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(54) **Title:** REAGENTS AND METHODS FOR USING HUMAN EMBRYONIC STEM CELLS TO EVALUATE TOXICITY OF PHARMACEUTICAL COMPOUNDS & OTHER CHEMICALS

(57) **Abstract:** The invention provides biomarker profiles of cellular metabolites and methods for screening chemical compounds including pharmaceutical agents, lead and candidate drug compounds and other chemicals using human embryonic stem cells (hESC) or lineage-specific cells produced therefrom. The inventive methods are useful for testing toxicity, particularly developmental toxicity and detecting teratogenic effects of such chemical compounds.

Reagents and Methods for Using Human Embryonic Stem Cells to Evaluate Toxicity of Pharmaceutical Compounds & Other Chemicals

This application claims the priority benefit of U.S. provisional patent applications, Serial Nos. 60/790,647, filed April 10, 2006, and 60/822,163, filed August 11, 2006, the entirety of which are incorporated by reference herein.

Background of the Invention

Field of the Invention

This invention provides methods for toxicological screening of pharmaceuticals and other chemical compounds. The invention specifically provides reagents that are human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, as well as methods for using these cells to detect developmental toxicity or teratogenic effects of pharmaceutical compounds and other chemicals. More particularly, the invention provides an *in vitro* means for analyzing toxicity of compounds predictive of their toxicity during human development. Candidate predictive biomarkers for toxic or teratogenic effects are also identified and provided herein.

Background of Invention

Birth defects are a major cause of infant morbidity in the United States, affecting 1 in every 33 infants born (Brent & Beckman, 1990, *Bull NY Acad Med* 66: 123-63; Rosano *et al.*, 2000, *J Epidemiology Community Health* 54:660-66), or approximately 125,000 newborns per year. It is understood that developmental toxicity can cause birth defects, and can generate embryonic lethality, intrauterine growth restriction (IUGR), dysmorphogenesis (such as skeletal malformations), and functional toxicity, which can lead to cognitive disorders such as autism. There is an increasing concern about the role that chemical exposure can play in the onset of these

disorders. Indeed, it is estimated that 5% to 10% of all birth defects are caused by *in utero* exposure to known teratogenic agents (Beckman & Brent, 1984, *Annu Rev Pharmacol* 24: 483-500).

Concern exists that chemical exposure may be playing a significant and preventable role in producing birth defects (Claudio *et al.*, 2001, *Environm Health Perspect* 109: A254-A261). This concern has been difficult to evaluate, however, since the art has lacked a robust and efficient model for testing developmental toxicity for the more than 80,000 chemicals in the market, plus the new 2,000 compounds introduced annually (General Accounting Office (GAO), 1994, *Toxic Substances Control Act: Preliminary Observations on Legislative Changes to Make TSCA More Effective,* Testimony, 07/13/94, GAO/T-RCED-94-263). Fewer than 5% of these compounds have been tested for reproductive outcomes and even fewer for developmental toxicity (Environmental Protective Agency (EPA), 1998, *Chemical Hazard Data Availability Study*, Office of Pollution Prevention and Toxins). Although some attempts have been made to use animal model systems to assess toxicity (Piersma, 2004, *Toxicology Letters* 149:147-53), inherent differences in the sensitivity of humans *in utero* have limited the predictive usefulness of such models. Development of a human-based cell model system would have an enormous impact in drug development and risk assessment of chemicals.

Toxicity, particularly developmental toxicity, is also a major obstacle in the progression of compounds through the drug development process. Currently, toxicity testing is conducted on animal models as a means to predict adverse effects of compound exposure, particularly on development and organogenesis in human embryos and fetuses. The most prevalent models that contribute to FDA approval of investigational new drugs are whole animal studies in rabbits and rats (Piersma, 2004,

Toxicology Letters 149:147-53). *In vivo* studies rely on administration of compounds to pregnant animals at different stages of pregnancy and embryonic/fetal development (first week of gestation, organogenesis stage and full gestation length). However, these *in vivo* animal models are limited by a lack of robustness between animal and human responses to chemical compounds during development. Species differences are often manifested in trends such as dose sensitivity and pharmacokinetic processing of compounds. At present, animal models are only 50% efficient in predicting human developmental response to compounds (Greaves *et al.*, 2004, *Nat Rev Drug Discov* 3:226-36). Thus, human-directed predictive *in vitro* models present an opportunity to reduce the costs of new drug development and enable safer drugs.

In vitro models have been employed in the drug industry for over 20 years (Huuskonen, 2005, *Toxicology & Applied Pharm* 207:S495-S500). Many of the current *in vitro* assays involve differentiation models using primary cell cultures or immortalized cells lines (Huuskonen, 2005, *Toxicology & Applied Pharm* 207:S495-S500). Unfortunately, these models differ significantly from their *in vivo* counterparts in their ability to accurately assess development toxicity. In particular, the ECVAM initiative (European Center for Validation of Alternative Methods) has used mouse embryonic stem cells as a screening system for predictive developmental toxicology. The embryonic stem cell test (EST) has shown very promising results, with a 78% statistically significant correlation to *in vivo* studies, and the test was able to differentiate strong teratogens from moderate/weak or non-embryotoxic compounds (Spielmann *et al.*, 1997, *In Vitro Toxicology* 10:119-27). This model is limited in part because toxicological endpoints are defined only for compounds that impair cardiac differentiation. This model also fails to account for interspecies developmental

differences between mice and humans, and so does not fully address the need in the art for human-specific model systems.

Thus there remains a need in this art for a human-specific *in vitro* method for reliably determining developmental toxicity in pharmaceutical agents and other chemical compounds. There also is a need in the art to better understand human development and its perturbation by toxins and other developmental disrupting agents, to assist clinical management of acquired congenital disorders and the many diseases that share these biochemical pathways, such as cancer.

The present invention provides for the assessment of a plurality of small molecules, preferably secreted or excreted by hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, and is determined and correlated with health and disease or insult state. Similar analyses have been applied to other biological systems in the art (Want *et al.*, 2005 *Chem Bio Chem* 6: 1941-51), providing biomarkers of disease or toxic responses that can be detected in biological fluids (Sabatine *et al.*, 2005 *Circulation* 112:3868-875).

Summary of the Invention

The present invention provides reagents and methods for *in vitro* screening of toxicity and teratogenicity of pharmaceutical and non-pharmaceutical chemicals using undifferentiated human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells. The invention provides human-specific *in vitro* methods for reliably determining toxicity, particularly developmental toxicity and teratogenicity, of pharmaceuticals and other chemical compounds using human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural

cells. As provided herein, hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, are useful for assessing toxic effects of chemical compounds, particularly said toxic and teratogenic effects on human development, thus overcoming the limitations associated with interspecies animal models. In particular, the invention demonstrates that metabolite profiles of hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells are altered in response to known disruptors of human development.

The invention shows that the hESC metabolome is a source of human biomarkers for disease and toxic response. In particular embodiments, exposure of hESC to valproate induced significant changes in different metabolic pathways, consistent with its known activity as a human teratogen. In other embodiments, hESC exposure to varying levels of ethanol induced significant alterations in metabolic pathways consistent with alcohol's known effects on fetal development.

In one aspect, the invention provides methods for using undifferentiated pluripotent human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, for *in vitro* evaluation. In the inventive methods, undifferentiated hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells are exposed to test compounds, preferably at concentrations reflective of *in vivo* levels or at levels found in maternal circulation. Further embodiments of this aspect of the invention provide for determination of the capacity of the test compound to induce differentiation of pluripotent hESC into particular cell types. In other embodiments, the inventive methods are provided using pluripotent, non-lineage restricted cells. The benefit of utilizing pluripotent stem cells is they permit analysis of global toxic

response(s) and are isolated from the physiological target of developmental toxicity, *i.e.* the human embryo. In addition, because these cells have not differentiated into a specific lineage, the potential for false negatives is reduced. In yet further embodiments are provided methods using hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, for assessing toxicity and particularly developmental toxicity and teratogenicity.

In another aspect the invention provides methods for identifying predictive biomarkers of toxic responses to chemical compounds, particularly pharmaceutical and non-pharmaceutical chemicals, and particularly to known teratogens. In embodiments of this aspect, a dynamic set representative of a plurality of cellular metabolites, preferably secreted or excreted by hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, is determined and correlated with health and disease or toxic insult state. Cellular metabolites according to this aspect of the invention generally range from about 10 to about 1500 Daltons, more particularly from about 100 to about 1000 Daltons, and include but are not limited to compounds such as sugars, organic acids, amino acids, fatty acids and signaling low-molecular weight compounds. Said biomarker profiles are diagnostic for toxicity of chemical compounds, particularly pharmaceutical and non-pharmaceutical chemicals, that participate in and reveal functional mechanisms of cellular response to pathological or toxic chemical insult, thus serving as biomarkers of disease or toxic response that can be detected in biological fluids. In particularly preferred embodiments of this aspect of the invention, these biomarkers are useful for identifying active (or activated) metabolic pathways following molecular changes predicted, *inter alia*, by other methods (such as transcriptomics and proteomics).

The invention thus also provides biomarker and pluralities of biomarkers, in some instances associated with metabolites from particular metabolic pathways, that are indicative of toxic or teratogenic insult. Said markers as provided by the invention are used to identify toxic and teratogenic insult, and in particular embodiments are used to characterize the amount or extent of said insult by being correlated with the amount or extent of the particular biomarker or plurality of biomarkers detected in cell culture media. In particular embodiments, said plurality of biomarkers provide a diagnostic pattern of toxic or teratogenic insult, more particularly identifying one or a multiplicity of specific metabolic pathways comprising metabolites detected after toxic or teratogenic insult.

The present invention is advantageous compared with *inter alia* the ECVAM mouse model because toxicity testing and biomarker identification are performed with human cells, specifically human embryonic stem cells (hESC). Human embryonic stem cells are able to recapitulate mammalian organogenesis *in vitro* (Reubinoff *et al.*, 2000, *Nature Biotechnology* 18:399-404; He *et al.*, 2003, *Circ Res* 93:32-9; Zeng *et al.*, 2004, *Stem Cells* 22:925-40; Lee *et al.*, 2000, *Mol Genet* Metab 86:257-68; Yan *et al.*, 2005, *Stem Cells* 22:781-90) because they are pluripotent and self-renewing cells. Thus, hESCs can reveal mechanisms of toxicity, particularly developmental toxicity, and identify developmental pathways that are particularly sensitive to chemicals during early human development. The "human for human" embryonic model provided by the inventive methods disclosed herein permits a better understanding of the pathways associated with developmental toxicity, as this is a system developed directly from the target organism, as well as being a more accurate and sensitive assay for toxic or teratogenic insult in human development.

The methods of the invention provide further advantages in identifying important biomarkers for toxicity and teratogenicity by functional screening of hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells. These biomarkers advantageously identify metabolic and cellular pathways and mechanisms of toxicity, particularly developmental toxicity. Importantly, these biomarkers may also assist in the evaluation of toxic effects of chemicals on the developing human embryo.

In yet another aspect of the invention, differentially-detected secreted or excreted cellular products identified by methods of the invention include those associated with neurodevelopmental disorders and alterations in associated metabolic pathways, and include but are not limited to kynurenine, glutamate, pyroglutamic acid, 8-methoxykynurenate, N'-formylkynurenine 5-hydroxytryptophan, N-acetyl-D-tryptophan and other metabolites in the tryptophan and glutamate metabolic pathways.

Functional toxicity in post-natal life can be predicted using hESC since differentiated cells with critical *in vivo* properties can be generated *in vitro*. hESCs can be used to produce lineage-specific cells, including lineage-specific stem cells, precursor cells and terminally-differentiated cells, providing therein enriched populations of cells typically present *in vivo* in mixtures of different cell types comprising tissues. The invention thus provides methods for using hESCs to produce said enriched and developmental stage-specific populations of cells for toxicity screening of chemical compounds, particularly drugs, drug lead compounds and candidate compounds in drug development, to identify human-specific toxicities of said chemical compounds. These aspects of the methods of the invention are advantageous over art-recognized *in vitro* and *in vivo* animal model systems.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

Brief Description of the Drawings

These and other objects and features of this invention will be better understood from the following detailed description taken in conjunction with the drawing wherein:

Figures 1A through 1C are profiles of secreted cellular metabolite biomarkers produced after contacting hESCs with 1mM valproate. These profiles were produced using liquid chromatography/electrospray ionization-time of flight (TOF) mass spectrometry (LC/ESI-TOF-MS) after treating the cells with valproate for 24 hours (Figure 1A), four days (Figure 1B) and eight days (Figure 1C). Secreted small molecules from treated (blue) and untreated (red) human embryonic stem cells were measured.

Figures 2A through 2D are profiles of secreted/excreted cellular metabolite biomarkers produced after contacting hESCs with 1mM valproate. These profiles were produced using liquid chromatography/electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS) after treating cells with valproate for 24 hours (Figure 2A), four days (Figure 2B), eight days (Figure 2C), and comparative metabolic profiling of hES cells (blue) and conditioned media (yellow) (Figure 2D).

Figures 3A through 3D are photomicrographs of cellular morphology showing the pluripotent embryonic stem cells following extended culture. The marker Oct-4 was retained in a similar manner as untreated controls (Figure 3A=5 days valproate, Figure 3B=5 days control, Figure 3C-8 days valproate, Figure 3D-8 days control).

Figure 4 shows the results of comparative mass spectrometry in the presence of chemical standards confirming the chemical identity of folic acid (exact mass 441.14), pyroglutamic acid (exact mass 129.04), glutamate (exact neutral mass 147.05) and kynurenine (exact mass 208.08).

Figure 5 represents the kynurenine metabolism pathway of tryptophan in humans (Wang *et al.*, 2006, *J Biol Chem* 281: 22021-22028, published electronically on June 5, 2006).

Figure 6 illustrates a hierarchical clustering of fold-change differences from 22,573 unique masses and is representative of multiple independent experiments in which hESCs and neural precursors produced from hESCs were treated with 1mM valproate. Non-embryonic cells (human fibroblasts) were used as controls (data not shown). Positive fold changes are red, negative fold changes are green, and missing data is grey.

Figure 7 shows the relative expression of enzymes in the kynurenine and serotonin synthesis pathways in hES cells. INDO, indoleamine 2,3 dioxygenase, TDO or TDO2, tryptophan 2,3-dioxygenase. (TDO2 was upregulated in valproate-treated hES cells in comparison to controls.) AFMID, arylformamidase, TPH1, tryptophan hydroxylase, AADAT, aminoadipate aminotransferase, KYNU, kynunreninase, GAPDH, glyceraldehyde 3-phosphate dehydrogenase, housekeeping control gene. KMO, kynurenine 3-monooxygenase, was not expressed in valproate-treated cells or controls.

