCF7L2
Opioid Signaling Pathway	3.72	ADCY4, CACNA1D, GNG7, GRK5, MAP2K5, NOS3, PRKAR1B, PRKCE, RPS6KA2, TCF7L2
Oxytocin Signaling Pathway	3.71	CACNA1D, CAMKK2, GNG7, MAP2K5, MYO18A, NFATC1, NOS3, PIK3R1, PRKAR1B, PRKCE
White Adipose Tissue Browning Pathway	3.68	ADCY4, ANGPT2, CACNA1D, CAMKK2, CTBP1, PRDM16, PRKAR1B
Estrogen Receptor Signaling	3.56	ADCY4, CACNA1D, CTBP1, GNG7, IGF1R, MMP14, NCOR2, NOS3, NOTCH1, PIK3R1, PRKAR1B, PRKCE
Transcriptional Regulatory Network in Embryonic Stem Cells	2.88	CDYL, FOXC1, GBX2, SOX2
Cellular Effects of Sildenafil (Viagra)	2.68	ADCY4, CACNA1D, MYO18A, NOS3, PDE2A, PRKAR1B
Relaxin Signaling	2.59	ADCY4, GNG7, NOS3, PDE2A, PIK3R1, PRKAR1B
eNOS Signaling	2.54	ADCY4, FLT1, NOS3, PIK3R1, PRKAR1B, PRKCE
Netrin Signaling	2.42	ABLIM2, CACNA1D, NFATC1, PRKAR1B
VEGF Family Ligand-Receptor Interactions	2.19	FLT1, NOS3, PIK3R1, PRKCE
Endothelin-1 Signaling	1.55	ADCY4, CASP2, NOS3, PIK3R1, PRKCE
HIF1 α Signaling	1.43	FLT1, MAP2K5, MMP14, PIK3R1, PRKCE
HUMAN IMMUNE		
Biological Pathways and Functions	-log(p-value)	Molecules
Proliferation of immune cells	10.46	CARMIL2, CD37, CD6, CD79B, CR2, CUL3, DEF6, EFNB2, ETS1, GFI1, IL15, IL6R, ITGA2B, JAK3, LCP1, MAD1L1, MAP4K1, MBL2, NCKAP1L, NCOR2, NFATC1, OSM, PLA2G6, PTPN6, PTPRCAP, RPS6KA1, RPTOR, RUNX3, S100B, S1PR4, SKAP1, SOCS3, STING1

Proliferation of mononuclear leukocytes	10.43	CARMIL2, CD37, CD6, CD79B, CR2, CUL3, DEF6, EFNB2, ETS1, GFI1, IL15, IL6R, ITGA2B, JAK3, LCP1, MAD1L1, MAP4K1, MBL2, NCKAP1L, NCOR2, NFATC1, OSM, PLA2G6, PTPN6, PTPRCAP, RPTOR, RUNX3, S100B, S1PR4, SKAP1, SOCS3, STING1
Differentiation of progenitor cells	10.31	CD6, CD79B, DOCK1, FGF6, GFI1, GNA15, HOXA3, HOXA5, IL15, IL6R, ITGA2B, JAK3, KCNAB2, MAD1L1, MAFK, MAP4K1, OLIG2, OSM, PTPN6, RPS6KA1, RPTOR, RXRA, S1PR4, SOCS3, TEK
Proliferation of lymphocytes	9.93	CARMIL2, CD37, CD6, CD79B, CR2, CUL3, DEF6, EFNB2, ETS1, GFI1, IL15, IL6R, ITGA2B, JAK3, LCP1, MAD1L1, MAP4K1, NCKAP1L, NCOR2, NFATC1, OSM, PLA2G6, PTPN6, PTPRCAP, RPTOR, RUNX3, S100B, S1PR4, SKAP1, SOCS3, STING1
Proliferation of blood cells	9.87	CARMIL2, CD37, CD6, CD79B, CR2, CUL3, DEF6, EFNB2, ETS1, GFI1, HOXA5, IL15, IL6R, ITGA2B, JAK3, LCP1, MAD1L1, MAP4K1, MBL2, NCKAP1L, NCOR2, NFATC1, OSM, PLA2G6, PTPN6, PTPRCAP, RPS6KA1, RPTOR, RUNX3, S100B, S1PR4, SKAP1, SOCS3, STING1
Cell proliferation of T lymphocytes	9.42	CARMIL2, CD37, CD6, CUL3, DEF6, EFNB2, ETS1, GFI1, IL15, IL6R, ITGA2B, JAK3, LCP1, MAD1L1, MAP4K1, NCKAP1L, NCOR2, OSM, PTPN6, PTPRCAP, RPTOR, RUNX3, S100B, S1PR4, SKAP1, SOCS3, STING1
Quantity of mononuclear leukocytes	9.24	C1QTNF6, CD6, CD79B, CR2, DEF6, EFNB2, ETS1, FGF6, GFI1, HOXA3, HVCN1, IL15, IL6R, JAK3, JARID2, LGMN, LSP1, MBL2, NEDD9, NFATC1, NKX2-3, OSM, PTPN6, RASAL3, RPTOR, RUNX3, SERPINB6, SIPA1, SOCS3, STING1, TEK
Quantity of lymphatic system cells	8.91	C1QTNF6, CD6, CD79B, CR2, DEF6, EFNB2, ETS1, GFI1, HOXA3, HVCN1, IL15, IL6R, JAK3, LGMN, LSP1, MBL2, NEDD9, NFATC1, NKX2-3, OSM, PTPN6, PTPRCAP, RASAL3, RFTN1, RPTOR, RUNX3, SERPINB6, SIPA1, SOCS3, STING1, TEK
Cell movement of blood cells	8.86	C1QTNF6, CAMK2D, CD37, CD6, CR2, DEF6, DOCK1, EFNB2, ETS1, EVL, IL15, IL6R, ITGA2B, JAK3, LCP1, LGMN, LSP1, MAP4K1, NCKAP1L, NEDD9, NFATC1, NKX2-3, OSM, PLA2G6, PTPN6, RPTOR, RUNX3, RXRA, S100B, S1PR4, SKAP1, SOCS3, STING1, TEK, ZBP1
Differentiation of hematopoietic cells	8.73	CD6, CD79B, DOCK1, GFI1, GNA15, HOXA3, HOXA5, IL15, IL6R, ITGA2B, JAK3, KCNAB2,

		MAD1L1, MAFK, MAP4K1, OSM, PTPN6, RPS6KA1, RXRA, TEK
Quantity of lymphocytes	8.45	C1QTNF6, CD6, CD79B, CR2, DEF6, EFNB2, ETS1, GFI1, HOXA3, HVCN1, IL15, IL6R, JAK3, LGMN, LSP1, MBL2, NEDD9, NFATC1, NKX2-3, OSM, PTPN6, RASAL3, RPTOR, RUNX3, SERPINB6, SIPA1, SOCS3, STING1, TEK
Leukocyte migration	8.38	C1QTNF6, CAMK2D, CD37, CD6, CR2, DEF6, DOCK1, EFNB2, ETS1, EVL, IL15, IL6R, ITGA2B, JAK3, LCP1, LGMN, LSP1, MAP4K1, NCKAP1L, NEDD9, NFATC1, NKX2-3, OSM, PLA2G6, PTPN6, RPTOR, RUNX3, RXRA, S100B, S1PR4, SKAP1, SOCS3, STING1, TEK
Differentiation of hematopoietic progenitor cells	8.12	CD6, CD79B, DOCK1, GFI1, GNA15, HOXA3, HOXA5, IL15, IL6R, ITGA2B, KCNAB2, MAD1L1, MAFK, MAP4K1, OSM, PTPN6, RPS6KA1, RXRA, TEK
Development of hematopoietic system	8.03	AGO2, CD79B, ETS1, GFI1, HOXA5, IL15, IL6R, ITGA2B, JAK3, LGMN, MAFK, OSM, PLA2G6, PTPN6, RPS6KA1, RPTOR, RUNX3, SOCS3, TEK
Lymphopoiesis	7.18	CARMIL2, CD6, CD79B, CR2, DEF6, EFNB2, ETS1, GFI1, IL15, IL6R, JAK3, LCP1, LSP1, MAD1L1, MAP2K2, NCKAP1L, NFATC1, NKX2-3, OSM, PTGS1, PTPN6, RFTN1, RPTOR, RUNX3, SOCS3
Binding of leukocytes	7.12	CD37, CD6, CR2, EVL, IL15, IL6R, JAK3, LCP1, LSP1, MAP4K1, MBL2, NCKAP1L, NEDD9, OSM, PLA2G6, PTPN6, SIPA1, SKAP1
Development of bone marrow	6.98	GFI1, HOXA5, IL15, IL6R, ITGA2B, LGMN, MAFK, OSM, PLA2G6, PTPN6, RPS6KA1, RPTOR, RUNX3, TEK
T cell development	5.90	CARMIL2, CD6, CD79B, DEF6, EFNB2, ETS1, GFI1, IL15, IL6R, JAK3, MAD1L1, NCKAP1L, NFATC1, OSM, PTGS1, PTPN6, RFTN1, RPTOR, RUNX3, SOCS3
TGF- β Signaling	3.21	MAP2K2, MAP4K1, PMEPA1, RUNX3, SMAD6
Role of JAK family kinases in IL-6-type Cytokine Signaling	3.14	IL6R, OSM, SOCS3
Th1 and Th2 Activation Pathway	2.84	GFI1, IL6R, JAK3, NFATC1, RUNX3, SOCS3
Th1 Pathway	2.74	IL6R, JAK3, NFATC1, RUNX3, SOCS3
JAK/STAT Signaling	2.56	JAK3, MAP2K2, PTPN6, SOCS3
PI3K Signaling in B Lymphocytes	2.46	CAMK2D, CD79B, CR2, MAP2K2, NFATC1
CXCR4 Signaling	2.14	DOCK1, FBNP1, GNA15, GNG7, MAP2K2
<u>MOUSE CARDIOMYOCYTE</u>		