Detailed Description of Preferred Embodiments

The invention provides reagents, including human embryonic stem cells (hESC) or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells produced therefrom, for assessing developmental

toxicity using the human embryonic stem cell metabolome. Human embryonic stem cells are pluripotent, self-renewing cells isolated directly from preimplantation human embryos that recapitulate organogenesis *in vitro*. Lineage-specific precursor cells are derived from hES cells and have entered a specific cellular lineage, but yet remain multipotent with regard to cell type within that specific lineage. For example, neural precursors have committed to neural differentiation but yet remain unrestricted as to its neural cell type. Also within the scope of the inventive methods are terminally-differentiated cell types, such as neurons. Biochemical pathways of human development and disease are active in hESCs and or hESC-derived lineage-specific cells, because they recapitulate differentiation into functional somatic cells. Disruption of these pathways during development contributes to disorders such as neural tube defects (NTDs) and cognitive impairment. Environmental agents, namely chemicals or drugs, participate in the ontogenesis of certain acquired congenital disorders. The question of which pathways during early human development are particularly susceptible to the effects of the environment remains unsolved.

The metabolome, defined as the total dynamic set of cellular metabolites present in cells, is a product of health or disease/insult states. Metabolomics is particularly sensitive to environmental effects in comparison to other "omic" areas of study, such as genomics and proteomics. Cellular metabolites include but are not limited to sugars, organic acids, amino acids and fatty acids, particularly those species secreted or excreted from cells, that participate in functional mechanisms of cellular response to pathological or chemical insult. These cellular metabolites serve as biomarkers of disease or toxic response and can be detected in biological fluids (Soga *et al.*, 2006, *J Biol Chem* 281:16768-78; Zhao *et al.*, 2006, *Birth Defects Res A Clin Mol Teratol* 76:230-6), including hESC culture media. Importantly, metabolomic

profiling may confirm functional changes that are often predicted by transcriptomics and proteomics.

However, because it was known that hESCs are highly sensitive to the culture microenvironment (Levenstein *et al.*, 2005, *Stem Cells* 24: 568-574; Li *et al.*, 2005, *Biotechnol Bioeng* 91:688-698.), their application as a source of predictive biomarkers in response to chemical compounds, including toxins, teratogens and particularly pharmaceutical agents, drug lead compounds and candidate compounds in drug development, and their usefulness in establishing *in vitro* models of disease and development was uncertain, *inter alia* because those of skill in the art could anticipate that exposure to an exogenous chemicals could be highly detrimental to survival of hES cells and preclude obtaining useful information from them. This concern has turned out not to be justified.

As used herein, the term "human embryonic stem cells (hESCs)" is intended to include undifferentiated stem cells originally derived from the inner cell mass of developing blastocysts, and specifically pluripotent, undifferentiated human stem cells and partially-differentiated cell types thereof (*e.g.*, downstream progenitors of differentiating hESC). As provided herein, *in vitro* cultures of hESC are pluripotent and not immortalized, and can be induced to produce lineage-specific cells and differentiated cell types using methods well-established in the art. In preferred embodiments, hESCs useful in the practice of the methods of this invention are derived from preimplantation blastocysts as described by Thomson *et al.*, in co-owned U.S. Patent No. 6,200,806. Multiple hESC cell lines are currently available in US and UK stem cell banks.

The terms "stem cell progenitor," "lineage-specific cell," "hESC derived cell" and "differentiated cell" as used herein are intended to encompass lineage-specific

cells that are differentiated from hES cells such that the cells have committed to a specific lineage of diminished potency. In some embodiments, these lineage-specific precursor cells remain undifferentiated with regard to final cell type. For example, neuronal stem cells are derived from hESCs and have differentiated enough to commit to neuronal lineage. However, the neuronal precursor retains 'stemness' in that it retains the potential to develop into any type of neuronal cell. Additional cell types include terminally-differentiated cells derived from hESCs or lineage-specific precursor cells, for example neural cells.

The term "cellular metabolite" as used herein refers to any small molecule secreted and/or excreted by a hESC or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, produced therefrom. In preferred embodiments, cellular metabolites include but are not limited to sugars, organic acids, amino acids, fatty acids, hormones, vitamins, oligopeptides (less than about 100 amino acids in length), as well as ionic fragments thereof. Cells may also be lysed in order to measure cellular products present within the cell. In particular, said cellular metabolites are from about 10 to about 3600 Daltons in molecular weight, more particularly about 10 to about 1500 Daltons, and yet more particularly from about 100 to about 1000 Daltons.

hESCs are cultured according to the methods of the invention using standard methods of cell culture well-known in the art, including, for example those methods disclosed in Ludwig *et al.* (2006, :Feeder-independent culture of human embryonic stem cells,: *Nat Methods* 3: 637-46.). In preferred embodiments, hESCs are cultured in the absence of a feeder cell layer during the practice of the inventive methods; however, hESCs may be cultured on feeder cell layer prior to the practice of the methods of this invention.

The term "administering" as used herein refers to contacting *in vitro* cultures of hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells produced therefrom with a toxic, teratogenic, or test chemical compound. In a preferred embodiment the dosage of the compound is administered in an amount equivalent to levels achieved or achievable *in vivo*, *for example*, in maternal circulation.

The phrases "identifying cellular metabolites that are differentially produced" or "detecting alterations in the cells or alternations in cell activity" as used herein include but are not limited to comparisons of treated hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, to untreated (control) cells (*i.e.*, cells cultured in the presence (treated) or absence (untreated) of a toxic, teratogenic, or test chemical compound). Detection or measurement of variations in cellular metabolites, excreted or secreted therefrom, between treated and untreated cells is included in this definition. In a preferred embodiment, alterations in cells or cell activity are measured by determining a profile of changes in cellular metabolites having a molecular weight of less than 3000 Daltons, more particularly between 10 and 1500 Daltons, and even more particularly between 100 and 1000 Daltons, in a treated versus untreated cell as illustrated in Figures 1A through 1C.

The term "correlating" as used herein refers to the positive correlation or matching of alterations in cellular metabolites including but not limited to sugars, organic acids, amino acids, fatty acids, and low molecular weight compounds excreted or secreted from hES cells or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, to an *in vivo* toxic response. The screened cellular metabolites can be involved in a wide range of biochemical

pathways in the cells and related to a variety of biological activities including, but not limited to inflammation, anti-inflammatory response, vasodilation, neuroprotection, oxidative stress, antioxidant activity, DNA replication and cell cycle control, methylation, and biosynthesis of, *inter alia*, nucleotides, carbohydrates, amino acids and lipids, among others. Alterations in specific subsets of cellular metabolites can correspond to a particular metabolic or developmental pathway and thus reveal effects of a test compound on *in vivo* development.

The term "physical separation method" as used herein refers to any method known to those with skill in the art sufficient to produce a profile of changes and differences in small molecules produced in hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, contacted with a toxic, teratogenic or test chemical compound according to the methods of this invention. In a preferred embodiment, physical separation methods permit detection of cellular metabolites including but not limited to sugars, organic acids, amino acids, fatty acids, hormones, vitamins, and oligopeptides, as well as ionic fragments thereof and low molecular weight compounds (preferably with a molecular weight less than 3000 Daltons, more particularly between 10 and 1500 Daltons, and even more particularly between 100 and 1000 Daltons). In particular embodiments, this analysis is performed by liquid chromatography/ electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS), however it will be understood that cellular metabolites as set forth herein can be detected using alternative spectrometry methods or other methods known in the art for analyzing these types of cellular compounds in this size range.

Data for statistical analysis were extracted from chromatograms (spectra of mass signals) using the Agilent Mass Hunter software (Product No. G3297AA,

Agilent Technologies, Inc., Santa Clara, CA); it will be understood that alternative statistical analysis methods can be used. Masses were binned together if they were within 10 ppm and eluted within a 2 minutes retention time window. A binned mass was considered to be the same molecule across different LC/ESI-TOF-MS analyses (referred to herein as an "exact mass," which will be understood to be ±10ppm). Binning of the data is required for statistical analysis and comparison of masses across the entire experiment. If multiple peaks with the same mass at the same retention time within a single sample were detected by Mass Hunter, they were averaged to assist data analysis. Masses lacking a natural isotopic distribution or with a signal-to-noise ratio of less than 3 were removed from the data prior to analysis. One of skill in the art will appreciate that the results from this assay provide relative values that are assessed according to annotated values within 10ppm to provide an identity for the molecular weight detected. Thus, a mass shift within 10 ppm is considered consistent with determining the identity of a specific cellular metabolite annotated known in the art due to differences in ionization source and instrumentation, *e.g.* between different experiments or using different instruments.

As used herein, a mass was considered to be the same across LC/ESI-TOF-MS runs using a simple algorithm that first sorts the data by mass and retention time. After sorting, a compound was considered unique if it had a retention time difference of less than or equal to three minutes and a mass difference less than or equal the weighted formula (0.000011 x mass). If a series of measurements fit this definition it was considered to be from the same compound. If either the mass or the retention time varied by more than the limits listed above it was considered to be a different compound and given a new unique designation.

Significance tests were determined by performing ANOVAs on the log base 2 transformed abundance values of unique compounds present in treated and untreated

media at each time point. A randomized complete block design was used with the ANOVA model including the effects of treatment, experiments, and a residual term, with the following formula: $Log_2(abundance_{ib}) = treatment_i + experiment_b + error_{ib}$.

Missing data were omitted from the test changing the degrees of freedom rather than assuming the missing data were absent. This assumption was made because the extensive filtering performed by the Mass Hunter software may miss or filter certain peaks because they are below a certain abundance threshold and not zero. The ANOVA F-test was considered significant if its *p*-value was less than 0.05. Fold changes were calculated using the least squared means for a given time and treatment.

The term "biomarker" as used herein refers to cellular metabolites that exhibit significant alterations between treated and untreated controls. In preferred embodiments, biomarkers are identified as set forth above, by methods including LC/ESI-TOF-MS. Metabolomic biomarkers are identified by their unique molecular mass and consistency with which the marker is detected in response to a particular toxic, teratogenic or test chemical compound; thus the actual identity of the underlying compound that corresponds to the biomarker is not required for the practice of this invention. Alternatively, certain biomarkers can be identified by, *for example*, gene expression analysis, including real-time PCR, RT-PCR, Northern analysis, and *in situ* hybridization, but these will not generally fall within the definition of the term "cellular metabolites" as set forth herein.

The basal metabolome of undifferentiated hESCs served as a collection of biochemical signatures of functional pathways that are relevant for stemness and self-renewal. Metabolite profiling was conducted on excreted or secreted cellular metabolites as opposed to intracellular compounds. Ultimately, biomarkers discovered *in vitro* are expected to be useful for analyzing *in vivo* biofluids such as

serum, amniotic fluid and urine, complex mixtures of extracellular biomolecules. This is advantageous over invasive procedures such as tissue biopsies because small molecules in biofluids can be detected non-invasively (in contrast to intracellular compounds). In addition, processing cellular supernatant for mass spectrometry is more robust and less laborious than cellular extracts. However, cellular extracts (from, for example, lysed cells) can be utilized in the methods of the invention.

The term "biomarker profile" as used herein refers to a plurality of biomarkers identified by the inventive methods. Biomarker profiles according to the invention can provide a molecular "fingerprint of the toxic and teratogenic effects of a test compound and convey what cellular metabolites, specifically excreted and secreted cellular metabolites, were significantly altered following test compound administration to hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells,. In these embodiments, each of the plurality of biomarkers is characterized and identified by its unique molecular mass and consistency with which the biomarker is detected in response to a particular toxic, teratogenic or test chemical compound; thus the actual identity of the underlying compound that corresponds to the biomarker is not required for the practice of this invention.

The term "biomarker portfolio" as used herein refers to a collection of individual biomarker profiles. The biomarker portfolios may be used as references to compare biomarker profiles from novel or unknown compounds. Biomarker portfolios can be used for identifying common pathways, particularly metabolic or developmental pathways, of toxic or teratogenic response.

These results set forth herein demonstrated that human embryonic stem cell metabolomics, and metabolomics from hESC-derived lineage-specific cells, such as

neural stem cells, neural precursor cells and neural cells, can be used in biomarker discovery and pathway identification. Metabolomics detected small molecules secreted by hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, produced therefrom and the identified biomarkers can be used for at least two purposes: first, to determine specific metabolic or developmental pathways that respond to or are affected by toxin or teratogen exposure, particularly said pathways utilized or affected during early development that are sensitive to toxic, teratogenic or test chemical compounds that are developmental disruptors and participate in the ontogenesis of birth defects; and second, to provide cellular metabolites that can be measured in biofluids to assist management and diagnosis of toxic exposure, birth defects or other disease.

A biomarker portfolio from hESCs or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, produced therefrom can also serve as a high throughput screening tool in preclinical phases of drug discovery. In addition, this approach can be used to detect detrimental effects of environmental (heavy metals, industrial waste products) and nutritional chemicals (such as alcohol) on human development. Ultimately, the methods of this invention utilizing the hESC metabolome or the metabolome of or hESC-derived lineage-specific cells, such as neural stem cells, neural precursor cells and neural cells, can assist pharmaceutical, biotechnology and environmental agencies on decision-making towards development of compounds and critical doses for human exposure. The integration of chemical biology to embryonic stem cell technology also offers unique opportunities to strengthen understanding of human development and disease. Metabolomics of cells differentiated from hESC should serve similar roles and be useful for elucidating mechanisms of toxicity and disease with greater sensitivity for

particular cell or tissue types, and in a human-specific manner. For example, key metabolic pathways, including as set forth herein folate, glutamate and tryptophan synthesis and degradation, may be differentially disrupted in earlier *versus* later stages of human development. In addition, metabolite profiles of neural precursor cells or neuronal cell populations can reveal biomarkers of neurodevelopmental disorders in target cell types. The association of metabolomics to stem cell biology can inform the mechanisms of action of folic acid and neural tube defects in the early human embryo.

Biomarker portfolios produced using the hESC-dependent and hESC-derived lineage-specific cell-dependent methods of this invention can also be used in high throughput screening methods for preclinical assessment of drug candidates and lead compounds in drug discovery. This aspect of the inventive methods produces minimal impact on industry resources in comparison to current developmental toxicology models, since implementation of this technology does not require experimental animals. The resulting positive impact on productivity enables research teams in the pharmaceutical industry to select and advance compounds into exploratory development with greater confidence and decreased risk of encountering adverse developmental effects.

The term "developmental pathway" as used herein refers to developmental or metabolic pathways in embryonic and fetal development.

"Supernatant" as used herein may include but is not limited to extracellular media, co-cultured media, cells, or a solution of fractionated or lysed cells.

Cellular metabolite profiles obtained from analysis of toxins, teratogens, alcohol, and test chemical compounds can be used to compose a library of biomarker portfolios. These portfolios can then be used as a reference for toxicological analysis of unknown chemical compounds. A similar strategy has been validated as a means

to determine cellular changes that arise in response to chemicals in non-hESC systems (Daston & Nacliff, 2005, *Reprod Toxicology* 19:381-94; Fella *et al.*, 2005, *Proteomics* 5:1914-21). Metabolic profiles of novel compounds can be compared to known biomarker portfolios to identify common mechanisms of toxic response. This approach can reveal functional markers of toxic response, which serve as screening molecules that are shared at least in part as a consequence of exposure to various different toxic and teratogenic compounds. Such hESC-derived small molecules can be used as measurable mediators of toxic response that refine or replace costly and complex screening systems (such as *in vivo* animal models) and have the additional advantage of being specific for human cells and human metabolic and developmental pathways.