Biological Pathways and Functions	-log(p-value)	Molecules
cardiac cell development	82.47	Actc1, Actn2, Alpk3, Atg5, Atg7, Bmp10, Cav3, Cdk1, Csrp3, Fhl2, Fhod3, Lrrc10, Map2k4, Mef2a, Myh6, Myl2, Mylk3, Myo18b, Mypn, Nexn, Nppb, Pcd4, Pitx2, Popdc2, Prox1, Slc8a1, Speg, Tcap, Tgfr3, Ttn, Vegfa, Xirp1
myofibril assembly	81.23	Actc1, Actn2, Casq2, Csrp3, Fhod3, Foxp1, Ldb3, Lmod2, Mef2a, Mybpc3, Myh6, Myl2, Mylk3, Myom1, Myom2, Myoz1, Myoz2, Myoz3, Mypn, Prkar1a, Prox1, Tcap, Tmod1, Tnnt2, Tpm1, Ttn, Xirp1
actomyosin structure organization	79.42	Actc1, Actn2, Casq2, Cdc42bpa, Csrp3, Ebp411l, Ebp4113, Ebp4114a, Ebp4114b, Fhod3, Foxp1, Frmd5, Frmd6, Itgb5, Ldb3, Limch1, Lmod2, Mef2a, Mybpc3, Myh6, Myl2, Mylk3, Myo18a, Myom1, Myom2, Myoz1, Myoz2, Myoz3, Mypn, Pcd6ip, Phactr1, Prkar1a, Prox1, Sorbs1, Tcap, Tmod1, Tnnt2, Tpm1, Trpm7, Ttn, Xirp1
striated muscle cell development	77.01	Actc1, Actn2, Alpk3, Atg5, Atg7, Bmp10, Capzb, Casq2, Cav3, Cdk1, Csrp3, Fhl2, Fhod3, Flnc, Foxp1, Homer1, Ldb3, Lef1, Lmod2, Lrrc10, Map2k4, Mef2a, Mybpc3, Myh6, Myl2, Mylk3, Myo18b, Myom1, Myom2, Myoz1, Myoz2, Myoz3, Mypn, Nexn, Nfatc2, Nppb, Pitx2, Popdc2, Prkar1a, Prox1, Ptdc2, Slc8a1, Smyd3, Speg, Tcap, Tmod1, Tnnt2, Tpm1, Ttn, Vegfa, Wfikk2, Xirp1
cardiac muscle contraction	75.80	Actc1, Ank2, Cacna1c, Cacna1d, Cacna1g, Cacna2d1, Camk2d, Casq2, Cav3, Csrp3, Gja5, Gpd1l, Kcnd3, Kcne1l, Kcne2, Kcnh2, Kcnj2, Kcnj5, Kcnj8, Kcnn2, Kcnq1, Mybpc3, Myh6, Myh7, Myl2, Myl3, Pkp2, Ryr2, Scn3b, Scn5a, Slc8a1, Snta1, Tcap, Tnnc1, Tnni3, Tnnt2, Tpm1, Ttn
cardiocyte differentiation	58.31	Acadm, Actc1, Actn2, Akap13, Alpk3, Atg5, Atg7, Bmp10, Bmp2, Cacybp, Cav3, Cdk1, Cited2, Csrp3, Ctnnb1, Eomes, Fhl2, Fhod3, Foxp1, Gata4, Gata6, Hand2, Hes1, Isl1, Lrp6, Lrrc10, Map2k4, Mef2a, Myh6, Myl2, Mylk3, Myo18b, Myocd, Mypn, Nexn, Nppb, Pax3, Pcd4, Pitx2, Popdc2, Prok2, Prox1, Rarb, Rbpj, Rxrb, Slc8a1, Sox6, Speg, T, Tbx2, Tcap, Tenm4, Tgfb2, Tgfr3, Ttn, Twist1, Vegfa, Xirp1

Morphology of heart	26.40	ACACB, ACADL, ACADM, ACTC1, ADAMTS9, ADCY5, ADCY6, ADGRL2, ADRA1B, ADRA2A, ADRB1, AGTR1, AKAP13, ALKBH7, ANGPT1, ANK3, ANKRD1, ATE1, ATP2A2, BMP10, C10orf71, CABIN1, CACNA1C, CAMK2D, CASP7, CASQ2, CAST, CASZ1, CAV3, CCN2, CD36, CDH2, CISD2, CKM, CKMT2, CORIN, CREB1, CRYAB, CSRP3, CTNNA3, CTSD, CXCL12, DAG1, DDIAS, DES, DHRS3, DOCK1, DTNBP1, DYRK1A, EDN1, EGLN1, EIF4EBP1, ERBB4, ERBIN, ETV6, F3, FABP3, FASN, FAT4, FBXO32, FGF9, FHL2, FHOD3, FNIP1, Foxp1, FXR1, GATA4, GHR, GJA1, GJC1, GRB2, GRK5, HDAC4, HDAC9, HGF, HIF1A, HSPB7, HSPG2, IGF1R, IL17RA, IL1B, INSR, JPH2, KCNE2, KCNJ11, KCNQ1, KIDINS220, LAMA4, LIF, LMOD2, LRRC10, LTBP1, MAPKAPK2, MB, MBNL2, MEF2D, mir-1, mir-133, mir-208, MMP15, MORF4L1, Morrbid, MRTFB, MYBPC3, MYH6, MYL2, MYLK3, MYOCD, MYOM1, MYOZ2, MYZAP, NCOA2, NDUFS6, NEXN, NF1, Nppb, NR2F2, NT5E, NTF3, NTRK3, PARK7, PARP1, PDCD5, PDGFA, PDLIM5, PFKM, PIK3R1, PIM1, PITX2, PKP2, Pln, POSTN, PPARGC1A, PRKAA2, PRKAR1A, PRKCE, PRKG1, PROX1, PTH1R, PTHLH, RAF1, RARB, RBPJ, RCAN2, RGS2, RGS6, ROCK1, ROCK2, RPS6KA2, RPS6KB2, RRM2B, RXRB, RYR2, SEMA3A, SGCD, SGCG, SIRPA, SLC8A1, SMAD3, SMAD7, SMYD1, SP3, SPEG, SRSF10, STAB2, TAB1, TEAD1, TERT, TGFB2, TGFB2, TGFB3, TGM2, TLL1, TMEM38A, TMOD1, TNNI3, TNNT2, TNS1, Tpm1, TRIM54, TRIM55, TRIM63, TRPC3, TTN, UTRN, VAV2, VAV3, VEGFA, XIRP1, ZFPM2
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Abnormal morphology of cardiovascular system	25.50	ACACB, ACADL, ACADM, ACTC1, ADAMTS9, ADCY5, ADCY6, ADGRL2, ADRA1B, ADRA2A, ADRB1, ADTRP, AGTR1, AKAP13, ANGPT1, ANK3, ANKRD1, ARID4B, ATE1, ATP2A2, BCAM, BMP10, BMP2, CABIN1, CACNA1C, CAMK2D, CASP7, CASQ2, CAST, CAV3, CBS/CBSL, CCN2, CD36, CDH2, CISD2, CKM, CKMT2, CORIN, CREB1, CRHR2, CRYAB, CSRP3, CTNNA3, CTSD, CXCL12, DAG1, DDIAS, DES, DHRS3, DOCK1, DYRK1A, E2F8, EDN1, EGLN1, EIF4EBP1, ERBB4, ERBIN, ETV6, F3, FABP3, FASN, FAT4, FBLN1, FBN1, FBXO32, FGF9, FHL2, FHOD3, FNIP1, Foxp1, FXR1, FZD5, GAB1, GATA4, GDNF, GHR, GJA1, GRB2, GRK5, GSC, HDAC4, HDAC9, HGF, HIF1A, HSPB7, HSPG2, IGF1R, IL17RA, IL1B, INSR, JPH2, KCNE2, KCNJ11, KCNQ1, KIDINS220, KLF2, LAMA4, LIF, LPL, LRRC10, LTBP1, MAPKAPK2, MB, MBNL2, MEF2D, mir-1, mir-122, mir-133, mir-208, MMP15, MORF4L1, Morrbid, MRTFB, MYBPC3, MYH6, MYL2, MYLK3, MYOCD, MYOM1, MYOZ2, MYZAP, NCOA2, NDUFS6, NEXN, NF1, Nppb, NR2F2, NT5E, NTF3, NTRK3, PARK7, PARP1, PDGFA, PDLIM5, PFKM, PIK3R1, PIM1, PITX2, PKP2, Pln, PLPP3, POSTN, PPARGC1A, PRKAA2, PRKAR1A, PRKCE, PRKG1, PROX1, RAF1, RARB, RBPJ, RCAN2, RGS2, ROCK1, ROCK2, RPS6KA2, RPS6KB2, RRM2B, RXRB, RYR2, SEMA3A, SETD2, SGCD, SGCG, SIRPA, SLC8A1, SMAD3, SMAD7, SMYD1, SP3, SPEG, SRSF10, STAB2, TAB1, TEAD1, TERT, TGFB2, TGFB2, TGFB2, TGFB2, TGM2, TLL1, TMEM38A, TNNI3, TNNT2, TNS1, Tpm1, TRIM54, TRIM55, TRIM63, TRPC3, TTN, UTRN, VAV2, VAV3, VEGFA, XIRP1, ZFPM2
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Enlargement of heart	25.12	ACTC1, ADCY5, ADCY6, ADRA2A, ADRB1, AGTR1, AKAP13, ANGPT1, ANK3, ANKRD1, ATP2A2, BMP10, CABIN1, CACNA1C, CAMK2D, CASQ2, CAST, CAV3, CCN2, CD36, CDH2, CKM, CKMT2, CORIN, CREB1, CRYAB, CSRP3, CTNNA3, CTSD, CXCL12, DAG1, DDIAS, DES, DYRK1A, EDN1, EGLN1, EIF4EBP1, ERBIN, F3, FABP3, FASN, FBXO32, FGF9, FHL2, FHOD3, FNIP1, GATA4, GJA1, GRB2, GRK5, HDAC4, HDAC9, HGF, HIF1A, IGF1R, IL17RA, IL1B, INSR, KCNE2, KCNJ11, KCNQ1, LAMA4, LIF, LRRC10, MAPKAPK2, MB, MBNL2, MEF2D, mir-1, mir-133, mir-208, MORF4L1, Morrbid, MYBPC3, MYH6, MYL2, MYLK3, MYOCD, MYOM1, MYOZ2, MYZAP, NCOA2, NDUFS6, NEXN, NF1, NT5E, NTF3, NTRK3, PARK7, PARP1, PDGFA, PDLIM5, PFKM, PIK3R1, PIM1, PITX2, Pln, POSTN, PPARGC1A, PRKAA2, PRKAR1A, PRKCE, PRKG1, PROX1, RAF1, RCAN2, RGS2, ROCK1, ROCK2, RYR2, SEMA3A, SGCD, SIRPA, SLC8A1, SMAD3, SMYD1, SP3, SPEG, STAB2, TERT, TGFB2, TGFB3, TNNT2, TNNI3, TNNI3, Tpm1, TRIM54, TRIM55, TRIM63, TRPC3, TTN, UTRN, VAV2, VAV3, VEGFA, XIRP1
Cardiogenesis	19.82	ACADM, ACTC1, ACTN2, ADGRL2, ADRA1B, AGTR1, AKAP13, ALPK3, ANGPT1, ARID2, ASB2, ATE1, ATP2A2, BICC1, BMP10, BMP2, C10orf71, C1orf127, CASP7, CASQ2, CASZ1, CAV3, CBS/CBSL, CCDC39, CCN1, CSRP3, CTBP2, CXCL12, DHRS3, DLC1, DSP, DTNBP1, EDN1, EGLN1, ERBB4, ESRRG, F2RL2, FAT4, FGF20, FGF9, FHOD3, FLRT3, Foxp1, FREM2, GAB1, GATA4, GJA1, GJC1, GLI2, GSC, HDAC4, HDAC9, HEG1, HIF1A, HSPG2, IL1B, INSR, JPH2, KCNQ1, LDB3, LIF, LMOD2, LRP6, LRRC10, LTBP1, MB, mir-1, mir-133, mir-208, MORF4L1, MRTFB, MYBPC3, MYH6, MYL2, MYLK3, MYO18B, MYOCD, MYOZ1, MYOZ2, NCOA2, NDST1, NF1, NR2F2, NTF3, NTRK3, PAX3, PCSK5, PCSK6, PDE2A, PDGFA, PITX2, PKP2, Pln, PPARGC1A, PRICKLE1, PRKAR1A, PROK2, PROX1, PTC2, RBPJ, ROBO1, RPS6KA2, RXRB, SCN5A, SETD2, SGCD, SGCG, SLC8A1, SMAD3, SMAD7, SMYD1, SPEG, SYNPO2L, TAB1, TBXT, TCAP, TEAD1, TGFB2, TGFB3, TLL1, TMOD1, TNNI3, TNNI3, TP53BP2, Tpm1, TRIM63, TTN, UTRN, VEGFA, VGLL4, WNT11, XIRP1, ZFPM2

Function of cardiac muscle	14.36	ADCY6, ADRA1B, ADRB1, ASPH, ATE1, ATP1A1, ATP2A2, C10orf71, CACNA2D1, CASQ2, CAV3, CKM, CORIN, CRHR2, CSRP3, CTNNA3, DES, DSG2, DTNBP1, EDN1, ESRRG, GAB1, GATA4, KCNIP2, LAMA4, LIF, LRRC10, MB, mir-1, mir-133, mir-208, MYBPC3, MYH6, MYLK3, NEXN, PDLIM5, Pln, PPARGC1A, PRKG1, PTH1R, RBPJ, RYR2, SGCD, SLC8A1, SLN, SMAD7, SOD1, SRL, SRSF10, TMEM38A, TNNT2, Tpm1, TRIM54, TRIM55, TRIM63, TTN, VAV3, VEGFA, XIRP1
Dilated Cardiomyopathy Signaling Pathway	11.60	ABCC9, ACTC1, ADCY5, ADCY6, ADCY9, ADRB1, ATP2A2, BAD, BAG3, CACNA1C, CACNA2D1, CAMK2D, DES, DSG2, GAB1, ITPR1, KDM1A, LAMA4, MYBPC3, MYH6, MYH7, MYH7B, MYL2, MYL3, MYO10, MYO18A, MYO18B, PDE2A, PRKAR1A, PRKAR2A, PRKCE, RBM20, RYR2, SCN5A, SGCD, TNNC1, TNNI3, TNNT1, TNNT2, TNNT3, TTN
Morphology of cardiomyocytes	11.26	ACADM, ACTC1, ADCY6, ADRB1, ATE1, ATP2A2, C10orf71, CABIN1, CASQ2, CAV3, CCN2, CDH2, CSRP3, CTNNA3, DES, DTNBP1, EDN1, FHL2, FXR1, GATA4, HDAC9, HGF, HSPB7, IL1B, INSR, JPH2, LAMA4, LIF, LRRC10, MB, mir-1, mir-133, mir-208, MORF4L1, MYBPC3, MYH6, MYLK3, MYOCD, MYOZ2, Nppb, Pln, RAF1, RPS6KA2, RYR2, SLC8A1, TAB1, TERT, TGFB3, TMEM38A, TNNI3, TNNT2, TRIM55, TRIM63, TTN, VAV2, VAV3, VEGFA
Cardiac Hypertrophy Signaling (Enhanced)	6.71	ACVR2A, ADCY5, ADCY6, ADCY9, ADRA1B, ADRA2A, ADRB1, AGTR1, AKAP13, ATP2A2, CACNA1C, CACNA2D1, CAMK2D, EDN1, EIF4E, EIF4EBP1, FGF13, FGF20, FGF6, FGF9, FGFR3, FZD5, FZD9, GATA4, GHR, GNB4, HDAC4, HDAC9, HSPB1, HSPB7, IGF1R, IL13, IL17RA, IL1B, IL20RA, IL21R, IL9R, ITGA2B, ITGA9, ITGAD, ITGAL, ITGAX, ITGB6, ITPR1, LIF, MAP3K20, MAP3K4, MAPK10, MAPKAPK2, MAPKAPK3, MEF2D, MYOCD, PDE10A, PDE2A, PDE4B, PDE7A, PDE7B, PIK3R1, PIK3R3, PIK3R4, PLCB2, PLCD3, PLCZ1, PRKAR1A, PRKAR2A, PRKCE, PRKCH, PRKG1, RAF1, RALB, RAP2A, RASD1, ROCK1, ROCK2, RPS6KB2, RYR2, TGFB2, TGFB3, TGFB3, WNT11