Examples

The Examples which follow are illustrative of specific embodiments of the invention, and various uses thereof. They are set forth for explanatory purposes only, and are not to be taken as limiting the invention.

Example 1

Developmental Toxicology Screening

To demonstrate the efficacy of hESCs as a model system for developmental toxicity testing, hESCs were treated with a known teratogen, valproate (VPA). Valproate is a common mood stabilizer and anti-convulsant drug with clinical indications in epilepsy and bipolar disorder (Williams *et al.*, 2001, *Dev Med Child Neuro* 43:202-6) that has been associated with developmental abnormalities (Meador *et al.*, 2006, *Neurology* 67: 407-412). The mechanism by which valproate produces developmental defects, however, is not fully understood, despite the increased

susceptibility of the nervous system (Bjerkedal *et al.*, 1982, *Lance* 2:109: Wyszynski *et al.*, 2005, *Neurology* 64:961-5; Rasalam *et al.*, 2005, *Dev Med Child Neuro* 47:551-555). Exposure to valproate results in a pronounced increase in spina bifida and neural tube defects (NTDs; Bjerkedal *et al.*, 1982, *Lancet* 2:109) at ten-to-twenty times that of the general population, as well as cognitive disorders such as autism (Adab *et al.*, 2004, *J Neurol Neurosurg Psychiatry* 75:1575-83). However, since VPA is an anti-convulsant drug with clinical indications in epilepsy and bipolar disorder (Williams *et al.*, 2001, *Dev Med Child Neurol* 43:202-06), treatment generally must be sustained throughout pregnancy.

Folic acid supplementation prior to pregnancy reduces the incidence of spina bifida by 70% (Shaw *et al.*, 1995, *Epidemiology* 6:219-226) although its precise mechanism of action is unknown. In addition, homocysteine and glutathione have also been implicated in NTDs (Zhao *et al.*, 2006, *Birth Defects Res A Clin Mol Teratol* 76:230-6). Thus, metabolite profiles of folate and related pathways were candidates for changes in response to valproate. In the results set forth herein, folic acid was significantly increased (by 16%) in the extracellular media of hES cells treated with valproate ($p = 0.022$ at eight days, Table 3 and Figure 4) but not its derivative dihydrofolate. Since mammalian cells do not synthesize folic acid, valproate may act by interfering with cellular uptake of folic acid.

Exposure of hESCs was performed as follows. H1 hESC (passage 41) were cultured on Matrigel (BD Scientific, San Jose, CA) in the absence of a feeder layer. hESCs were maintained in conditioned medium (CM) collected from mouse embryonic fibroblasts (MEFs) (80% DMEM/F12, Invitrogen, Carlsbad, CA) and 20% KNOCKOUT serum replacement (Invitrogen) supplemented with 1 mM L-glutamine (Invitrogen), 1% MEM non-essential amino acids (Invitrogen), and 0.1 mM 2-

mercaptoethanol (Sigma, Chemical Co., St. Louis, MO). Prior to feeding hESCs, the culture medium was supplemented with 4 ng/mL human recombinant basic fibroblast growth factor (Invitrogen). hESCs were passaged when the wells were ~80% confluent. To passage, hESCs were incubated in a 1 mg/mL dispase (Invitrogen)/DMEM/F12 solution for 7-10 minutes at 37°C. After this treatment hESCs were washed and seeded on fresh Matrigel coated plates. In parallel studies disclosed herein, H1 and H9 cells were cultured in defined medium known as TeSR (Ludwig *et al.*, 2006, *Id.*).

H1 and H9 (equivalent to NIH code WA01/WA09) hESC were treated with valproate (VPA) (22μM and 1mM) (Sigma # P4543) according to the procedure outlined below; each experiment involved three separate VPA treatments, and each treatment group had a parallel control group with a total of six 6-well culture dishes (Nunc, Naperville, IL) per experiment (two 6-well culture dishes per treatment). Treatment 1 (labeled 24H) exposed hESC cells to 1mM VPA (Sigma) for 24 hours followed by collection of supernatant and cell pellets. In a second treatment group (labeled 4D), hESC cells were exposed to 22μM or 1mM VPA for 4 days and harvested on day 4. In a third treatment group (labeled extended culture, EC), hESC cells received 22μM or 1mM VPA for 4 days followed by culture in standard hESC media for an additional four days. For this group, cells and supernatant were harvested on day eight.

To assess the effects of teratogenic VPA treatment on hESCs, the treated cells were analyzed as set forth below to determine changes in a total dynamic set of small molecules present in cells according to health and disease or insult states. Small molecules including but not limited to sugars, organic acids, amino acids, fatty acids, hormones, vitamins, oligopeptides (less than about 100 amino acids in length), as well

as ionic fragments thereof and signaling low molecular weight compounds were known to participate in and reveal functional mechanisms of cellular response to pathological or chemical insult. These analyses were also used to identify active pathways following molecular changes predicted by other analyses including *for example* transcriptomics and proteomics.

Supernatant from VPA-treated and control hESCs were subjected to liquid chromatography and electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS) to assess changes and differences in small molecules (as defined herein) produced by the cells in the presence and absence of VPA treatment. Supernatant was collected from control and treated plates of hESCs at 24H, 4D, and 8D, and CM was collected as a "no treatment" control. The supernatant and media were stored at -80°C until preparation for mass spectrometry analysis. For analysis, samples were prepared in a 20% Acetonitrile (Fisher Scientific Co., Pittsburgh, PA) solution (comprising 500μL of supernatant, 400μL acetonitrile and 1.1 mL distilled water) and centrifuged through a Millipore 3kDa Centricon column (Millipore, Billerica, MA) for 3 hours at 4575 x *g* to remove proteins. The flow-through was retained for analysis, as it contains small molecules free of high molecular weight compounds such as proteins. In each analysis, three replicates for each sample were injected into a 2.1 x 200mm C18 column using a 90 minute gradient from 5% Acetonitrile, 95% Water, 0.1% Formic Acid to 100% Acetonitrile, 0.1% Formic Acid at a flow rate of 40μL/min. ESI-TOF-MS (TOF) was performed on the flow-through using an Agilent ESI-TOF mass spectrometer. Data was collected from 100-3600 m/z, and particularly in the 0-1500 m/z range. The raw data was analyzed to identify the separated small molecules using a computer compilation and analysis program (Mass Hunter) provided by the manufacturer and according to manufacturer's

instructions (Agilent; statistical analyses were performed as described above in the Detailed Description and Preferred Embodiments. This analysis generated lists of retention time/accurate mass pair feature. Another program (Mass Profiler, Agilent) was used to compare multiple run sets to find ion intensity changes of features that changed between the sample conditions. Significance tests were determined by performing ANOVAs on the log base 2 transformed abundance values of unique compounds present in treated and untreated media at each time point.

The plurality of small molecules identified using these methods were then compared with exact mass and retention time from ESI-TOF-MS using public databases (for example, at http://metlin.scripps.edu.,www.nist.gov/srd/chemistry.htm; http://www.metabolomics.ca/). Mass spectrometry analysis also included predicted chemical structures of small molecules based upon exact mass, although currently-available public databases do not in every instance include matching small molecules due to database limitations. In addition, more comprehensive private databases are available for comparative analysis, such as the NIST/EPA/NIH Mass Spectral Library: 05. NIST ASCII Version.

The results of these analyses are shown in Figures 1A through 1C and Figure 2A through 2C. In Figures 1A through 1C each feature on the plot corresponds to a small molecule with specific exact mass and retention time. The plots summarize significant differences found between treated (blue) and untreated (red) groups at different time points. As shown in the Figure, at 24 hours (24H) there was consistent down-regulation of the secreted biomolecules in treated (blue) cells in comparison to untreated (red) controls. At four days (4D) and eight days (EC), treated (blue) cells secreted a higher number of small molecules in comparison to untreated cells (red); said small molecules were thus considered as candidate biomarkers. In particular,

metabolites from the folate pathway, including tetrahydrofolate (exact mass 444) and dihydrofolate (exact mass 441) were detected. These findings were considered significant, since they show for the first time that hESCs contacted with a known teratogen (VPA) that causes a birth defect (spina bifida) respond by up-regulating a metabolic pathway that produces a compound (folate) known to ameliorate the effects of the teratogen when administered to a woman bearing a developing embryo or fetus.

Further, the results shown in Figures 1A through 1C revealed approximately 40 small molecules that were absent in treated groups, suggesting that multiple cellular pathways were "silenced" in response to VPA at 24 hours in comparison to untreated controls. At four and eight days after treatment, however, multiple candidate biomarkers were upregulated in treated versus untreated human embryonic stem cells; these results are shown in Table 1. Candidate biomarkers were identified as small molecules showing a change in treated versus untreated cells measured to be at least a two-fold difference. In many instances, these small molecules are absent or detected at very low concentrations in untreated human embryonic stem cells.

These studies demonstrated that the claimed methods for assessing developmental toxicity and the identification of biomarkers using hESCs provided robust information on changes in small molecule content of cells in response to being contacted with a known teratogen, VPA. The results concerning a compound (VPA) that is involved in the etiology of spina bifida and neural tube defects (NTDs)(Bjerkedal *et al.*, 1982, *Lancet* 2:109) when exposed to a developing human conceptus are particularly striking. The results shown here indicated a marked increase (2 to 8 fold) in key metabolites of the folate pathway (dihydrofolic acid, tetrahydrofolic acid, *S*-adenosylmethionine) following treatment with VPA (in comparison to untreated cells). These methods were reproducible, having been

repeated with consistent results obtained in three independent studies using hESCs and on non-embryonic cells (human fibroblasts) as controls (data not shown), and suggested a heretofore unknown adaptive response of the fetus to the chemical/environmental insult and identified sensitive markers for said insult(s).

The mechanism for VPA developmental defects, however, is not fully understood despite the fact that the nervous system is particularly sensitive to its effects (Bjerkedal *et al.*, 1982, *Lancet* 2:109; Narita *et al.*, 2000, *Pediatric Res* 52:576-79; Rasalam *et al.*, 2005, *Dev Med Child Neurol* 47:551-55). Folic acid supplementation prior to pregnancy prevents the incidence of spina bifida by 70% (Shaw *et al.*, 1995, *Epidemiology* 6:219-226), although the exact mechanism of action is also unknown. The information obtained herein can be used to elucidate mechanisms of action of folic acid and neural tube defects in the early human embryo. These methods can also be applied to other known teratogens, such as retinoic acid, warfarin, and thalidomide (Franks *et al.*, 2004, *Lancet* 363:1802-11) to validate the predictive ability of hESCs using the methods of the invention.

Table 1: Candidate small molecules (biomarkers) of developmental toxicity detected in undifferentiated human embryonic stem cells treated with 1mM valproate in comparison to untreated controls.

Change in VPA Treated hESCs in comparison to untreated controls					
Exact mass	RT	24 Hours	4 Days	8 Days	Candidate Biomarker
355.066	16		UP		**SAM S-ADENOSYLMETHIONINAMINE**
355.12	30		UP		**SAM**
381.1574	12		UP	UP	**GLUTATHIONE**
398.21	39	DOWN	UP		**SAM OXOBUTANOATE**
441.8831	12			UP	**DIHYDROFOLIC ACID**
444.1729	17		UP	UP	**TETRAHYDROFOLIC ACID**
472.16	17	ZERO	UP	UP	**TETRAHYDROFOLATE**
612.15	17	DOWN	DOWN	UP	**GLUTATHIONE OXIDIZED**

RT = retention time
Small molecule detection was conducted with LC/ESI-TOF-MS in triplicate samples of supernatant processed independently.

As discussed above, metabolite profiles were determined at 24 hours, four days and eight days after valproate treatment. At four days after treatment, multiple candidate biomarkers were upregulated in treated versus untreated human embryonic stem cells (shown in Figures 2A through 2C). In addition to the results set forth above regarding increased levels of certain metabolites, multiple metabolite peaks were down-regulated in response to valproate at 24 hours in comparison to untreated controls (Figures 2A through 2C).

hESCs were cultured in conditioned media from mouse embryonic fibroblasts, which generated 1277 of the 3241 measured compounds. Many metabolites in human development and disease are likely present in conditioned media from mouse embryonic fibroblasts due to common metabolic pathways. Rigorous investigation is required to validate candidate biomarkers that are not exclusive to hESCs and are also present in the media.

Example 2

Gene Expression Analysis

The efficacy of the analysis shown in Example 1 was confirmed by gene expression studies, wherein changes in gene expression were observed following VPA treatment of hESCs. VPA treatment was not detrimental to hESCs, which remained viable for multiple passages following teratogen exposure, thus enabling gene expression analysis to be performed.

Treated and control H1/NIH code WA01 hES cells (passage 41) were analyzed by real-time PCR, and each treatment group was paired with a corresponding control group that received the standard growth media combination of CM+bFGF without VPA. In these studies, total cellular RNA was extracted from

cells harvested at 24 hours (24H), 4 days (4D) and 8 days (EC) using the RNA Easy Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Expression levels of candidate test genes and a housekeeping gene (Beta-2-microglobulin) were evaluated by quantitative real-time PCR using a DNA Engine–Opticon 2 Detection System (MJ Research, Watertown, MA). The housekeeping gene acts as an internal control for normalization of RNA levels. The primers used for real-time PCR reactions were designed using Beacon Designer software (Premier Biosoft International, Palo Alto, CA). RNA was reverse transcribed using iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA), wherein each cDNA synthesis reaction (20 μL) included 4 μL of 5x iScript reaction mix, 1 μL of iScript reverse transcriptase, and 2 μL of RNA. PCR was performed on cDNA in PCR reaction mixtures (25 μL) each containing 12.5 μL of Supermix (contains dNTPs, Taq DNA polymerase, SYBR Green I, and fluorescein), 250 nM forward primer, 250 nM reverse primer, and 1.6 μL RT-PCR products. Melting curve analysis and agarose gel electrophoresis were performed after real-time PCR reaction to monitor PCR specificity, wherein PCR products were detected with SYBR Green I using the iQ SYBR Green Supermix kit (Bio-Rad).

Quantifying the relative expression of real-time PCR was performed using the 2-ΔΔCt method (Livak & Schmittgen, 2001, *Methods* 25:402-8), and a general linear model was employed to fit the expression data. The PROC GLM procedure in SAS (version 8.2; SAS Institute, Cary, NC) was used to estimate least squares means in expression between treated and untreated hESCs and $P < 0.05$ was considered statistically significant

Real-time PCR was conducted on samples from 24 hours (24H), 4 days (4D) and 8 days (EC) after VPA treatment to investigate expression levels of epigenetic

regulators (such as DNA methyltransferase-1, DNMT-1, BMI-1, EED) and critical transcription factors responsible for embryonic patterning and neurodevelopment (RUNX2, BMP7, FGF8, CBX2, GLI3, SSH and SP8) in human embryonic stem cells. These experiments showed hESCs treated with VPA were subject to marked changes in their transcriptional activity following teratogen treatment. VPA induced overall marked (2 to 30 fold) downregulation of transcription levels as early as 24 hours after exposure in all genes tested (with the exception of DNMT-1 and Shh). At 4 days after treatment, however, expression of the ubiquitous DNA methyltransferase-1 was almost abolished, and sonic hedgehog, which is absolutely critical for neurogenesis (Ye *et al.*, 1998, *Cell* 93:755-66), was down-regulated five-fold in comparison to untreated controls. At 8 days after VPA treatment, the majority of the genes were upregulated in comparison to untreated controls.