Actin Cytoskeleton Signaling	6.24	ACTC1, ACTN2, ACTR3, ARHGEF7, DOCK1, EZR, FGF13, FGF20, FGF6, FGF9, GRB2, ITGA2B, ITGA9, ITGAD, ITGAL, ITGAX, ITGB6, MYH6, MYH7, MYH7B, MYL2, MYL3, MYLK3, MYO10, MYO18A, MYO18B, PDGFA, PIK3R1, PIK3R3, PIK3R4, PPP1R12A, Ppp1r12b, RAF1, RALB, RAP2A, RASD1, ROCK1, ROCK2, SSH2, TLN2, TRIO, TTN, VAV2, VAV3
Calcium Signaling	5.02	ACTC1, ASPH, ATP2A2, ATP2B1, ATP2C1, CABIN1, CACNA1C, CACNA2D1, CAMK2D, CASQ2, CREB1, HDAC4, HDAC9, ITPR1, MEF2D, MYH6, MYH7, MYH7B, MYL2, MYL3, MYO10, MYO18A, MYO18B, PRKAR1A, PRKAR2A, RAP2A, RCAN2, RYR2, SLC8A1, TNNC1, TNNI3, TNNT1, TNNT2, TNNT3, TP63, Tpm1, TRPC3
Factors Promoting Cardiogenesis in Vertebrates	4.49	ACVR2A, BMP10, BMP2, CAMK2D, CREB1, FZD5, FZD9, GATA4, LRP6, MAPK10, MYH6, MYH7, MYL2, MYOCD, PLCB2, PLCD3, PLCZ1, PRKCE, PRKCH, ROCK1, ROCK2, SCN5A, TCF4, TCF7L2, TGFB2, TGFB2, TGFB3, WNT11
Cardiac Hypertrophy Signaling	4.42	ADCY5, ADCY6, ADCY9, ADRA1B, ADRA2A, ADRB1, CACNA1C, CACNA2D1, CREB1, EIF4E, GATA4, GNB4, GRB2, HSPB1, IGF1R, MAP3K4, MAPK10, MAPKAPK2, MAPKAPK3, MEF2D, MYL2, MYL3, PIK3R1, PIK3R3, PIK3R4, PLCB2, PLCD3, PLCZ1, PRKAR1A, PRKAR2A, RAF1, RALB, RAP2A, RASD1, RHOBTB1, RHOBTB2, ROCK1, ROCK2, TAB1, TGFB2, TGFB2
White Adipose Tissue Browning Pathway	3.83	ADCY5, ADCY6, ADCY9, CACNA1C, CACNA2D1, CAMP, CREB1, CTBP2, FGFR3, FNDC5, ITPR1, LDHB, PPARGC1A, PRKAA2, PRKAB1, PRKAB2, PRKAR1A, PRKAR2A, PRKG1, RARB, RUNX1T1, RXRB, RXRG, SLC16A1, VEGFA
Role of NFAT in Cardiac Hypertrophy	3.75	ADCY5, ADCY6, ADCY9, CABIN1, CACNA1C, CACNA2D1, CAMK2D, GATA4, GNB4, GRB2, HDAC4, HDAC9, IGF1R, ITPR1, LIF, MAPK10, MEF2D, PIK3R1, PIK3R3, PIK3R4, PLCB2, PLCD3, PLCZ1, PRKAR1A, PRKAR2A, PRKCE, PRKCH, RAF1, RALB, RAP2A, RASD1, RCAN2, SLC8A1, TGFB2, TGFB2
Thrombin Signaling	3.19	ADCY5, ADCY6, ADCY9, ARHGEF16, ARHGEF3, CAMK2D, CREB1, F2RL2, GATA4, GNB4, GRB2, ITPR1, MYL2, MYL3, PIK3R1, PIK3R3, PIK3R4, PLCB2, PLCD3, PLCZ1, PPP1R12A, Ppp1r12b, PRKCE, PRKCH, RAF1, RALB, RAP2A, RASD1, RHOBTB1, RHOBTB2, ROCK1, ROCK2

Cardiac β -adrenergic Signaling	2.97	ADCY5, ADCY6, ADCY9, ADRB1, AKAP1, AKAP13, AKAP6, ATP2A2, CACNA1C, CACNA2D1, GNB4, PDE10A, PDE2A, PDE4B, PDE7A, PDE7B, PKIG, PPP1R12A, PPP1R14C, PPP1R3A, PPP1R3D, PPP2R2B, PPP2R3A, PRKAR1A, PRKAR2A, RYR2, SLC8A1
Apelin Cardiomyocyte Signaling Pathway	1.77	ATP2A2, HIF1A, ITPR1, MAPK10, MYL2, MYL3, PIK3R1, PIK3R3, PIK3R4, PLCB2, PLCD3, PLCZ1, PRKCE, PRKCH, SLC8A1
Cardiomyocyte Differentiation via BMP Receptors	1.63	BMP10, BMP2, GATA4, MYH7, MYL2
Nitric Oxide Signaling in the Cardiovascular System	1.46	ADRB1, ATP2A2, CACNA1C, CACNA2D1, ITPR1, PDE2A, PIK3R1, PIK3R3, PIK3R4, PRKAR1A, PRKAR2A, PRKCE, PRKCH, PRKG1, RYR2, VEGFA
MOUSE HEPATOCYTE		
Biological Pathways and Functions	-log(p-value)	Molecules
Concentration of lipid	14.21	ABCC4, ABCG8, ACLY, ADCY10, AGPAT2, AGT, ANGPTL8, APOA1, Apoc1, APOE, AQP12A/AQP12B, C3, CAMP, CERS2, CFB, CIDEC, COL18A1, CPE, CRHR1, CYP17A1, CYP8B1, DHCR24, EGFR, ELOVL2, EPAS1, ESR2, F2, FABP1, FASN, FFAR4, FOXA2, GC, GCGR, GCK, GDF15, GNAT1, Gucy2g, Gulo, HP, HPN, HRH1, IRS2, KL, KLF15, LCAT, LDLRAP1, LEPR, LPAR3, LRP1, LRP5, LSR, mir-290, mir-33, MPC1, MRAP, NR0B2, NR1I3, NSMF, NXPH4, OXSM, PCK1, PEMT, PLIN2, PLPP3, PNPLA3, PON1, PRF1, PSEN2, PTPN1, RXRA, SCARB1, SCD, SEC14L2, SERPINA12, SFTPB, SGMS1, SLC37A4, WNT4, WWOX
FXR/RXR Activation	12.50	ABCG8, AGT, AMBP, APOA1, APOE, APOF, APOH, C3, CYP8B1, FASN, FOXA2, GC, ITIH4, LCAT, NR0B2, PCYOX1, PON1, RXRA, SCARB1, SERPINF2, SLC27A5, VTN
Quantity of carbohydrate	11.83	ABCC4, AGPAT2, AMBP, APOA1, APOE, ATF6, C3, CFB, CIDEC, CPE, EGFR, ESR2, F2, FABP1, FFAR4, FOXA2, Foxp1, GCGR, GCK, GCKR, GDF15, GLS2, GNMT, Gulo, HPN, IKBKB, IL6ST, IRS2, LCAT, LEPR, LIFR, LRP1, LRP5, MRAP, NR0B2, NR1I3, PCK1, PEMT, PER2, PLIN2, PON1, PRF1, PSEN2, PTPN1, SCARB1, SCD, SFTPB, Sik1, SLC23A1, SLC37A4, SPHK2, STX1A, USF2, WWOX

Quantity of steroid	11.36	ABCC4, ABCG8, ADCY10, AGPAT2, AGT, APOA1, Apoc1, APOE, AQP12A/AQP12B, CRHR1, CYP17A1, CYP8B1, DHCR24, EGFR, ESR2, FABP1, FASN, FFAR4, GC, GCGR, GCK, GDF15, Gucy2g, Gulo, HP, HPN, IRS2, KL, LCAT, LDLRAP1, LEPR, LRP1, LRP5, LSR, mir-33, MPC1, MRAP, NR0B2, NR1I3, NSMF, PEMT, PLIN2, PON1, PSEN2, PTPN1, RXRA, SCARB1, SCD, SEC14L2, SERPINA12, SLC37A4, WNT4
LXR/RXR Activation	10.80	ABCG8, AGT, AMBP, APOA1, APOE, APOF, APOH, C3, FASN, GC, IL1RAP, ITIH4, LCAT, PCYOX1, PON1, RELA, RXRA, SCD, SERPINF2, VTN
Morphology of liver	5.02	ABCD3, AGPAT2, AGT, APOA1, APOE, BCR, C3, CYP2E1, EGFR, ESR2, FFAR4, GCGR, GNMT, IKBKB, IL6ST, LEPR, LRP1, MST1, MTF1, NR0B2, NR1I3, PCK1, PEMT, RELA, RXRA, SERPINA12, SLC37A4, TGFA, TMPRSS6, WWOX
Liver lesion	4.90	AGT, APOA1, APOE, BCL6, C3, CCN1, CDK1, CERS2, CIDEC, CYP2E1, EGFR, FASN, FGFR2, GADD45B, GNMT, HP, IKBKB, IL6ST, ITIH4, LRP1, Meg3, mir-26, mir-455, MST1, MTF1, NFKBIB, NINJ1, NR0B2, NR1I3, PEMT, PIM3, PNPLA3, PRF1, PTPN1, RELA, RXRA, SCD, SERPINC1, SLC26A1, SNAI1, TGFA
LPS/IL-1 Mediated Inhibition of RXR Function	4.84	ABCC4, ABCG8, ACSL5, ALAS1, ALDH3A2, ALDH7A1, ALDH8A1, APOE, Cyp2d26, CYP2E1, FABP1, IL1RAP, NR0B2, NR1I3, PAPSS2, RXRA, SCARB1, SLC27A5, SULT4A1
PXR/RXR Activation	4.56	ALAS1, ALDH3A2, NR0B2, NR1I3, PAPSS2, PRKAG2, RELA, RXRA, SCD
Histidine Degradation III	4.50	AMDHD1, FTCD, MTHFD1, UROC1
Acute Phase Response Signaling	4.20	AGT, AMBP, APOA1, APOH, C3, CFB, F2, HP, IKBKB, IL1RAP, IL6ST, ITIH4, NFKBIB, RELA, SERPINF2
Proliferation of liver cells	4.06	AGT, C3, CCN1, CERS2, EGFR, FGFR2, IKBKB, IL6ST, ITIH4, LEPR, MST1, NR1I3, PIM3, RELA, RXRA, TGFA, TOP1MT
Proliferation of hepatocytes	3.47	AGT, C3, CCN1, CERS2, EGFR, FGFR2, IKBKB, IL6ST, ITIH4, MST1, RELA, RXRA, TGFA, TOP1MT
Response of liver	3.42	APOA1, APOE, BCL6, C3, CCN1, CIDEC, GNMT, HP, IKBKB, IL6ST, NFKBIB, NINJ1, NR0B2, PRF1, RELA, RXRA, SCD, TGFA
Extrinsic Prothrombin Activation Pathway	3.16	F12, F2, F7, SERPINC1

Fatty Acid Biosynthesis Initiation II	3.15	FASN, OXSM
Iron homeostasis signaling pathway	3.09	ACO1, BMP1, BMP6, EGFR, EPAS1, GDF15, HP, LRP1, SLC11A2, TFRC, TMPRSS6
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.81	AGT, COL15A1, COL18A1, CYP2E1, ECE1, EGFR, FGFR2, IFNGR2, IL1RAP, LEPR, PROK1, RELA, TGFA
Morphology of hepatocytes	2.80	CYP2E1, EGFR, IKBKB, LRP1, MST1, PEMT, RELA, RXRA, TMPRSS6
Complement System	2.78	C3, C8A, C8B, CFB, MASP2
Coagulation System	2.66	F12, F2, F7, SERPINC1, SERPINF2
Hepatic Cholestasis	2.49	ABCG8, ADCY10, CYP8B1, GCGR, IKBKB, IL1RAP, NFKBIB, NR0B2, PRKAG2, PRKD3, RELA, RXRA
PPAR α /RXR α Activation	2.37	ADCY10, APOA1, FASN, GPD1, IKBKB, IL1RAP, ITGB5, NFKBIB, NR0B2, PRKAG2, RELA, RXRA
TR/RXR Activation	1.71	FASN, HP, NCOA4, PCK1, RXRA, SCARB1
Hepatic Fibrosis Signaling Pathway	1.62	AGT, CACNG3, COL18A1, IKBKB, IL1RAP, IRS2, ITGB5, LEPR, LRP1, LRP5, NFKBIB, PRKAG2, PRKD3, PROK1, RELA, SNAI1, TFRC, WNT4
<u>MOUSE LUNG EPITHELIAL</u>		
Biological Pathways and Functions	-log(p-value)	Molecules
vasculature development	48.31	Acvr1l, Adam15, Adamts6, Adamts9, Ahr, Aldh1a2, Aqp1, Arhgef15, Bcas3, Bmper, Bmpr2, Calcr1, Casp8, Cav1, Ccbe1, Cd34, Cdh13, Cdh2, Cdh5, Chd7, Cited2, Clic4, Col4a2, Col5a1, Ctnnb1, Cxcl12, Cxcr4, Cyp1b1, Cyr61, Dll4, Dlx3, E2f7, Efnb2, Egfl7, Egr3, Elk3, Emcn, Eng, Epas1, Esm1, Ets1, Fgfr2, Flt1, Flt4, Fmn13, Fn1, Foxc2, Foxf1, Foxo1, Fzd4, Fzd8, Gata4, Gata6, Gbx2, Gja4, Grem1, Has2, Hdac7, Hectd1, Heg1, Hhex, Hif1a, Immp2l, Itgb1, Itgb3, Jag1, Jun, Kdr, Lama1, Ltbp1, Luzp1, Mcam, Mef2c, Meis1, Mkl2, Mmp2, Myh10, Myocd, Nkx3-1, Nodal, Notch1, Nr2f2, Nr4a1, Nrcam, Nrp1, Nrp2, Ntrk2, Osr1, Pcsk5, Pdgfb, Pdgfra, Pecam1, Plcd3, Plg, Plpp3, Plxnd1, Pnpla6, Prcp, Prdm1, Prickle1, Prok2, Prox1, Prrx1, Pten, Ptk2, Ptprb, Qk, Ramp2, Rapgef1, Rapgef2, Rasip1, Reck, Rhoj, Robo4, Rora, Rxra, Slpr1, Sema3c, Sema5a, Slc4a7, Smad5, Smad6, Smad7, Smarca2, Sox18, Sox4, Syk, T, Tbx1, Tbx2, Tbx3, Tek, Tgfb2, Tgfbi, Tgfbr3, Thsd7a, Tmem100, Tmem204, Tnfaip2, Tspan12, Ubp1, Vav3, Vegfc, Wt1, Zfp361l, Zfp362, Zmiz1

cardiovascular system development	47.65	Abl2, Acvrl1, Adam15, Adamts6, Adamts9, Ahr, Aldh1a2, Aqp1, Arhgef15, Bcas3, Bmper, Bmpr2, Calcr1, Casp8, Cav1, Ccbe1, Cd34, Cdh13, Cdh2, Cdh5, Chd7, Cited2, Clic4, Col4a2, Col5a1, Ctnnb1, Cxcl12, Cxcr4, Cyp1b1, Cyr61, Dll4, Dlx3, E2f7, Efnb2, Egfl7, Egr3, Elk3, Emcn, Eng, Epas1, Esm1, Ets1, Fgfr2, Flt1, Flt4, Fmnl3, Fn1, Foxc2, Foxf1, Foxo1, Foxp1, Fzd4, Fzd8, Gata4, Gata6, Gbx2, Gja4, Grem1, Has2, Hdac7, Hectd1, Heg1, Hhex, Hif1a, Immp2l, Itgb1, Itgb3, Jag1, Jun, Kenj1, Kdr, Lama1, Ltbp1, Luzp1, Mcam, Mef2c, Meis1, Mkl2, Mmp2, Myh10, Myocd, Nkx3-1, Nodal, Notch1, Nr2f2, Nr4a1, Nrcam, Nrp1, Nrp2, Ntrk2, Osr1, Pcsk5, Pdgfb, Pdgfra, Pecam1, Plcd3, Plg, Plpp3, Plxnd1, Pnpla6, Prcp, Prdm1, Prickle1, Prok2, Prox1, Prrx1, Pten, Ptk2, Ptprb, Qk, Ramp2, Rapgef1, Rapgef2, Rasip1, Reck, Rhoj, Robo4, Rora, Rxra, S1pr1, Sema3c, Sema5a, Slc4a7, Smad5, Smad6, Smad7, Smarca2, Sox18, Sox4, Syk, T, Tbx1, Tbx2, Tbx3, Tek, Tgfb2, Tgfbi, Tgfbr3, Thsd7a, Tmem100, Tmem204, Tnfaip2, Tspan12, Ubp1, Vav3, Vegfc, Wt1, Zfp361l, Zfp362l, Zmiz1
blood vessel development	45.04	Acvrl1, Adam15, Adamts6, Adamts9, Ahr, Aldh1a2, Aqp1, Bcas3, Bmper, Bmpr2, Calcr1, Casp8, Cav1, Ccbe1, Cd34, Cdh13, Cdh2, Cdh5, Chd7, Cited2, Clic4, Col4a2, Col5a1, Ctnnb1, Cxcl12, Cxcr4, Cyp1b1, Cyr61, Dll4, Dlx3, E2f7, Efnb2, Egfl7, Egr3, Elk3, Emcn, Eng, Epas1, Esm1, Ets1, Fgfr2, Flt1, Flt4, Fmnl3, Fn1, Foxc2, Foxf1, Foxo1, Fzd4, Fzd8, Gata4, Gata6, Gbx2, Gja4, Grem1, Has2, Hdac7, Hectd1, Heg1, Hhex, Hif1a, Itgb1, Itgb3, Jag1, Jun, Kdr, Lama1, Ltbp1, Luzp1, Mcam, Mef2c, Meis1, Mkl2, Mmp2, Myh10, Myocd, Nkx3-1, Notch1, Nr2f2, Nr4a1, Nrcam, Nrp1, Nrp2, Ntrk2, Osr1, Pcsk5, Pdgfb, Pdgfra, Pecam1, Plcd3, Plg, Plpp3, Plxnd1, Pnpla6, Prcp, Prdm1, Prickle1, Prok2, Prox1, Prrx1, Pten, Ptk2, Ptprb, Qk, Ramp2, Rapgef1, Rapgef2, Rasip1, Reck, Robo4, Rora, Rxra, S1pr1, Sema3c, Sema5a, Smad5, Smad6, Smad7, Sox18, Sox4, Syk, T, Tbx1, Tbx2, Tbx3, Tek, Tgfb2, Tgfbi, Tgfbr3, Thsd7a, Tmem100, Tnfaip2, Tspan12, Ubp1, Vav3, Vegfc, Wt1, Zfp361l, Zfp362l, Zmiz1