These results embodied two major implications for developmental toxicology. First, VPA induced persistent changes in key epigenetic modulators that also participate in differentiation of other tissues, such as DNMT-1 and the polycomb family member EED. Second, the effects of teratogens persisted in hESCs during critical stages of neurogenesis and organogenesis. For example, genes whose expression was affected as shown herein (including sonic hedgehog and FGF-8) are known to be master regulators of differentiation of serotonergic neurons in the brain (Ye *et al.*, 1998, *Cell* 93:755-66). Of particular notice is the fact that DNMT-1 expression is almost abolished at four days after treatment. *In vivo*, disruption of this enzyme is lethal to embryos, since it is the major maintenance methyltransferase during DNA replication (Li *et al.*, 1992, *Cell* 69:915-26).

Following teratogen exposure, temporal-specific alterations in developmental gene expression were observed. Developmental genes differ in their susceptibility to

teratogens at different times. This indication may be critical to understanding specificity of epigenetic disruptors on certain organs or tissues. RUNX2, for example, is a transcriptional activator of bone development (Napierala *et al.*, 2005, *Mol Genet Metab* 86:257-68), and is more sensitive to VPA-mediated up-regulation at very early or late stages following exposure. Real-time PCR results from hESCs disclosed herein were correlated to previous findings *in vivo* in mice (Okada *et al.*, 2004, *Birth Defects Res A Clin Mol Teratol* 70:870-879) and rats (Miyazaki *et al.*, 2005, *Int J Devl Neuroscience* 23:287-97). In these animal studies, VPA inhibited the expression of Polycomb genes, Eed, Bmi1 and Cbx2 and induced downregulation of Shh while FGF8 levels remained unchanged. The results shown here at four days following VPA treatment (Table 2) were consistent with these observations using other developmental model systems.

Table 2: 1mM VPA treatment resulted in marked changes in the expression of epigenetic regulators and developmental genes that are critical for embryonic patterning and differentiation of neurons.

Gene	*1-24H control*	*2-24H VPA treated*	*3-4D control*	*4-4D VPA treated*	*5-EC control*	*6-EC VPA treated*
BMI-1	0.543	0.252	1.651	0.112	1.020	1.071
DNMT1	0.664	0.624	1.742	0.002	0.731	1.124
EED	0.757	0.342	1.501	0.185	1.381	1.769
H19	0.207	0.006	0.144	0.846	10.660	2.756
RUNX2	0.325	1.769	5.198	4.020	1.177	2.434
BMP7	0.511	0.093	0.397	0.342	0.731	1.664
FGF8	2.544	0.801	0.384	0.314	0.16	0.837
CBX2	1.113	0.245	1.221	1.1881	0.81	1.946
GLI3	0.202	0.015	0.016	0.774	0.950	1.918
Shh	0.562	0.562	2.772	0.533	1.369	---
SP8	0.49	0.235	0.25	1.703	2.132	5.808

Gene expression levels are relative to a housekeeping gene (target gene/Beta-2-Microglobulin).

Example 3

Human Embryonic Stem Cell Metabolome: Metabolite Profiles Following Teratogen Exposure

Exposure of hES cells to the teratogen valproate induced significant changes in different metabolic pathways, including pathways important during pregnancy and development. An alternative metabolic pathway activated during pregnancy are shown in Figure 5, wherein tryptophan is converted to kynurenine. To investigate this aspect of the invention, hESCs were cultured as described in Example 1, and the procedure for valproate treatment was performed as described therein. Treatment 1 (24 hours) exposed hES cells to 22μM valproate for 24 hours followed by collection of supernatant and cell pellets. In the second treatment group (4 days), hES cells were exposed to 22μM valproate for 4 days and harvested on day 4. In the third treatment or extended culture (EC, 8 days), hES cells received valproate for 4 days followed by culture in standard hES cell media for an additional four days. Cells and supernatant were harvested on day eight. Each treatment had a parallel control group with a total of six 6-well culture dishes per experiment (two 6-well culture dishes per treatment).

Metabolome analysis was performed as described in Example 1 (Wu and McAllister, 2003, *J Mass Spectrom* 38:1043-53). Complex mixtures were separated by liquid chromatography (LC) prior to electrospray ionization (ESI) time of flight (TOF) mass spectrometry according to the methods described in this Example and Example 1. Mass Hunter (Agilent) software was applied to deconvolute the data and determine the abundance of each mass. Data were extracted from the entire mass spectrum using the m/z range of 0 to 1500 and the top 2 million most abundant mass peaks from each sample were used for data deconvolution. The minimum signal-to-noise ratio was set to 5. The masses with a minimum relative abundance greater than 0.1% were exported from the Mass Hunter software and used for further analysis.

hESCs treated with 22μM valproate resulted in 3,241 detected mass signals 42 injections. Of the total of 3,241 mass signals detected in these experiments, 1,963 compounds were measured solely in hES cells and 1,278 compounds were also present in conditioned media; 443 of these were only measured in 1 of 42 injections. 110 compounds (3%) had statistically-significant differences in at least one time point in valproate-treated hESCs compared with control. Fold changes as high as seven- to thirteen-fold were measured after valproate treatment, but these mass signals exhibited high variability across experiments. Representative masses identified following treatment of cells with 1mM and 22 μM VPA are summarized in Tables 3 and 4, respectively. Several peaks (1,963) were detected in hES cells but not in conditioned media. One of these small molecules was kynurenine, a compound produced by an alternative tryptophan metabolic pathway, activated during pregnancy and immune response. The levels of kynurenine increased by 44% (p value = 0.004 at four days, Table 5) following valproate treatment. Kynurenine was detected exclusively in hES cells and absent in conditioned media. The chemical identity of this peak was confirmed by comparative mass spectrometry in the presence of the chemical standard (Figure 4).

The results of these experiments suggested that kynurenine is a candidate biomarker for neurodevelopmental disorders, in particular those originated by exposure of the human embryo to anti-epileptic drugs such as VPA (Ornoy *et al.*, 2006, *Reproductive Toxicol* 21:399-409). Strikingly, recent studies have suggested that kynurenine metabolism may be a novel target for the mechanism of action of anti-epileptic drugs (Kocki *et al.*, 2006, *Eur J Pharmacol* 542:147-51). Cognitive and behavioral disorders are known adverse effects of antiepileptic exposure during pregnancy. Tryptophan is the precursor of serotonin, a key neurotransmitter in the

pathogenesis of these and other diseases, such as depression. In addition, increased plasma levels of kynurenine have been linked to postpartum depression (Kohl *et al.*, 2005, *J Affect Disord* 86:135-42). The alteration in tryptophan metabolism detected herein is a means for examining novel mechanisms in pathogenesis of serotonin-related behavioral disorders such as autism (Chugani, 2004, *Ment Retard Dev Disabil Res Rev* 10:112-116). An increase in kynurenine levels during development may reduce the bioavailability of tryptophan and consequently serotonin, leading to cognitive dysfunction.

Glutamate and pyroglutamic acid were also elevated in hESCs treated with valproate. Glutamate and pyroglutamic acid were elevated in response to valproate (20% and 27%, respectively), although only pyroglutamic acid exhibited statistically significant changes (p=0.021 at 4 days, Figures 3A through 3D). Glutathione (GSH) is metabolized by gamma-glutamyltranspeptidase into glutamate, a neurotransmitter of NMDA receptors, and cysteinylglycine (Cys-Gly). Glutathione (exact neutral mass 612.15) and *S*-adenosyl-homocysteine (exact neutral mass 384.12) were detected at very low levels in comparison to other mass signals (data not shown). For these experiments for low level detection, small molecules were identified by comparative ESI-TOF-MS with chemical standards that were "spiked" into conditioned media at different concentrations and used to confirm neutral exact masses and retention times of experimental mass signals (Figures 3A through 3D). Neutral exact masses and/or empirical chemical formulas generated by ESI-TOF-MS were searched in public databases (including *for example* http://metlin.scripps.edu., www.nist.gov/srd/chemistry.htm, www.metabolomics.ca) for candidate compounds.

These results suggested that valproate affects the glutamate synthesis pathway in the developing human embryo. The affinity of anti-epileptic drugs towards

glutamate targets has been previously suggested (Rogawski and Loscher, 2004, *Nat Rev Neurosci* 2004 5:553-64). Abnormal levels of glutamate metabolites were measured in maternal serum and amniotic fluid of pregnant women whose infants were diagnosed spina bifida (Groenen *et al.*, 2004, *Eur J Obstet Gynecol Reprod Biol.;* 112:16-23) with nuclear magnetic resonance (NMR). The levels of glutamine and hydroxyproline were significantly higher in NTDs, and as a result the hESC methods provided herein provide a robust resource to model *in vivo* alterations of development.

Table 5. Changes in metabolic profiles of four compounds in hES cells treated with valproate versus untreated controls at 24 hours (24h), 4 days (4D), and eight days (8D) after treatment.

Molecule	24h P-value	24h fold	4D P-value	4D fold	8D P-value	8D fold	Mass	RT
Pyroglutamic acid	0.242	57% decrease	**0.021**	27% increase	0.917	3% decrease	129.0426	19.9
Folic acid	0.638	3% increase	0.626	4% increase	**0.022**	16% increase	441.1395	32.7
Glutamate	0.969	1% increase	0.108	24% increase	0.651	10% increase	147.0535	20.0
Kynurenine	0.087	29% increase	**0.004**	44% increase	N.D.	N.D.	208.0850	25.9

RT= retention time
Fold changes are represented as percent difference of the least squared means of valproate treated and untreated hES cells. *p*-values were determined by ANOVA. The mass is the average neutral mass detected by ESI-TOF-MS and the RT is the average retention time the molecule eluted at. P-values less than 0.05 are in bold.

Example 4

Kynurenine: Biomarker for Diagnosis and Treatment of Developmental Toxicity and CNS Disorders

Kynurenine was shown in Example 3 to be detected in valproate-treated hES cells. Kynurenine (along with glutamate and pyroglutamic acid) was differentially produced in valproate-treated human embryonic stem cells (hES) versus controls. Kynurenine is a novel biomarker useful for the identification of neurodevelopmental

disorders in infants and *in vitro* developmental toxicity of chemicals. This example describes the identification of biomarkers for neurodevelopmental disorders, including cellular products differentially produced in teratogen-treated hESCs.

The amino acid tryptophan (TRP) is a precursor of the neurotransmitter serotonin, a key mediator of numerous CNS disorders, such as depression, neurodegeneration and cognitive impairment. Tryptophan catabolism into kynurenic acid is an alternative route for tryptophan metabolism (Figure 5), that is activated in specific circumstances such as inflammatory response or pregnancy. Up-regulation of the kynurenine pathway is correlated with psychosis in adult diseases such as schizophrenia and bipolar disorder, an indication that increased levels of pathway intermediates may trigger psychotic features (Miller *et al.*, 2006, *Brain Res* 16:25-37). Significantly, metabolism using the kynurenine pathway is accompanied by decreased tryptophan metabolism using the serotonin pathway (in the absence of exogenous tryptophan, an essential amino acid not synthesized by mammals including man). An increase in kynurenine levels during development can reduce the bioavailability of tryptophan and consequently serotonin, leading to cognitive dysfunction.

In addition, kynurenic acid (KYNA), one of the end products of this tryptophan metabolic pathway, is an antagonist of glutamate neurotransmission and N-methyl-D-aspartate (NMDA) receptors. Recent studies have demonstrated that kynurenic acid is a druggable target via its role in the activation of the previously orphan GPCR receptor GPR35 (Wang *et al.*, 2006, *J Biol Chem* 281:22021-8). Quinolinic acid (QUIN), another end product of the pathway (Figure 5), and 3-hydroxy-kynurenine, an intermediate, act as neurotoxicants (Guillemin *et al.*, 2005, *J Neuroinflammation* 26:16; Chiarugui *et al.*, 2001, *J Neurochem* 77:1310-8). QUIN is involved in the pathogenesis of Alzheimer's disease where its neurotoxicity may be

involved in increased inflammation and in convulsions by interacting with the N-methyl-D-aspartate (NMDA) receptor complex, a type of glutamate receptor (Guillemin *et al.*, 2002, *J Neuroinflammation* 26:16,;Nemeth *et al.*, 2005, *Curr Neurovasc Res* 2:249-60). Kynurenin (KYN), another pathway intermediate, is synthesized in the brain and is transported across the blood-brain barrier (Nemeth *et al.*, 2005, *Curr Neurovasc Res* 2:249-60). KYN is metabolized to the neurotoxic quinolinic acid (QUIN) and the neuroprotective kynurenic acid (KYNA) (Figure 5). Increased serum levels of KYN have been correlated to clinical manifestation of depression with different etiologies, such as post-partum disorder (Kohl *et al.*, 2005, *J Affect Disord* 86:135-42) and interferon-alpha treatment (Capuron *et al.*, 2003, *Biol Psychiatry* 54:906-14).

Exposure of hES cells to valproate, a disruptor of human development, induced significant changes in different metabolic pathways, including the production of kynurenine (exact neutral mass 208.08), which was significantly upregulated in response to valproate as detected by liquid chromatography electrospray ionization time of flight mass spectrometry (LC/ESI-TOF-MS) as described in Example 4. Additionally, novel chemical entities, having exact neutral masses of 328.058, 336.163, 343.080, were detected and are not yet catalogued in public databases.

When neural precursors derived from hESCs were exposed to 1mM valproate, a marked decrease in both serotonin (176.0946) and indoleacetaldehyde (159.0689), a downstream sub-product of serotonin generated by monoaminoxoidase activity (MAO) was observed (Table 6). Glutamate and pyroglutamic acid or hydroxyproline (p=0.021) were also elevated in hES cells treated with valproate. These results suggest that valproate affects the glutamate synthesis pathway in the developing human embryo. This finding emulates *in vivo* neurophysiology, where compounds

from the kynurenine pathway modulate activity at NMDA glutamate receptors and produce epileptic phenotypes, including seizures (Perkins and Stone, 1982, *Brain Res* 247:184-187.).

As a consequence of the identification of kynurenine herein, chemical inhibitors of kynurenine synthesis can be used as novel therapeutics in mood disorders; for example, small molecules that antagonize indoleamine 2,3-dioxygenase (IDO) or kynurenine formylase activities, which converts tryptophan (TRP) into kynurenine (KYN). Inhibition of TRP catabolism to KYN can be used to ameliorate disease symptoms in cognitive and neurodegenerative disorders by increasing serotonin levels, via elevated synthesis of this neurotransmitter or reduced depletion through the kynurenine pathway.