angiogenesis	34.15	Acvrl1, Adam15, Ahr, Bcas3, Bmper, Calcr1, Casp8, Cav1, Ccbe1, Cd34, Cdh13, Clic4, Col4a2, Ctnnb1, Cxcl12, Cxcr4, Cyp1b1, Cyr61, Dll4, E2f7, Efnb2, Egfl7, Egr3, Elk3, Emcn, Eng, Epas1, Esm1, Ets1, Fgfr2, Flt1, Flt4, Fmn13, Fn1, Foxc2, Fzd8, Gbx2, Grem1, Hif1a, Itgb1, Itgb3, Jun, Kdr, Mcam, Meis1, Mmp2, Notch1, Nr4a1, Nrcam, Nrp1, Nrp2, Pdgfra, Pecam1, Plcd3, Plxnd1, Pnpla6, Prcp, Prok2, Pten, Ptk2, Ptpb, Ramp2, Rasip1, Robo4, Rora, Rxa, S1pr1, Sema5a, Smad5, Sox18, Syk, Tbx1, Tek, Tgfb1, Thsd7a, Tmem100, Tnfaip2, Tspan12, Ubp1, Vav3, Vegfc
Respiratory system development	14.13	ADGRF5, AFF4, AHR, AP3B1, ARRB1, BMP7, BMPR2, CAV1, CAV2, CCBE1, CHD7, DNAAF11, DPPA4, ECE1, ELK3, ELN, EPAS1, EPHA3, FAS, FAT4, Fendrr, FGF7, FGFR2, FLT4, FOXA2, FOXF1, Foxp1, GATA3, GIT1, HEG1, HTT, IGF1R, ISL1, ITGA3, ITGA6, KDR, LAMC1, LIPA, LRRK2, mir-126, mir-17, MMP2, NCOA2, NFIB, NKIRAS2, NKX2-6, NODAL, PCSK5, PECAM1, PLG, PTEN, Ptpd, RARB, RARG, RC3H1, RIPPLY3, ROCK2, SAV1, SERPINE1, SKI, SOX11, SPRY2, SPRY4, SYNE1, TBX1, TEK, TNS3, TSHZ3, WT1, ZNF521
Morphology of respiratory system	6.69	ADGRF5, AFF4, ARRB1, BMP7, BMPR2, CAV1, CAV2, CCBE1, DNAAF11, DPPA4, ELK3, ELN, EPAS1, EPHA3, FAS, FAT4, FGFR2, FLT4, FOXA2, Foxp1, GIT1, GPC6, HTT, IGF1R, ITGA3, ITGA6, LAMC1, LIPA, LRRK2, MMP2, NFIB, NKX2-6, Nrg1, PBX1, PECAM1, PLG, PTEN, Ptpd, RARB, RARG, RBL2, SERPINE1, SOX11, SOX7, SPRY2, SPRY4, SYNE1, TBX1, TSHZ1, TTLL1, ZNF521
Cell death of endothelial cells	6.29	ABL2, ACVRL1, ADAM15, BCL2L11, CASP8, CDH5, COL4A2, DUSP6, EPAS1, FAS, FLT1, FLT4, HGF, KDR, LIPA, MALAT1, MMP2, PECAM1, PLG, PPAR, PTEN, PTK2, SEMA3F, SEMA6A, SERPINE1, SOCS1
Remodeling of blood vessel	6.17	ACVRL1, AHR, BCL6B, BMPR2, CHD7, DLL4, EFN2, EPAS1, EXT1, JAG1, KCNK6, KL, MEF2C, SEMA3C, SERPINE1, TBX1, TEK, UBR4

Transcription of DNA	6.08	ABLIM1, ACVRL1, ADRB2, AEBP2, AFF3, AHR, ALX1, AP3B1, APBB2, ATOH1, BACH1, BACH2, BARX2, BCL6B, BMP7, BMPR2, CAV1, CAVIN2, CAVIN4, CBFA2T3, CHD7, COL4A2, CSRNPI, DACH1, DHX36, DLL4, DMRT1, E2F3, EBF1, EGR3, ELK3, EPAS1, ESRRG, ETS1, FGF7, FGFR2, FLI1, FOXA2, FOXF1, FOXK1, FOXL1, Foxp1, GATA2, GATA3, GMNN, HDAC7, HDAC9, HIVEP2, Hmga2, IGF1R, IL22, IRF1, IRF8, ISL1, JAG1, JAK2, JUN, KLF3, KLF4, LCOR, LPIN2, MAF, MAML3, MBD2, MDFIC, MECOM, MEF2A, MEF2C, Meis1, mir-467, MNT, MRTFB, NCOA2, NFATC2, NFIB, NFKB1, NODAL, Nrg1, PBX1, PCBP3, PPARD, PRDM1, PRMT6, PROX1, RALGAP1, RARB, RARG, RFX3, RIPPLY3, RUNX1, RYBP, SERTAD2, SETD3, SKI, SLC39A8, SMAD1, SMAD3, SOX11, SOX18, SOX7, TBX1, TBXT, TCF4, TLE4, TRAF7, TWIST2, WT1, WWOX, WWTR1, YES1, ZEB1, ZEB2, ZMIZ1, ZNF326, ZNF608, ZNF704
Expression of RNA	5.29	ABLIM1, ACVRL1, ADRB2, AEBP2, AFF3, AHR, ALX1, AMPH, Ank2, AP3B1, APBB2, ATF6B, ATOH1, ATP1B1, BACH1, BACH2, BARX2, BCL6B, BMP7, BMPR2, CAV1, CAVIN2, CAVIN4, CBFA2T3, CHD7, CLDN5, COL4A2, CSRNPI, DACH1, DHX36, DLL4, DMRT1, DNAJC1, DUSP4, E2F3, EBF1, EDN3, EGR3, ELK3, ELP1, EPAS1, ESRRG, ETS1, FGF7, FGFR2, FLI1, FOXA2, FOXF1, FOXK1, FOXL1, Foxp1, GATA2, GATA3, GMNN, HDAC7, HDAC9, HGF, HIP1, HIVEP2, Hmga2, HTT, IGF1R, IKBKB, IL22, IRF1, IRF8, ISL1, JAG1, JAK2, JUN, Kdm1b, KIT, KLF3, KLF4, LCOR, let-7, LPIN2, LRRK2, MAF, MAML3, MBD2, MDFIC, MECOM, MEF2A, MEF2C, Meis1, mir-146, mir-17, mir-181, mir-467, MNT, MRTFB, NCOA2, NFATC2, NFIB, NFKB1, NGF, NOCT, NODAL, NR2C2, Nrg1, NTRK2, PBX1, PCBP3, PMP22, POLR2C, PPARD, PPP1R15A, PRDM1, PRKAA2, PRMT6, PROX1, PTEN, QKI, RAC1, RALGAP1, RAMP2, RAP1A, RARB, RARG, RBL2, RC3H1, RELN, RFX3, RIPPLY3, ROCK2, RPS6KA2, RUNX1, RYBP, SERTAD2, SETD3, SKI, SLC39A8, SMAD1, SMAD3, SMAD6, SMARCA2, SOCS1, SOX11, SOX18, SOX7, TBX1, TBXT, TCF4, TLE4, TMEM135, TNC, TRAF7, TTC21B, TWIST2, VAV3, VIM, WT1, WWOX, WWTR1, YES1, ZEB1, ZEB2, ZMIZ1, ZNF326, ZNF608, ZNF704

Quantity of progenitor cells	5.28	AHR, ATP2B1, BCL2L11, BLK, CASP8, CAV2, CBFA2T3, CD247, CSK, DLL4, DUSP6, EBF1, EFNB2, EGR3, EPAS1, ETS1, FAS, FLI1, GATA2, HIVEP2, IGF1R, IL1R1, IRF1, IRF8, ITPR2, JAK2, KDR, KIT, KITLG, KL, KLF4, LAMC1, let-7, LTBR, Ly6a (includes others), MAF, MECOM, Meis1, mir-17, NFKB1, NR2C2, PBX1, PECAM1, PLCG1, PLXND1, PPARD, PRKCH, PRKDC, PTEN, RAC1, RARG, RBL2, RNASEL, RUNX1, SCARB1, SOCS1, TCF4, TCIM, THEMIS, TLE4, TMOD3, TNFRSF11B, VAV3, VCAM1, WIPF1, ZEB1
Angiogenesis of lesion	4.60	ACVRL1, CCN1, CDH5, DLL4, FGFR2, FLT1, FLT4, HGF, IKBKB, KDR, KITLG, LTBR, mir-17, mir-467, MMP2, PLG, S1PR1, SEMA6A, SERPINE1, TEK, VAV3, VEGFC, WT1
Quantity of airway epithelial cells	4.10	ABCG1, AP3B1, EPAS1, FGF7, LIPA, NDEL1, PTEN, RARB
Proliferation of vascular cells	4.07	ADAM15, BMPR2, CALCRL, CDH13, DLL4, E2F3, EFNB2, FLT1, HGF, IGF1R, IRF1, let-7, mir-126, PECAM1, PLG, PTEN, PTK2, RAC1, ROCK2, Rps4l, SEMA3F, SERPINE1, SMAD3, SOCS1, SPRY4
Morphology of germ layer	4.06	AFDN, BMPR2, DAD1, EXT2, FGF7, FOXA2, FOXF1, GMNN, HIRA, HTT, ISL1, LAMA1, MECOM, METAP2, PBX1, PLPP3, PTGIS, RAC1, SKI, SMAD1
Thrombin Signaling	3.14	ADCY1, ADCY9, ARHGEF15, ARHGEF3, F2RL2, F2RL3, GATA2, GATA3, GNA14, GNAT1, GNG7, IKBKB, ITPR2, MYL4, NFKB1, PLCD3, PLCG1, PRKCH, PTK2, RAC1, RAP1A, RHOJ, ROCK2
eNOS Signaling	2.84	ADCY1, ADCY9, AQP1, CASP8, CAV1, CCNA1, CHRNA10, FLT1, FLT4, HSPA4, ITPR2, KDR, PLCG1, PRKAA2, PRKAB1, PRKCH, SLC7A1, VEGFC
Pulmonary Healing Signaling Pathway	2.72	BLK, BMPR2, FGF7, FGFR2, JAG1, KDR, MMP2, NFKB1, PECAM1, PRKAA2, PRKAB1, PRKCH, RAC1, RAP1A, SMAD1, TCF4, TNFRSF11B, VEGFC, WNT2B, WWTR1, YES1
Apelin Endothelial Signaling Pathway	2.49	ADCY1, ADCY9, GNA14, GNAT1, GNG7, JUN, MEF2A, MEF2C, NFKB1, PRKAA2, PRKAB1, PRKCH, RAP1A, SMAD3, TEK, VCAM1
Cardiomyocyte Differentiation via BMP Receptors	2.46	BMP7, BMPR2, MEF2C, SMAD1, SMAD6
Pulmonary Fibrosis Idiopathic Signaling Pathway	2.15	AEBP2, BMPR2, CAV1, COL15A1, COL25A1, COL4A2, COL5A1, EFNB2, FGFR2, ITGA2, JAG1, JAK2, JUN, MMP2, NFKB1, PDGFD, PLG, PTEN,

		PTK2, RAP1A, ROCK2, RPS6KA2, SERPINE1, SMAD3, TCF4, VIM, WNT2B, WWTR1
Wound Healing Signaling Pathway	2.01	BMPR2, COL15A1, COL25A1, COL4A2, COL5A1, FGF7, FGFR2, IKBKB, IL1R1, ITGA3, ITGA6, JAK2, JUN, LAMA1, LAMC1, NFKB1, PDGFD, RAC1, RAP1A, TNFRSF11B, VEGFC, VIM
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	1.52	ELP1, IKBKB, IRF1, IRF8, JAK2, JUN, NFKB1, PLCG1, PON1, PPM1J, PPP2R2A, PRKCH, RAC1, RAP1A, RHOJ, TNFRSF11B
Opioid Signaling Pathway	1.50	ADCY1, ADCY9, ARRB1, BLK, CACNA1C, CACNA2D4, GNA14, GNAT1, GNG7, GRK5, ITPR2, KCNJ3, NFKB1, PRKCH, RAC1, RAP1A, RGS12, RGS3, RPS6KA2, SCN7A, TCF4, YES1
Sphingosine-1-phosphate Signaling	1.37	ADCY1, ADCY9, CASP7, CASP8, PDGFD, PLCD3, PLCG1, PTK2, RAC1, RHOJ, S1PR1
MOUSE IMMUNE		
Biological Pathways and Functions	-log(p-value)	Molecules
hematopoietic or lymphoid organ development	5.19	Carmil2, Cbfa2t3, Cd4, Cxcr5, Dll1, Gata2, Gfi1, H2-DMA, Mpl, Nkx2-3, Runx1, Runx3, Sema4a, Tgfb3, Tmem91
Abnormal morphology of lymphoid organ	4.96	GFI1, GIMAP1-GIMAP5, LAG3, LMNA, NKX2-3, NQO1, PIK3CD, RHOF, RUNX1, RUNX3, TBXA2R, TGFB3
immune system development	4.96	Bfsp2, Clic4, Dll1, Gja1, Hoxa5, Ripor2, Tjp2
Th2 Pathway	4.69	GFI1, HLA-DMB, IL12RB1, PIK3CD, RUNX3, TGFB3
Abnormal morphology of spleen	4.55	GIMAP1-GIMAP5, LAG3, LMNA, NKX2-3, NQO1, PIK3CD, RHOF, RUNX3, TBXA2R, TGFB3
hemopoiesis	4.41	Cbfa2t3, Cd4, Dll1, Gata2, Gfi1, H2-DMA, Mpl, Nkx2-3, Runx1, Runx3, Sema4a, Tgfb3, Tmem91
Th1 and Th2 Activation Pathway	4.11	GFI1, HLA-DMB, IL12RB1, PIK3CD, RUNX3, TGFB3
Quantity of lymphoid organ	3.42	GFI1, GIMAP1-GIMAP5, LAG3, NKX2-3, PIK3CD, RUNX1, RUNX3, TGFB3
Transcription of DNA	3.31	AFF3, AGAP2, ATF7IP, BHLHA15, CRY2, GATA2, GBX2, GFI1, HOXA5, NCOR2, NKX2-3, NR0B2, RUNX1, RUNX3, SGSM1, ZNF326
Differentiation of antigen presenting cells	3.07	GATA2, GFI1, NKX2-3, RUNX1, RUNX3, ZNF831
Quantity of leukocytes	3.00	GATA2, GFI1, GIMAP1-GIMAP5, IL12RB1, LAG3, NEDD4L, NKX2-3, NQO1, PIK3CD, PIM3,

		RHOF, RPS6KA4, RUNX1, RUNX3, SLC2A1, TBXA2R
IL-23 Signaling Pathway	2.91	IL12RB1, PIK3CD, RUNX1
Th1 Pathway	2.80	HLA-DMB, IL12RB1, PIK3CD, RUNX3
Cell death of hematopoietic progenitor cells	2.79	GATA2, GFI1, NQO1, PIK3CD, RUNX1, TBXA2R
Leukocyte Extravasation Signaling	2.77	ARHGAP9, CTNND1, CTTN, NCF4, PIK3CD
Quantity of myeloid cells	2.76	GATA2, GFI1, GIMAP1-GIMAP5, IL12RB1, NEDD4L, NQO1, PIK3CD, PIM3, RUNX1, RUNX3, TBXA2R
Leukotriene Biosynthesis	2.74	ALOX5AP, GGT1
Development of phagocytes	2.70	GATA2, GFI1, INSL3, RUNX1, RUNX3, ZNF831
Differentiation of conventional dendritic cells	2.69	GFI1, RUNX1, ZNF831
Eicosanoid Signaling	2.50	ALOX5AP, GGT1, TBXA2R
Quantity of B lymphocytes	2.48	GFI1, GIMAP1-GIMAP5, NKX2-3, NQO1, PIK3CD, PIM3, RHOF, SLC2A1
Apoptosis of T lymphocytes	2.42	GFI1, GIMAP1-GIMAP5, NQO1, PIK3CD, RUNX1, TBXA2R
T Cell Exhaustion Signaling Pathway	1.66	HLA-DMB, IL12RB1, LAG3, PIK3CD, TGFBR3
Senescence Pathway	1.39	CACNB2, PIK3CD, RPS6KA4, TGFBR3
GM-CSF Signaling	1.39	PIK3CD, RUNX1