Collectively, the metabolite changes detected in hES cells in response to valproate converge functionally towards folate, kynurenine and glutamate pathways. Figure 6 illustrates the hierarchical clustering of the fold change differences from 22,573 unique masses. Changes in the above-mentioned pathways were consistent and reproducible in multiple independent studies of 1mM VPA treated hESCs, and neural precursors produced from hESCs (Figure 6).

Example 5

Gene Expression Analysis of Kynurenine Pathway

The efficacy of the analysis in Example 4 was confirmed by gene expression studies, wherein changes in gene expression were observed following VPA treatment of hESC. Valproate treatment of human embryonic stem cells induced a marked upregulation in the small molecule kynurenine, an intermediate metabolite in the catabolism of tryptophan. Tryptophan is the precursor of the neurotransmitter

serotonin (5HT). Thus, whether expression of enzymes in the metabolism of tryptophan to kynurenine and its opposite route, serotonin synthesis, was altered in human embryonic stem cells was investigated to examine the mechanistic properties of the kynurenine pathway and its response to valproate.

Human embryonic stem cells treated with 1mM valproate and untreated controls were harvested at four days after treatment and stored at -80°C prior to RNA isolation using RNeasy (Qiagen). 5 μg of RNA templates were reverse transcribed and amplified (QIAGEN OneStep RT-PCR) according to the manufacturer's instructions using primers designed for transcribed human sequences of the following genes: INDO, indoleamine 2,3 dioxygenase, TDO or TDO2, tryptophan 2,3-dioxygenase, AFMID, arylformamidase, TPH1, tryptophan hydroxylase the rate-limiting enzyme in serotonin biosynthesis, AADAT, aminoadipate aminotransferase, KYNU, kynunreninase, KMO, kynurenine 3-monooxygenase, GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

The results of this study showed that the majority of enzymes in the kynurenine pathway and serotonin synthesis were expressed in hES cells at four days after treatment of hES cells with 1mM valproate (Figure 7). Indoleamine 2, 3 dioxygenase INDO, catabolizes tryptophan into the kynurenine pathway, and produces kynurenine as an end product. The expression of tryptophan 2,3 dioxygenase (TDO or TDO2) was also examined. TDO2, like INDO, catalyzes the first step in the kynurenine pathway. These data suggested that TDO2 expression was upregulated in hES cells treated with valproate in comparison to untreated controls. The rate limiting enzyme in 5HT synthesis, TPH1, was also expressed in hES cells (Figure 7). Expression of these enzymes supported the conclusion that hES cells recapitulate metabolic pathways of tryptophan catabolism and serotonin synthesis.

Interestingly, VPA induced pronounced expression of rate-limiting enzymes in this pathway.

Example 6

Developmental Toxicology Screening for Prenatal Alcohol Exposure

To identify differentially secreted metabolites in response to alcohol, as well as the pathways involved in fetal alcohol syndrome, human embryonic stem cells were treated with 0, 0.1 and 0.3% ethanol for four days followed by LC/ESI-TOF mass spectrometry according to the general methods described above for valproate in Example 1. Extracellular media was collected and processed at 24 hours and four days after treatment, and 49,481 mass signals were detected following three technical replications. Of the 49,481 mass signals, 1,860 compounds were significantly different ($p<0.05$) in at least one treatment and had a significant time change (<1 or >1). (Table 7). Binned masses were annotated *in silico* by querying the neutral masses in several different databases. These databases included Metlin, Biological Magnetic Resonance Data Bank (BMRB), NIST Chemistry WebBook, and the Human Metabolome Database. A mass was considered identified when its neutral mass was within 10 ppm of a known compound annotated in one of the databases listed above.

The putative kynurenine compound (measured exact neutral mass 208.0816) was upregulated three-fold at day four, but not 24 hours, in both treatments (0.1%, p = 0.001 and 0.3% p = 0.002, respectively). Another putative metabolite in the kynurenine pathway, 8-methoxykynurenate (219.0532) was also upregulated at four days in response to both 0.1% and 0.3% alcohol treatment ($p<0.05$). The analysis also detected a significant downregulation of 5-hydroxy-L-tryptophan (220.0848) at four

days following 0.3% alcohol treatment ($p<0.05$) in comparison to untreated controls. 5-hydroxy-L-tryptophan is the only intermediate metabolite between tryptophan and serotonin and its synthesis is mediated by tryptophan hydroxylase, the rate limiting enzyme in serotonin synthesis. These results suggest that alcohol exposure during human development can affect serotonin bioavailability due to upregulation of tryptophan catabolism into kynurenines. In addition, alcohol exposure induced significant changes in metabolic pathways and small molecules involved in neural development such as glutamate, gabapentin, adrenaline and glutathione.

Example 7

Developmental Toxicology Screening of Neuronal Precursor Cells

Metabolomic assessment of teratogens on embryonic development is not limited exclusively to hESCs. The methods of the invention are also useful with other progenitor stem cells, including lineage-restricted stem cells such as neural precursor cells. To illustrate the efficacy of toxicology screening on lineage-specific stem cells, neuronal precursors derived from hESCs were treated with 1mM valproate according to the methods described in Example 1.

Approximately 135 compounds were differentially secreted in VPA-treated neuronal precursors versus control. (See Table 6). The results of this study illustrated that the methods of the invention reveal alterations in the metabolic profile of lineage-specific stem cells in response to teratogen exposure.