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WHAT IS CLAIMED IS

1. A method of determining if a subject has suffered tissue damage from exposure to a toxic agent, the method comprising
 - (a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;
 - (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns;
 - (c) measuring the quantity of the cfDNA of the determined cellular origin, and
 - (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin;wherein an increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the subject has suffered or suffers tissue damage from the exposure.
2. A method of determining if a subject has suffered tissue damage from exposure to a toxic agent, the method comprising, at two or more time points,
 - (a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;
 - (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and
 - (c) measuring the quantity of the cfDNA of the determined cellular origin,wherein an increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the subject has suffered or suffers tissue damage from the exposure.
3. A method of treating a subject who has suffered tissue damage from exposure to a toxic agent, the method comprising administering a treatment for the tissue damage to the subject,wherein the subject is determined to have suffered from tissue damage by a method comprising:

(a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;

(b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns;

(c) measuring the quantity of the cfDNA of the determined cellular origin, and

(d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin;

wherein an increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the subject has suffered tissue damage.

4. A method of treating a subject who has suffered tissue damage from exposure to a toxic agent, the method comprising administering a treatment for the tissue damage to the subject,

wherein the subject is determined to have suffered from tissue damage by a method comprising, at two or more time points:

(a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;

(b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and

(c) measuring the quantity of the cfDNA of the determined cellular origin,

wherein an increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the subject has suffered tissue damage.

5. A method of treating tissue damage in a subject, the method comprising administering a treatment for the tissue damage to the subject and monitoring the tissue damage,

wherein the monitoring comprises:

(a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;

(b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns;

(c) measuring the quantity of the cfDNA of the determined cellular origin, and

(d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin;

wherein a decrease in the measured quantity of the cfDNA of the determined cellular origin as compared to the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is effective, and an increase or no change in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is not effective.

6. A method of treating tissue damage in a subject, the method comprising administering a treatment for the tissue damage to the subject and monitoring the tissue damage,

wherein the monitoring comprises, at two or more time points:

(a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;

(b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and

(c) measuring the quantity of the cfDNA of the determined cellular origin,

wherein a decrease in the measured quantity of the cfDNA of the determined cellular origin at later time point as compared to an earlier time point is indicative that the treatment is effective, and an increase or no change in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the treatment is not effective.

7. The method of claim 5 or 6, wherein the tissue damage is caused by exposure to a toxic agent.

8. The method of any one of claims 1-4 or 7, wherein the toxic agent comprises radiation.
9. The method of claim 8, wherein the radiation is for therapeutic purposes, accidental, or environmental.
10. The method of claim 8, wherein the radiation comprises a radioactive substance.
11. The method of claim 10, wherein the radioactive substance is ingested by the subject, inhaled by the subject, or absorbed through body surface contamination by the subject.
12. The method of any one of claims 1-4 or 7, wherein the toxic agent comprises a microorganism.
13. The method of claim 12, wherein the microorganism comprises a pathogen.
14. The method of claim 13, wherein the pathogen is selected from a bacterium and virus.
15. The method of any one of claims 1-4 or 7, wherein the toxic agent is from a synthetic chemical source or from a biological source.
16. The method of any one of claims 1-4 or 7, wherein the toxic agent comprises a pharmaceutical therapy.
17. The method of any one of claims 1-4 or 7, wherein the toxic agent comprises a chemical or biological or radioactive substance used as a weapon.
18. A method of treating a subject in need thereof, the method comprising administering a treatment to the subject and monitoring whether the treatment causes tissue damage in the subject,

wherein the monitoring comprises:

- (a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;
- (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns;
- (c) measuring the quantity of the cfDNA of the determined cellular origin, and
- (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin;

wherein an increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative that the treatment is causing tissue damage.

19. A method of treating a subject in need thereof, the method comprising administering a treatment to the subject and monitoring whether the treatment causes tissue damage in the subject,

wherein the monitoring comprises, at two or more time points:

- (a) sequencing cell-free DNA (cfDNA) in a biospecimen from the subject;
- (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and
- (c) measuring the quantity of the cfDNA of the determined cellular origin,

wherein an increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative that the treatment is causing tissue damage.

20. The method of any one of claims 1-19, further comprising adjusting the treatment administered to the subject when the treatment is indicated to be not effective or causing tissue damage.

21. The method of any one of claims 5-20, wherein the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a

population of individuals who were not exposed to the toxic agent, or who were not administered the treatment.

22. A method of treating a subject having a tumor, the method comprising

(A) monitoring a response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises:

- (i) determining whether there is an adverse reaction to the first treatment, comprising
 - (a) sequencing circulating tumor DNA (cfDNA) in a biospecimen from the subject;
 - (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns;
 - (c) measuring the quantity of the cfDNA of the determined cellular origin, and
 - (d) comparing the measured quantity of the cfDNA of the determined cellular origin with a normal quantity of cfDNA of the determined cellular origin, in which an increase in the measured quantity of the cfDNA of the determined cellular origin over the normal quantity of cfDNA of the determined cellular origin is indicative of an adverse reaction;
- (ii) determining whether there is a response to the first treatment, comprising:
 - (a) sequencing circulating tumor DNA (ctDNA) in a biospecimen from the subject,
 - (b) determining clonal heterogeneity of cells of the tumor by genotyping the ctDNA, in which the presence of more than one clone of the tumor cells or the presence of a tumor cell clone that has not been previously identified in the subject is indicative of an ineffective response to the first treatment; and

(B) either administering the same treatment as the first treatment when it is determined that there is no adverse reaction, that there is not an ineffective response, or a

combination thereof; or administering an adjusted treatment when it is determined that there is an adverse reaction, that there is an ineffective response, or a combination thereof.

23. The method of claim 22, wherein the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who do not have a tumor.

24. The method of claim 22, wherein the normal quantity of cfDNA comprises a quantity of cfDNA for the determined cellular origin that is generated in a population of individuals who did not receive the first treatment.

25. A method of treating a subject having a tumor, the method comprising
(A) monitoring a response to a first treatment, an adverse reaction to the first treatment, or a combination thereof, in which the monitoring comprises, at two or more time points,

- (i) determining whether there is an adverse reaction to the first treatment, comprising
 - (a) sequencing cell-free (cfDNA) in a biospecimen from the subject;
 - (b) determining cellular origin of the cfDNA by identifying the methylation patterns in one or more portions of the sequence of the cfDNA that contains methylation sites, wherein the cellular origin of the cfDNA is determined when the methylation pattern in the one or more portions is the same as a known cell-type specific methylation patterns; and
 - (c) measuring the quantity of the cfDNA of the determined cellular origin, wherein an increase in the measured quantity of the cfDNA of the determined cellular origin at a later time point as compared to an earlier time point is indicative of an adverse reaction; and
- (ii) determining whether there is a response to the first treatment, comprising
 - (a) sequencing circulating tumor (ctDNA) in a biospecimen from the subject,
 - (b) determining clonal heterogeneity of cells of the tumor by genotyping the ctDNA, wherein the presence of more than one clone of the tumor cells or the presence of a tumor cell clone in a subsequent time point that has not

been identified at a previous time point is indicative of an ineffective response to the first treatment; and

(B) either administering the same treatment as the first treatment when it is determined that there is no adverse reaction, that there is not an ineffective response, or a combination thereof; or administering an adjusted treatment when it is determined that there is an adverse reaction, that there is an ineffective response, or a combination thereof.

27. The method of any one of claims 1-26, wherein the biospecimen comprises a biological fluid.

28. The method of claim 27, wherein the biological fluid is selected from blood, serum, plasma, cerebrospinal fluid, saliva, urine, and sputum.

29. The method of claim 27, wherein the biological fluid comprises blood, serum, or plasma.

30. The method of any one of claims 1-29, wherein the methylation pattern comprises a segment of nucleotide sequence containing at least 3 CpG dinucleotides.

31. The method of any one of claims 1-30, wherein the known methylation patterns are set forth in Table 2.