The results disclosed herein are set forth in the following tables

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	8.31910526	103.056358	4D	1mM VPA	1.987286671	0.01734377	gamma-Aminobutryic acid	
1mM VPA	6.78779869	103.098349	4D	1mM VPA	2.143101233	0.00047552	2-Aminoisobutyric acid	
1mM VPA	8.39854546	113.082093	4D	1mM VPA	16.4054129	0.01342684	1-Pyrroline-5-carboxylic acid	
1mM VPA	11.7534444	120.043328	4D	1mM VPA	1.355758298	0.03951319	3,4-Dihydroxybutyric acid	
1mM VPA	85.7330833	121.088708	24H	1mM VPA	10.30519572	0.02442001	Phenylethylamine	
1mM VPA	7.307128	122.071261	4D	1mM VPA	-2.2989897	0.01870947	Unknown	
1mM VPA	38.1047857	129.070626	8D	1mM VPA	3.023878137	0.03988213	2-Ketobutyric acid; 2-Oxobutyric acid; alpha-Ketobutyric acid; alpha-Ketobutyrate	
1mM VPA	14.2761702	136.038753	8D	1mM VPA	2.696709281	0.00083818	Hypoxanthine	Allopurinol
1mM VPA	31.3610896	141.114252	8D	1mM VPA	1.865419366	0.02273706	Unknown	
1mM VPA	43.9201842	143.095482	8D	1mM VPA	2.064797071	0.03120757	1-Aminocyclohexanecarboxylic acid	
1mM VPA	51.283453	144.113677	24H	1mM VPA	4.820891632	0.02775836	Caprylic acid	Valproic acid
1mM VPA	51.283453	144.113677	4D	1mM VPA	8.26720694	0.0011089	Caprylic acid	Valproic acid
1mM VPA	16.307931	147.068314	4D	1mM VPA	-2.05380612	0.03872229	3-Methyloxindole	
1mM VPA	16.307931	147.068314	24H	1mM VPA	-1.80875876	0.037812	3-Methyloxindole	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	22.5095926	153.079352	4D	1mM VPA	-2.65737163	0.01268393	Dopamine	
1mM VPA	5.36288806	155.068364	8D	1mM VPA	-1.34957018	0.0476914	L-Histidine	
1mM VPA	20.6395854	155.072941	4D	1mM VPA	3.82298622	0.00676609	L-Histidine	
1mM VPA	14.2071091	160.060653	8D	1mM VPA	12.348809	1.23E-06	Unknown	
1mM VPA	14.3670392	161.081539	8D	1mM VPA	4.777314979	0.0001252	Unknown	
1mM VPA	44.8165285	162.067353	4D	1mM VPA	1.640573238	0.01289447	Unknown	
1mM VPA	31.5154611	162.124096	24H	1mM VPA	1.911228139	0.01933211	Unknown	
1mM VPA	31.3624074	165.079533	8D	1mM VPA	1.705269784	0.00206584	4-(3-Pyridyl)-butanoic acid	
1mM VPA	32.0426154	167.094942	8D	1mM VPA	-2.20640862	0.01874726	Methyldopamine	
1mM VPA	20.0098065	173.083484	4D	1mM VPA	5.458861144	0.00198632	2-Oxoarginine	
1mM VPA	15.3496532	177.082617	8D	1mM VPA	2.397781171	0.01291216	Unknown	
1mM VPA	24.1314286	179.094351	4D	1mM VPA	1.851635336	0.03464009	Salsolinol	Homophenylalanine
1mM VPA	21.8046482	187.064183	8D	1mM VPA	2.839427352	0.00590774	Unknown	
1mM VPA	21.8046482	187.064183	4D	1mM VPA	3.356831218	1.24E-05	Unknown	
1mM VPA	23.317	187.08492	4D	1mM VPA	3.781608467	0.03987705	6-Acetamido-3-oxohexanoate	
1mM	27.7865556	189.042631	8D	1mM	2.865724657	0.02214848	Kynurenic acid	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
VPA				VPA				
1mM VPA	61.5462473	196.090684	4D	1mM VPA	3.519815968	0.01102697	Unknown	
1mM VPA	24.0551398	197.105003	4D	1mM VPA	2.231478645	0.00076838	L-Metanephrine	
1mM VPA	20.0989286	197.175657	8D	1mM VPA	4.237754463	0.00034728	Unknown	
1mM VPA	73.9582188	198.16015	4D	1mM VPA	2.039619449	0.01232495	5-Dodecenoic acid	
1mM VPA	29.2935714	201.100569	4D	1mM VPA	5.966976107	0.00328699	Unknown	
1mM VPA	28.6036393	203.115212	8D	1mM VPA	1.872544495	0.00040874	L-Glutamic acid n-butyl ester	Acetylcarnitine
1mM VPA	9.07926923	209.06985	8D	1mM VPA	-12.4054314	0.01848957	4-Carboxyphenylglycine	
1mM VPA	48.3434453	213.079468	8D	1mM VPA	2.512458907	0.02116099	Unknown	
1mM VPA	44.6001887	214.064259	4D	1mM VPA	1.446032522	0.02767084	Unknown	
1mM VPA	44.6001887	214.064259	8D	1mM VPA	1.783609761	0.00082206	Unknown	
1mM VPA	69.6687917	214.064356	24H	1mM VPA	1.316493137	0.0171064	Unknown	
1mM VPA	18.7504371	216.094569	4D	1mM VPA	2.349086763	0.01043169	Unknown	
1mM VPA	30.5773235	216.100485	4D	1mM VPA	2.050108123	0.04358571	Unknown	
1mM VPA	6.39474737	218.076897	4D	1mM VPA	1.737243521	0.02003307	Unknown	
1mM VPA	6.68071429	219.144891	24H	1mM VPA	-1.58008262	0.02066251	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	10.5609423	220.085746	4D	1mM VPA	-2.39827983	1.63E-06	5-Hydroxytryptophan	
1mM VPA	53.8814512	222.078039	4D	1mM VPA	2.882259036	0.02550855	Unknown	
1mM VPA	73.0997018	223.049411	8D	1mM VPA	-4.31392173	0.04819747	7,8-Dihydro-7,8-dihydroxykynurenate	
1mM VPA	6.45641791	229.095757	8D	1mM VPA	2.4471457	0.00094873	Malonylcarnitine	
1mM VPA	23.7927189	229.095855	4D	1mM VPA	2.057653416	0.00038761	Malonylcarnitine	
1mM VPA	55.5371429	229.145042	4D	1mM VPA	2.032140286	0.00236036	Unknown	
1mM VPA	19.9783175	229.164555	8D	1mM VPA	3.779774064	1.34E-08	Unknown	
1mM VPA	32.6065714	229.201979	4D	1mM VPA	3.088058322	0.00439209	Unknown	
1mM VPA	9.79255	230.080163	4D	1mM VPA	-1.383766	0.02197836	Unknown	
1mM VPA	7.80846914	233.123128	8D	1mM VPA	6.784300156	0.00043647	Unknown	
1mM VPA	14.3537949	236.080213	4D	1mM VPA	-3.35334287	0.00032832	N'-Formylkynurenine	
1mM VPA	45.2867439	238.12327	8D	1mM VPA	3.718961983	0.01466842	2-Amino-3-methylbutyric acid	
1mM VPA	12.1171264	244.109135	4D	1mM VPA	1.659329044	0.01962434	Unknown	
1mM VPA	12.127642	245.119507	4D	1mM VPA	2.061936638	0.02383097	Unknown	
1mM VPA	19.8036863	246.100428	4D	1mM VPA	4.924577653	0.02636633	N-Acetyl-D-tryptophan	
1mM	8.947	247.140975	8D	1mM	2.877468231	0.01777412	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
VPA				VPA				
1mM VPA	19.7590488	247.173942	8D	1mM VPA	3.071620539	3.15E-06	Unknown	
1mM VPA	48.7837308	248.191881	4D	1mM VPA	2.692786782	0.00724013	Unknown	
1mM VPA	8.00665714	249.119037	4D	1mM VPA	1.948008537	0.01865508	Unknown	
1mM VPA	53.1723271	256.1093	8D	1mM VPA	3.068428571	0.00024804	D-2-Amino-3-hydroxybutyric acid	gamma-Amino-beta-hydroxybutyric acid
1mM VPA	23.22678	257.099256	8D	1mM VPA	2.601240877	0.00033049	5-Methylcytidine	
1mM VPA	5.57045	257.891738	24H	1mM VPA	-1.17015529	0.01640996	Unknown	
1mM VPA	7.08067539	258.019715	24H	1mM VPA	1.315125063	0.02206679	Unknown	
1mM VPA	22.8759189	258.121153	4D	1mM VPA	1.510263204	0.01588551	Unknown	
1mM VPA	28.8676889	258.133722	24H	1mM VPA	5.578974665	0.03000729	Unknown	
1mM VPA	47.6525584	258.133727	4D	1mM VPA	-1.91733177	0.01442461	Unknown	
1mM VPA	18.7499759	259.11867	4D	1mM VPA	1.504620863	0.02037249	N-(gamma-L-Glutamyl)amino-D-proline	
1mM VPA	13.2221957	260.083648	8D	1mM VPA	2.984522231	0.02760558	Unknown	
1mM VPA	22.373	264.109282	4D	1mM VPA	-1.74811488	0.01791457	Acetyl-N-formyl-5-methoxykynurenamine	
1mM VPA	58.8093824	265.132541	8D	1mM VPA	2.363623094	0.03359569	(2R,3S)-rel-2,3-dihydroxy--Butanoic acid	
1mM	27.779593	271.112691	4D	1mM	1.685060044	0.03867385	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
VPA				VPA				
1mM VPA	24.7259575	272.124009	4D	1mM VPA	2.036511555	0.02960407	Unknown	
1mM VPA	44.0582051	272.168272	8D	1mM VPA	2.056227653	0.00325332	Unknown	
1mM VPA	41.65612	272.211662	8D	1mM VPA	-5.12943527	0.00643051	3-Oxo-delta1-steroid	
1mM VPA	39.378469	273.105953	4D	1mM VPA	2.674742484	0.00030929	Unknown	
1mM VPA	8.93373171	276.136639	4D	1mM VPA	5.215118375	0.0006752	Unknown	
1mM VPA	67.6599775	280.237772	24H	1mM VPA	2.086232575	0.04410145	Linoleic acid	Octadecadienoic acid
1mM VPA	14.2211017	281.125398	8D	1mM VPA	8.170361997	1.56E-06	1-Methyladenosine	
1mM VPA	71.5568571	282.225649	4D	1mM VPA	4.282045127	0.00101203	Unknown	
1mM VPA	72.7757434	282.253397	8D	1mM VPA	-1.86257691	0.03054583	Oleic acid	Elaidic acid
1mM VPA	59.4208378	284.19613	4D	1mM VPA	5.253576839	0.0351483	Unknown	
1mM VPA	5.70108824	284.980449	4D	1mM VPA	1.366608495	0.03819988	Unknown	
1mM VPA	6.9018125	285.140063	4D	1mM VPA	15.96344365	0.00293691	Unknown	
1mM VPA	64.3362162	286.186749	4D	1mM VPA	2.524154118	0.04701735	N-Acetyl-leucyl-leucine	
1mM VPA	64.3362162	286.186749	24H	1mM VPA	2.577549261	0.00532464	N-Acetyl-leucyl-leucine	
1mM VPA	75.3938769	288.263273	4D	1mM VPA	2.556553115	0.0003234	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	26.6263806	289.137569	8D	1mM VPA	7.105814367	0.00077408	Unknown	
1mM VPA	15.0419293	289.139413	8D	1mM VPA	3.953690666	0.00671585	Unknown	
1mM VPA	15.8950204	295.128678	8D	1mM VPA	2.286752138	1.23E-06	N6,N6-Dimethyladenosine	
1mM VPA	6.02850649	301.172858	4D	1mM VPA	5.302600282	0.00165331	Unknown	
1mM VPA	59.5394364	301.222733	4D	1mM VPA	4.091131755	0.01328711	Unknown	
1mM VPA	72.4551579	304.237816	8D	1mM VPA	-5.09223853	0.00158305	Arachidonic acid	
1mM VPA	44.6849231	305.936181	8D	1mM VPA	2.13864941	0.00372138	3-Iodo-4-hydroxyphenylpyruvate	
1mM VPA	7.93489796	306.092269	24H	1mM VPA	-2.59907813	0.00116532	Unknown	
1mM VPA	22.339	306.121765	24H	1mM VPA	2.666042908	0.00540921	Z-Gly-Pro; Z-Gly-Pro-OH	
1mM VPA	59.461	306.180711	4D	1mM VPA	2.390810858	0.01473431		
1mM VPA	12.9788342	307.161748	8D	1mM VPA	5.76411852	0.00135244	Unknown	
1mM VPA	4.76167568	308.158497	8D	1mM VPA	3.212062578	7.02E-05	Unknown	
1mM VPA	7.58973529	316.131974	4D	1mM VPA	1.861931503	0.01887819	Unknown	
1mM VPA	66.9950694	316.200989	4D	1mM VPA	2.178145003	0.00392399	Gibberellin A12 aldehyde	
1mM VPA	62.665	319.244008	24H	1mM VPA	3.088914632	0.0457215	Unknown	
1mM	19.019754	320.137541	4D	1mM	2.083198045	0.04299189	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
VPA				VPA				
1mM VPA	67.8541343	320.230187	4D	1mM VPA	1.784846494	0.03271491	Unknown	
1mM VPA	67.8541343	320.230187	24H	1mM VPA	1.981647012	0.01061379	Unknown	
1mM VPA	10.672	321.168775	24H	1mM VPA	2.153375627	0.00361195	Unknown	
1mM VPA	35.4656491	324.169472	4D	1mM VPA	2.684214566	0.00585101	Unknown	
1mM VPA	63.932859	326.0008	24H	1mM VPA	1.479797739	0.00340947	Unknown	
1mM VPA	63.932859	326.0008	4D	1mM VPA	1.541142217	0.01010729	Unknown	
1mM VPA	62.4897344	328.242558	4D	1mM VPA	1.831213495	0.03531113	Docosahexaenoic acid	
1mM VPA	55.092	329.001202	24H	1mM VPA	1.889887032	0.01315641	Unknown	
1mM VPA	6.02840404	330.105879	8D	1mM VPA	4.856779538	0.01414085	Unknown	
1mM VPA	12.8257065	330.153322	4D	1mM VPA	-1.46094311	0.02278769	Unknown	
1mM VPA	47.63185	330.240548	4D	1mM VPA	2.777910272	0.01585359	Unknown	
1mM VPA	67.7267647	330.242694	4D	1mM VPA	3.168939244	0.02399703	Unknown	
1mM VPA	9.01253333	331.103633	8D	1mM VPA	5.010657754	0.00879812	Unknown	
1mM VPA	18.8430244	334.151446	24H	1mM VPA	7.598422851	0.04853637	Unknown	
1mM VPA	4.05694118	336.031706	4D	1mM VPA	-23.1557728	5.36E-05	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	6.7701658	336.15353	4D	1mM VPA	2.062222503	0.01789166	Unknown	
1mM VPA	54.915974	347.982073	4D	1mM VPA	16.74896451	0.02976287	Unknown	
1mM VPA	45.6079091	348.203076	4D	1mM VPA	3.375263185	0.00190076	Unknown	
1mM VPA	6.04629167	349.134979	8D	1mM VPA	1.449645356	0.02177991	Unknown	
1mM VPA	67.3780816	352.221576	24H	1mM VPA	2.329951622	0.01190993	Prostaglandin	
1mM VPA	22.8247245	353.157641	4D	1mM VPA	3.01822425	0.00974537	2-Keto-3-Methylvaleric acid	
1mM VPA	19.103773	353.158931	4D	1mM VPA	2.134354771	0.00286811	Unknown	
1mM VPA	29.94892	355.242828	4D	1mM VPA	3.751584361	0.01212028	Unknown	
1mM VPA	15.1070313	356.156972	4D	1mM VPA	5.181249294	0.00024122	l-Glutamic-gamma-semialdehyde	
1mM VPA	59.1895507	358.229755	4D	1mM VPA	4.389936283	0.00450968	Unknown	
1mM VPA	7.78296	359.071286	8D	1mM VPA	3.504721971	0.02819084	Unknown	
1mM VPA	27.8286847	359.198793	4D	1mM VPA	2.67566964	0.04513681	Unknown	
1mM VPA	7.67294845	362.15214	24H	1mM VPA	3.677690313	0.01613594	Aminohexanoic acid	
1mM VPA	6.16412	364.18324	4D	1mM VPA	4.422922613	0.01590116	Unknown	
1mM VPA	19.3098372	364.185514	8D	1mM VPA	2.529233091	0.00135633	Gibberellin A44	
1mM	53.9854054	366.239292	4D	1mM	6.176116644	3.03E-05	3b-Allotetrahydrocortisol	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
VPA				VPA				
1mM VPA	17.7238836	372.188649	4D	1mM VPA	3.32395304	2.43E-07	Ornithine	
1mM VPA	11.4481111	374.168865	8D	1mM VPA	3.707636994	0.00715743	Unknown	
1mM VPA	13.8172619	374.207426	8D	1mM VPA	2.50185816	0.03397986	Unknown	
1mM VPA	17.6750468	388.183189	8D	1mM VPA	3.124878291	0.00060074	Malic acid	Diglycolic acid
1mM VPA	21.3150329	392.209899	4D	1mM VPA	2.402938958	0.02146723	Unknown	
1mM VPA	79.7277263	404.258123	4D	1mM VPA	2.231633324	0.02122436	7a,12a-Dihydroxy-3-oxo-4-cholenoic acid	
1mM VPA	24.7042427	407.206755	4D	1mM VPA	2.564362115	0.03457095	Unknown	
1mM VPA	16.9907536	408.172332	8D	1mM VPA	1.47549599	0.01779219	4-Hydroxyphenylacetaldehyde;	
1mM VPA	16.9907536	408.172332	4D	1mM VPA	1.84894204	0.00218606	4-Hydroxyphenylacetaldehyde;	
1mM VPA	29.02944	411.227331	4D	1mM VPA	3.412904392	0.00663705	Gln His Lys	
1mM VPA	31.0764706	411.788303	4D	1mM VPA	4.812211329	0.01959219	Unknown	
1mM VPA	9.74277941	412.191819	8D	1mM VPA	4.778970957	0.02280244	Unknown	
1mM VPA	24.0870602	416.213608	8D	1mM VPA	1.904483779	0.00013882	Unknown	
1mM VPA	13.7165	420.160178	4D	1mM VPA	4.500233939	0.04166145	Unknown	
1mM VPA	27.4410904	421.219685	4D	1mM VPA	2.92999865	0.01453378	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	84.9993429	424.278966	4D	1mM VPA	2.302178983	0.02079967	1b,3a,7a,12a-Tetrahydroxy-5b-cholanoic acid	
1mM VPA	84.9993429	424.278966	24H	1mM VPA	2.318995467	0.00016902	1b,3a,7a,12a-Tetrahydroxy-5b-cholanoic acid	
1mM VPA	54.5975206	429.099036	4D	1mM VPA	3.524698852	2.05E-05	Unknown	
1mM VPA	25.5684706	430.183077	4D	1mM VPA	1.148698355	0.00012401	Unknown	
1mM VPA	47.39725	432.071275	4D	1mM VPA	2.645059178	0.01175067	Unknown	
1mM VPA	14.5288987	445.217914	8D	1mM VPA	2.820595921	0.00824753	Unknown	
1mM VPA	7.5585	445.286693	4D	1mM VPA	-1.13705339	0.04453994	Unknown	
1mM VPA	19.9357624	455.226453	8D	1mM VPA	2.768491323	0.0146344	Adipate	
1mM VPA	84.7522195	470.350048	4D	1mM VPA	3.767741534	0.00027941	Unknown	
1mM VPA	8.479	471.146232	24H	1mM VPA	2.569343893	0.02598742	10-Formyldihydrofolate	
1mM VPA	22.7650396	471.202698	4D	1mM VPA	2.257302866	1.30E-06	Unknown	
1mM VPA	15.4262845	491.253192	8D	1mM VPA	4.916392167	0.00321994	Unknown	
1mM VPA	60.5202059	493.458959	4D	1mM VPA	2.789487333	0.00131052	Unknown	
1mM VPA	44.8878444	502.216027	4D	1mM VPA	1.922921676	0.00968802	Unknown	
1mM VPA	14.5675854	502.227438	8D	1mM VPA	3.101787817	0.00862582	Unknown	
1mM	31.0262667	504.2848	4D	1mM	5.119489655	7.48E-05	Unknown	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
VPA				VPA				
1mM VPA	17.4495833	516.244788	4D	1mM VPA	2.722628233	0.00035163	Unknown	
1mM VPA	44.14925	527.321492	8D	1mM VPA	2.213454933	0.00740287	Unknown	
1mM VPA	70.8363171	528.362659	4D	1mM VPA	2.941801698	0.03554601	Unknown	
1mM VPA	74.25245	530.344375	24H	1mM VPA	3.680750602	0.03848341	Unknown	
1mM VPA	9.15167857	532.249475	8D	1mM VPA	7.170133597	0.00736475	Unknown	
1mM VPA	29.9863488	535.254098	8D	1mM VPA	6.049014001	0.00097203	Unknown	
1mM VPA	87.9394	535.392963	4D	1mM VPA	1.80125196	0.03183737	Unknown	
1mM VPA	8.02928261	549.20135	8D	1mM VPA	11.08087574	0.00035145	Unknown	
1mM VPA	19.5000458	550.228302	4D	1mM VPA	2.35969435	0.00064579	Unknown	
1mM VPA	24.7983934	551.248871	24H	1mM VPA	1.396388132	0.01890342	Unknown	
1mM VPA	74.3730889	552.326244	24H	1mM VPA	1.885569072	0.031072	Lithocholate 3-O-glucuronide	
1mM VPA	75.3072222	561.322428	4D	1mM VPA	5.181249294	0.00798648	Unknown	
1mM VPA	14.1881491	565.230107	4D	1mM VPA	2.651300141	0.04312251	Unknown	
1mM VPA	5.97658065	574.262774	8D	1mM VPA	2.982867719	0.00682514	Unknown	
1mM VPA	70.4439429	594.37144	4D	1mM VPA	2.591881931	0.04970674	2-Hydroxyadenine	

Table 3: Cellular metabolites measured in human embryonic stem cells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	Probt	annotation.1	annotation.2
1mM VPA	15.895551	598.283549	8D	1mM VPA	4.074717385	0.00446383	2-Hydroxyadenine	
1mM VPA	76.5242159	599.574322	8D	1mM VPA	2.569165805	2.63E-05	Unknown	
1mM VPA	76.2647	600.576755	4D	1mM VPA	1.998614186	0.00464652	Unknown	
1mM VPA	76.2647	600.576755	8D	1mM VPA	2.690920931	0.00059418	Unknown	
1mM VPA	79.7894576	613.589997	8D	1mM VPA	3.099853425	0.01314731	Unknown	
1mM VPA	8.59329167	658.254492	8D	1mM VPA	13.32441233	0.02367066	Unknown	
1mM VPA	60.8503	688.51026	4D	1mM VPA	3.690969971	0.00301918	Unknown	
1mM VPA	69.7080175	690.409258	4D	1mM VPA	3.89061979	0.001728	Unknown	
1mM VPA	65.32996	738.583348	4D	1mM VPA	3.877159268	0.00021681	Unknown	
1mM VPA	69.7792917	810.640892	4D	1mM VPA	3.934008296	0.00141534	Unknown	
1mM VPA	21.6729286	921.002586	24H	1mM VPA	10.91318268	0.00761215	Unknown	
1mM VPA	5.86856	1007.84992	4D	1mM VPA	23.49041018	0.04324009	3-Dehydrocarnitine	

Table 4: Cellular metabolites produced in hESCs treated with 22μM valproate

cpdID	RT	MASSavg	time	trt	Fold	P-value	Compound 1	Compound2
77	28.21	99.0681	4 days	VPA	-1.81	**0.020**	N-Methyl-2-pyrrolidinone	
103	12.00	103.0991	4 days	VPA	-2.24	**0.028**	Gamma-Aminobutryic acid	2-Aminoisobutyric acid
141	34.03	113.0840	4 days	VPA	-1.43	**0.013**	Unknown	
189	12.08	119.0473	8 days	VPA	1.22	**0.040**	4-Amino-3-hydroxybutanoate	
210	96.44	120.0436	4 days	VPA	-4.22	**0.006**	3,4-Dihydroxybutyric acid	
263	19.93	129.0426	4 days	VPA	-1.27	**0.021**	Pyroglutamic acid	1-Pyrroline-4-hydroxy-2-carboxylate
323	29.83	134.0939	4 days	VPA	-1.43	**0.034**	Unknown	
329	16.96	136.0384	24 hours	VPA	-2.09	**0.038**	Hypoxanthine	Allopurinol
343	12.98	141.0412	4 days	VPA	-1.24	**0.011**	1,4,4,6-Tetrahydro-6-oxonicotinate	2-Aminomuconate semialdehyde
362	11.40	141.9381	4 days	VPA	1.19	**0.034**	Unknown	
396	11.97	146.0683	4 days	VPA	-1.02	**0.002**	Glutamine	
413	44.84	148.0638	8 days	VPA	-2.72	**0.004**	Unknown	
444	12.37	144.0687	8 days	VPA	-1.42	**0.014**	Unknown	
449	20.19	146.0066	8 days	VPA	-1.24	**0.002**	2,4-dicarboxylic acid	
496	30.40	161.0688	8 days	VPA	-1.23	**0.019**	4-Methyl-L-glutamate	2,2'-Iminodipropanoate
431	24.83	164.4009	4 days	VPA	-1.17	**0.049**	Unknown	
603	72.83	173.9844	8 days	VPA	-1.80	**0.017**	Unknown	
604	20.34	174.0160	4 days	VPA	-1.36	**0.003**	cis-Aconitate	Dehydroascorbate
636	42.18	178.0994	8 days	VPA	1.47	0.002	Phenylvaleric acid	
646	24.00	181.0740	4 days	VPA	-1.11	**0.004**	Salsolinol	Homophenylalanine
671	24.90	187.0609	4 days	VPA	-1.40	**0.018**	Unknown	
674	36.08	187.0973	4 days	VPA	2.14	**0.042**	Unknown	
812	29.93	204.0899	8 days	VPA	1.08	**0.034**	L-Tryptophan	

cpdID	RT	MASSavg	time	trt	Fold	P-value	Compound 1	Compound2
843	24.94	208.0840	4 days	VPA	-1.14	0.004	Kynurenine	Formyl-4-hydroxykynurenamine
893	44.64	214.1680	8 days	VPA	-2.11	0.005	Fenamic acid	
1089	47.40	242.0808	8 days	VPA	1.87	0.02	Unknown	
1104	7.98	243.9760	4 days	VPA	1.92	0.032	Unknown	
1282	44.30	274.0947	8 days	VPA	1.78	0.019	3-Oxo-delta4-steroid	
1447	43.40	300.2784	8 days	VPA	-2.22	0.033	Unknown	
1440	27.94	314.2032	4day	VPA	-1.12	0.012	Unknown	
1637	24.91	330.1480	4day	VPA	-1.42	0.004	Unknown	
1684	11.94	336.1634	4 days	VPA	-1.13	0.023	Unknown	
1691	34.87	338.0974	4 days	VPA	1.74	0.033	Unknown	
1776	39.67	342.1130	4day	VPA	1.46	0.044	Unknown	
1816	24.40	348.1139	8 days	VPA	2.56	0.025	Unknown	
1838	11.00	361.9194	4 days	VPA	-1.08	0.004	Unknown	
1948	12.00	384.1664	8 days	VPA	1.38	0.018	Unknown	
1949	14.98	387.1498	4 days	VPA	-1.26	0.001	Unknown	
2084	64.24	414.2934	4 days	VPA	-1.20	0.031	Unknown	
2131	88.14	426.2983	4 days	VPA	1.85	0.022	Cholanoic acid	
2134	74.20	427.1200	8day	VPA	-1.88	0.044	Unknown	
2138	26.91	428.2423	8day	VPA	1.70	0.003	Unknown	
2144	34.14	431.2733	4 days	VPA	-1.24	0.041	Unknown	
2186	32.68	441.1394	8 days	VPA	-1.16	0.022	Folate	Folic acid
2191	64.67	442.2934	8 days	VPA	1.79	0.001	Unknown	
2214	92.89	440.3448	8 days	VPA	-1.84	0.037	Unknown	
2233	12.00	444.0841	8 days	VPA	-1.30	0.041	Unknown	
2244	64.41	449.3198	24 hours	VPA	-1.78	0.024	Unknown	
2291	87.74	470.3249	4 days	VPA	-0.18	0.002	Unknown	
2244	30.91	467.2631	8 days	VPA	1.69	0.004	Unknown	
743	13.81	197.0186	8day	VPA	-1.39	0.016	Unknown	

cpdID	RT	MASSavg	time	trt	Fold	P-value	Compound 1	Compound2
636	42.18	178.0994	8 days		1.47	**0.010**	Unknown	

Table 6: Cellular metabolites measured in neural precursors derived from hESells treated with 1mM of valproate

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	36.648	102.0322438	2d	NS 1mM VPA	-2.23119	2-Ketobutyric acid	Acetoacetic acid
NS 1mM VPA	36.648	102.0322438	4d	NS 1mM VPA	-1.83846	2-Ketobutyric acid	Acetoacetic acid
NS 1mM VPA	9.33225	119.958645	4d	NS 1mM VPA	6.98086	Unknown	
NS 1mM VPA	12.2841	121.0621387	4d	NS 1mM VPA	3.502341	Unknown	
NS 1mM VPA	24.10558	125.0838833	2d	NS 1mM VPA	1.79316	Unknown	
NS 1mM VPA	30.43985	125.08394	2d	NS 1mM VPA	1.529012	1-Methylhistamine	
NS 1mM VPA	30.43985	125.08394	4d	NS 1mM VPA	1.576622	1-Methylhistamine	
NS 1mM VPA	23.33772	129.0573222	2d	NS 1mM VPA	1.543375	Pyroglutamic acid	
NS 1mM VPA	23.33772	129.0573222	4d	NS 1mM VPA	1.663008	Pyroglutamic acid	
NS 1mM VPA	12.13216	131.0941359	4d	NS 1mM VPA	1.577796	L-Isoleucine	Aminocaproic acid
NS 1mM VPA	12.13216	131.0941359	2d	NS 1mM VPA	2.474877	L-Isoleucine	Aminocaproic acid
NS 1mM VPA	8.881211	136.0366263	2d	NS 1mM VPA	2.287439	Erythronic acid	Erythronic acid
NS 1mM VPA	8.881211	136.0366263	4d	NS 1mM VPA	2.653537	Erythronic acid	Erythronic acid

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	12.00967	136.0376917	2d	NS 1mM VPA	2.346054	Erythronic acid	Erythronic acid
NS 1mM VPA	12.00967	136.0376917	4d	NS 1mM VPA	2.914393	Erythronic acid	Erythronic acid
NS 1mM VPA	3.9669	138.04396	2d	NS 1mM VPA	1.521407	Urocanic acid	Nicotinamide N-oxide
NS 1mM VPA	3.9669	138.04396	4d	NS 1mM VPA	2.642558	Urocanic acid	Nicotinamide N-oxide
NS 1mM VPA	4.28225	141.9392625	2d	NS 1mM VPA	1.077336	5,10-Methylenetetrahydrofolate	
NS 1mM VPA	4.28225	141.9392625	4d	NS 1mM VPA	1.111532	5,10-Methylenetetrahydrofolate	
NS 1mM VPA	23.33784	143.0734947	2d	NS 1mM VPA	1.728349	Unknown	
NS 1mM VPA	23.33784	143.0734947	4d	NS 1mM VPA	1.986515	Unknown	
NS 1mM VPA	55.5845	144.1153313	4d	NS 1mM VPA	10.83178	Caprylic acid	Valproic acid
NS 1mM VPA	55.5845	144.1153313	2d	NS 1mM VPA	11.64535	Caprylic acid	Valproic acid
NS 1mM VPA	5.609182	145.1572818	2d	NS 1mM VPA	1.00993	Spermidine	
NS 1mM VPA	5.609182	145.1572818	4d	NS 1mM VPA	1.117314	Spermidine	
NS 1mM VPA	33.6357	148.03738	4d	NS 1mM VPA	-5.75294	Citramalic acid	Hydroxyglutaric acid
NS 1mM VPA	33.6357	148.03738	2d	NS 1mM VPA	-1.49356	Citramalic acid	Hydroxyglutaric acid
NS 1mM VPA	62.42614	152.1201762	4d	NS 1mM VPA	2.50602	Unknown	
NS 1mM VPA	62.42614	152.1201762	2d	NS 1mM VPA	3.371426	Unknown	
NS 1mM VPA	8.862333	158.0177333	2d	NS 1mM VPA	1.596402	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	8.862333	158.0177333	4d	NS 1mM VPA	2.236076	Unknown	
NS 1mM VPA	8.269857	158.1374571	4d	NS 1mM VPA	3.106829	Unknown	
NS 1mM VPA	8.269857	158.1374571	2d	NS 1mM VPA	3.626498	Unknown	
NS 1mM VPA	10.07033	159.0688667	2d	NS 1mM VPA	-2.87026	Indoleacetaldehyde	
NS 1mM VPA	10.07033	159.0688667	4d	NS 1mM VPA	-1.35298	Indoleacetaldehyde	
NS 1mM VPA	12.85888	161.0509118	2d	NS 1mM VPA	2.601443	Unknown	
NS 1mM VPA	12.85888	161.0509118	4d	NS 1mM VPA	5.136057	Unknown	
NS 1mM VPA	6.713565	166.0840609	4d	NS 1mM VPA	33.80296	Unknown	
NS 1mM VPA	6.713565	166.0840609	2d	NS 1mM VPA	90.97629	Unknown	
NS 1mM VPA	23.58909	168.0687909	2d	NS 1mM VPA	4.649885	Unknown	
NS 1mM VPA	23.58909	168.0687909	4d	NS 1mM VPA	5.02165	Unknown	
NS 1mM VPA	31.08471	171.1250706	4d	NS 1mM VPA	1.626943	Unknown	
NS 1mM VPA	62.57554	172.1454	4d	NS 1mM VPA	1.745543	Capric acid	Decanoic acid
NS 1mM VPA	62.57554	172.1454	2d	NS 1mM VPA	1.8794	Capric acid	Decanoic acid
NS 1mM VPA	20.68721	175.0830857	2d	NS 1mM VPA	1.153935	N-Carboxyethyl-gamma-aminobutyric acid	
NS 1mM VPA	20.68721	175.0830857	4d	NS 1mM VPA	2.294392	N-Carboxyethyl-gamma-aminobutyric acid	
NS 1mM VPA	41.71109	176.0946	2d	NS 1mM VPA	-1.60014	Serotonin	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	41.71109	176.0946	4d	NS 1mM VPA	-1.23797	Serotonin	
NS 1mM VPA	25.29	177.0469231	2d	NS 1mM VPA	3.379693	N-Formyl-L-methionine	
NS 1mM VPA	25.29	177.0469231	4d	NS 1mM VPA	3.82485	N-Formyl-L-methionine	
NS 1mM VPA	26.75621	177.0789684	2d	NS 1mM VPA	1.26513	5-Hydroxytryptophol	
NS 1mM VPA	26.75621	177.0789684	4d	NS 1mM VPA	1.423219	5-Hydroxytryptophol	
NS 1mM VPA	8.503333	177.113375	2d	NS 1mM VPA	-6.74695	Unknown	
NS 1mM VPA	8.503333	177.113375	4d	NS 1mM VPA	-2.88736	Unknown	
NS 1mM VPA	27.53982	179.0938118	2d	NS 1mM VPA	-2.71897	Salsolinol	Homophenylalanine
NS 1mM VPA	27.53982	179.0938118	4d	NS 1mM VPA	-1.71231	Salsolinol	Homophenylalanine
NS 1mM VPA	55.15089	179.0949632	4d	NS 1mM VPA	-1.64525	Salsolinol	Homophenylalanine
NS 1mM VPA	55.15089	179.0949632	2d	NS 1mM VPA	-1.39458	Salsolinol	Homophenylalanine
NS 1mM VPA	37.08443	185.1406571	4d	NS 1mM VPA	-2.39254	Unknown	
NS 1mM VPA	23.33726	187.0635211	2d	NS 1mM VPA	1.674013	Unknown	
NS 1mM VPA	23.33726	187.0635211	4d	NS 1mM VPA	1.921765	Unknown	
NS 1mM VPA	28.77111	187.1206333	2d	NS 1mM VPA	2.457813	8-Amino-7-oxononanoic acid	
NS 1mM VPA	28.77111	187.1206333	4d	NS 1mM VPA	3.559361	8-Amino-7-oxononanoic acid	
NS 1mM VPA	62.50765	190.1720118	4d	NS 1mM VPA	1.758553	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	62.50765	190.1720118	2d	NS 1mM VPA	1.940004	Unknown	
NS 1mM VPA	7.850167	196.0933333	4d	NS 1mM VPA	1.937281	Unknown	
NS 1mM VPA	7.850167	196.0933333	2d	NS 1mM VPA	6.390957	Unknown	
NS 1mM VPA	45.36418	197.1060727	2d	NS 1mM VPA	1.280472	L-Metanephrine	
NS 1mM VPA	45.36418	197.1060727	4d	NS 1mM VPA	1.62879	L-Metanephrine	
NS 1mM VPA	8.363925	199.0952975	2d	NS 1mM VPA	10.44025	Unknown	
NS 1mM VPA	8.363925	199.0952975	4d	NS 1mM VPA	10.88407	Unknown	
NS 1mM VPA	22.22829	206.06375	4d	NS 1mM VPA	2.263839	Unknown	
NS 1mM VPA	22.22829	206.06375	2d	NS 1mM VPA	4.317383	Unknown	
NS 1mM VPA	62.46678	208.1829217	4d	NS 1mM VPA	2.052914	Unknown	
NS 1mM VPA	62.46678	208.1829217	2d	NS 1mM VPA	2.734885	Unknown	
NS 1mM VPA	9.2636	211.0349075	4d	NS 1mM VPA	1.516795	Creatine phosphate	
NS 1mM VPA	9.2636	211.0349075	2d	NS 1mM VPA	1.893074	Creatine phosphate	
NS 1mM VPA	44.11333	212.1400167	4d	NS 1mM VPA	-9.35257	Unknown	
NS 1mM VPA	44.11333	212.1400167	2d	NS 1mM VPA	-6.85493	Unknown	
NS 1mM VPA	7.746115	217.1048885	2d	NS 1mM VPA	2.916422	N-a-Acetylcitrulline	
NS 1mM VPA	7.746115	217.1048885	4d	NS 1mM VPA	23.86569	N-a-Acetylcitrulline	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	22.26729	217.1307097	2d	NS 1mM VPA	2.122093	Propionylcarnitine	
NS 1mM VPA	22.26729	217.1307097	4d	NS 1mM VPA	2.236406	Propionylcarnitine	
NS 1mM VPA	16.1278	220.0841	2d	NS 1mM VPA	-1.25214	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1mM VPA	16.1278	220.0841	4d	NS 1mM VPA	1.215413	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1mM VPA	9.809786	220.0845	2d	NS 1mM VPA	-1.06102	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1mM VPA	9.809786	220.0845	4d	NS 1mM VPA	1.371126	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1mM VPA	12.15958	220.0845895	2d	NS 1mM VPA	-1.54275	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1mM VPA	12.15958	220.0845895	4d	NS 1mM VPA	1.162644	5-Hydroxytryptophan	5-Hydroxy-L-tryptophan
NS 1mM VPA	8.4172	223.92951	2d	NS 1mM VPA	-3.24844	Unknown	
NS 1mM VPA	8.4172	223.92951	4d	NS 1mM VPA	-2.85631	Unknown	
NS 1mM VPA	22.009	225.62685	4d	NS 1mM VPA	2.716119	Unknown	
NS 1mM VPA	22.009	225.62685	2d	NS 1mM VPA	3.854852	Unknown	
NS 1mM VPA	10.0963	227.01938	4d	NS 1mM VPA	1.631698	L-Glutamic acid 5-phosphate	
NS 1mM VPA	6.0771	227.09052	4d	NS 1mM VPA	-5.41292	Deoxycytidine	
NS 1mM VPA	6.0771	227.09052	2d	NS 1mM VPA	-2.98012	Deoxycytidine	
NS 1mM VPA	14.51476	228.05894	4d	NS 1mM VPA	3.339111	Unknown	
NS 1mM VPA	14.51476	228.05894	2d	NS 1mM VPA	6.425869	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	67.13919	230.1515667	4d	NS 1mM VPA	-5.02528	Dodecanedioic acid	
NS 1mM VPA	67.13919	230.1515667	2d	NS 1mM VPA	-2.98776	Dodecanedioic acid	
NS 1mM VPA	19.28286	234.1010143	2d	NS 1mM VPA	-1.25569	5-Methoxytryptophan	
NS 1mM VPA	19.28286	234.1010143	4d	NS 1mM VPA	-1.18869	5-Methoxytryptophan	
NS 1mM VPA	10.51438	236.0815625	4d	NS 1mM VPA	1.367658	N'-Formylkynurenine	
NS 1mM VPA	17.826	238.0864167	2d	NS 1mM VPA	5.397657	Propanoic acid	
NS 1mM VPA	17.826	238.0864167	4d	NS 1mM VPA	5.703771	Propanoic acid	
NS 1mM VPA	7.8115	239.087425	4d	NS 1mM VPA	1.950568	Unknown	
NS 1mM VPA	7.8115	239.087425	2d	NS 1mM VPA	30.57925	Unknown	
NS 1mM VPA	42.05716	246.1469838	4d	NS 1mM VPA	-2.28801	3-Hydroxydodecanedioic acid	
NS 1mM VPA	42.05716	246.1469838	2d	NS 1mM VPA	-1.71537	3-Hydroxydodecanedioic acid	
NS 1mM VPA	30.669	256.09664	4d	NS 1mM VPA	1.692487	Aryl beta-D-glucoside	
NS 1mM VPA	30.669	256.09664	2d	NS 1mM VPA	1.966122	Aryl beta-D-glucoside	
NS 1mM VPA	59.82681	256.1080938	2d	NS 1mM VPA	2.962348	Unknown	
NS 1mM VPA	59.82681	256.1080938	4d	NS 1mM VPA	3.845884	Unknown	
NS 1mM VPA	69.57173	258.18226	4d	NS 1mM VPA	4.075358	Tetradecanedioic acid	
NS 1mM VPA	69.57173	258.18226	2d	NS 1mM VPA	5.744182	Tetradecanedioic acid	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	22.0274	264.11209	2d	NS 1mM VPA	1.230997	Acetyl-N-formyl-5-methoxykynurenamine	
NS 1mM VPA	22.0274	264.11209	4d	NS 1mM VPA	1.338241	Acetyl-N-formyl-5-methoxykynurenamine	
NS 1mM VPA	11.94583	268.0806	4d	NS 1mM VPA	3.240011	3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid	
NS 1mM VPA	11.94583	268.0806	2d	NS 1mM VPA	3.329815	3-Deoxy-D-glycero-D-galacto-2-nonulosonic acid	
NS 1mM VPA	20.58271	270.1203286	2d	NS 1mM VPA	1.808333	L-gamma-Glutamyl-L-hypoglycin;	
NS 1mM VPA	20.58271	270.1203286	4d	NS 1mM VPA	1.890677	L-gamma-Glutamyl-L-hypoglycin;	
NS 1mM VPA	56.5351	272.08566	4d	NS 1mM VPA	1.178071	5-S-Cysteinyldopamine	
NS 1mM VPA	56.5351	272.08566	2d	NS 1mM VPA	1.718998	5-S-Cysteinyldopamine	
NS 1mM VPA	64.11407	278.0251024	4d	NS 1mM VPA	5.17024	Unknown	
NS 1mM VPA	64.11407	278.0251024	2d	NS 1mM VPA	6.667641	Unknown	
NS 1mM VPA	28.90879	290.1501643	4d	NS 1mM VPA	3.329013	Unknown	
NS 1mM VPA	28.90879	290.1501643	2d	NS 1mM VPA	8.490919	Unknown	
NS 1mM VPA	26.42889	295.1063913	2d	NS 1mM VPA	7.265582	Unknown	
NS 1mM VPA	26.42889	295.1063913	4d	NS 1mM VPA	8.896581	Unknown	
NS 1mM VPA	73.28533	315.2406111	4d	NS 1mM VPA	2.599877	Decanoylcarnitine	
NS 1mM VPA	73.28533	315.2406111	2d	NS 1mM VPA	3.899691	Decanoylcarnitine	
NS 1mM VPA	77.38144	318.2193688	4d	NS 1mM VPA	1.932263	Leukotriene A4	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	77.38144	318.2193688	2d	NS 1mM VPA	2.342543	Leukotriene A4	
NS 1mM VPA	41.33024	324.1144588	4d	NS 1mM VPA	3.317923	Acetohexamide	
NS 1mM VPA	41.33024	324.1144588	2d	NS 1mM VPA	3.713445	Acetohexamide	
NS 1mM VPA	33.60576	330.1013765	4d	NS 1mM VPA	2.25561	Unknown	
NS 1mM VPA	33.60576	330.1013765	2d	NS 1mM VPA	2.310447	Unknown	
NS 1mM VPA	35.06233	331.1049867	4d	NS 1mM VPA	2.719086	Unknown	
NS 1mM VPA	35.06233	331.1049867	2d	NS 1mM VPA	3.041317	Unknown	
NS 1mM VPA	52.557	349.22592	4d	NS 1mM VPA	2.41993	Unknown	
NS 1mM VPA	52.557	349.22592	2d	NS 1mM VPA	6.652089	Unknown	
NS 1mM VPA	53.57858	350.2096	4d	NS 1mM VPA	1.508076	Prostaglandin E3	
NS 1mM VPA	53.57858	350.2096	2d	NS 1mM VPA	1.612834	Prostaglandin E3	
NS 1mM VPA	65.76353	356.2702895	4d	NS 1mM VPA	2.05875	Tetracosahexaenoic acid	
NS 1mM VPA	65.76353	356.2702895	2d	NS 1mM VPA	2.405825	Tetracosahexaenoic acid	
NS 1mM VPA	81.79271	369.2880824	4d	NS 1mM VPA	6.636435	cis-5-Tetradecenoylcarnitine	
NS 1mM VPA	81.79271	369.2880824	2d	NS 1mM VPA	9.965816	cis-5-Tetradecenoylcarnitine	
NS 1mM VPA	27.34076	374.1222235	4d	NS 1mM VPA	2.300022	Unknown	
NS 1mM VPA	27.34076	374.1222235	2d	NS 1mM VPA	2.889783	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	8.881	380.164	4d	NS 1mM VPA	2.519949	Unknown	
NS 1mM VPA	8.881	380.164	2d	NS 1mM VPA	2.633667	Unknown	
NS 1mM VPA	22.46583	385.1025667	4d	NS 1mM VPA	1.45497	S-Inosyl-L-homocysteine	
NS 1mM VPA	22.46583	385.1025667	2d	NS 1mM VPA	1.533705	S-Inosyl-L-homocysteine	
NS 1mM VPA	68.31689	386.23145	4d	NS 1mM VPA	6.386163	1-tridecanoyl-sn-glycero-3-phosphate	
NS 1mM VPA	68.31689	386.23145	2d	NS 1mM VPA	7.117538	1-tridecanoyl-sn-glycero-3-phosphate	
NS 1mM VPA	89.1946	390.27608	2d	NS 1mM VPA	1.682855	7-Hydroxy-3-oxocholanoic acid	
NS 1mM VPA	89.1946	390.27608	4d	NS 1mM VPA	3.296512	7-Hydroxy-3-oxocholanoic acid	
NS 1mM VPA	26.42904	394.2127308	2d	NS 1mM VPA	2.670186	Unknown	
NS 1mM VPA	26.42904	394.2127308	4d	NS 1mM VPA	3.50273	unknown	
NS 1mM VPA	76.06971	398.2439857	4d	NS 1mM VPA	2.845315	Unknown	
NS 1mM VPA	76.06971	398.2439857	2d	NS 1mM VPA	3.609136	Unknown	
NS 1mM VPA	33.53038	399.2100125	2d	NS 1mM VPA	6.345173	unknown	
NS 1mM VPA	33.53038	399.2100125	4d	NS 1mM VPA	8.777833	unknown	
NS 1mM VPA	8.9305	406.1058875	4d	NS 1mM VPA	1.976577	unknown	
NS 1mM VPA	8.9305	406.1058875	2d	NS 1mM VPA	2.00634	unknown	
NS 1mM VPA	65.76447	409.3155632	4d	NS 1mM VPA	4.662331	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	65.76447	409.3155632	2d	NS 1mM VPA	5.930421	Unknown	
NS 1mM VPA	18.93053	416.0834333	4d	NS 1mM VPA	3.527468	Unknown	
NS 1mM VPA	18.93053	416.0834333	2d	NS 1mM VPA	4.202864	Unknown	
NS 1mM VPA	8.6127	416.20208	2d	NS 1mM VPA	3.29783	Lactone	
NS 1mM VPA	8.6127	416.20208	4d	NS 1mM VPA	3.495266	Lactone	
NS 1mM VPA	14.963	420.05275	2d	NS 1mM VPA	-3.434	Unknown	
NS 1mM VPA	14.963	420.05275	4d	NS 1mM VPA	-3.09195	Unknown	
NS 1mM VPA	62.93221	427.1025357	2d	NS 1mM VPA	3.882014	Unknown	
NS 1mM VPA	62.93221	427.1025357	4d	NS 1mM VPA	6.045912	Unknown	
NS 1mM VPA	33.59771	430.1185143	2d	NS 1mM VPA	3.469797	N-Ethylmaleimide-S-glutathione	
NS 1mM VPA	33.59771	430.1185143	4d	NS 1mM VPA	3.98295	N-Ethylmaleimide-S-glutathione	
NS 1mM VPA	24.9718	434.19845	4d	NS 1mM VPA	3.713915	Unknown	
NS 1mM VPA	24.9718	434.19845	2d	NS 1mM VPA	3.76882	Unknown	
NS 1mM VPA	23.07	434.1985875	2d	NS 1mM VPA	2.416021	Unknown	
NS 1mM VPA	23.07	434.1985875	4d	NS 1mM VPA	3.44524	Unknown	
NS 1mM VPA	37.33089	438.1460579	2d	NS 1mM VPA	2.829002	Unknown	
NS 1mM VPA	37.33089	438.1460579	4d	NS 1mM VPA	3.253909	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	5.410909	441.9424	4d	NS 1mM VPA	-3.5345	Unknown	
NS 1mM VPA	5.410909	441.9424	2d	NS 1mM VPA	-2.45689	Unknown	
NS 1mM VPA	34.58505	443.2362947	2d	NS 1mM VPA	2.648862	Unknown	
NS 1mM VPA	34.58505	443.2362947	4d	NS 1mM VPA	3.427563	Unknown	
NS 1mM VPA	33.85267	445.1694	2d	NS 1mM VPA	-1.31487	Tetrahydrofolic acid	Tetrahydrofolate
NS 1mM VPA	33.85267	445.1694	4d	NS 1mM VPA	-1.04073	Tetrahydrofolic acid	Tetrahydrofolate
NS 1mM VPA	38.63717	449.1638333	2d	NS 1mM VPA	3.04704	Unknown	
NS 1mM VPA	38.63717	449.1638333	4d	NS 1mM VPA	4.206864	Unknown	
NS 1mM VPA	21.9772	456.2448	4d	NS 1mM VPA	4.145738	unknown	
NS 1mM VPA	21.9772	456.2448	2d	NS 1mM VPA	8.390862	unknown	
NS 1mM VPA	42.40369	467.1731077	2d	NS 1mM VPA	-10.3323	Unknown	
NS 1mM VPA	42.40369	467.1731077	4d	NS 1mM VPA	-4.42263	Unknown	
NS 1mM VPA	23.41189	474.1090778	4d	NS 1mM VPA	2.094815	Unknown	
NS 1mM VPA	23.41189	474.1090778	2d	NS 1mM VPA	2.928466	Unknown	
NS 1mM VPA	22.666	482.1554563	4d	NS 1mM VPA	2.075721	Unknown	
NS 1mM VPA	22.666	482.1554563	2d	NS 1mM VPA	2.519832	Unknown	
NS 1mM VPA	75.55014	493.3252405	4d	NS 1mM VPA	2.09415	1-(9E-hexadecenoyl)-sn-glycero-3-phosphocholine	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	75.55014	493.3252405	2d	NS 1mM VPA	2.34894	1-(9E-hexadecenoyl)-sn-glycero-3-phosphocholine	
NS 1mM VPA	37.34533	506.1856556	2d	NS 1mM VPA	2.031088	Unknown	
NS 1mM VPA	37.34533	506.1856556	4d	NS 1mM VPA	2.405509	unknown	
NS 1mM VPA	23.67792	514.162208	2d	NS 1mM VPA	2.2744	unknown	
NS 1mM VPA	23.67792	514.162208	4d	NS 1mM VPA	3.27837	unknown	
NS 1mM VPA	36.44195	514.2430667	4d	NS 1mM VPA	4.332807	Unknown	
NS 1mM VPA	36.44195	514.2430667	2d	NS 1mM VPA	4.565629	Unknown	
NS 1mM VPA	41.29621	527.3552786	2d	NS 1mM VPA	9.44893	Unknown	
NS 1mM VPA	41.29621	527.3552786	4d	NS 1mM VPA	12.3251	Unknown	
NS 1mM VPA	21.94381	534.2784846	4d	NS 1mM VPA	2.818876	unknown	
NS 1mM VPA	21.94381	534.2784846	2d	NS 1mM VPA	2.912693	unknown	
NS 1mM VPA	26.05061	546.3146929	2d	NS 1mM VPA	1.574871	Unknown	
NS 1mM VPA	26.05061	546.3146929	4d	NS 1mM VPA	3.130802	Unknown	
NS 1mM VPA	25.92929	556.13945	4d	NS 1mM VPA	4.837329	unknown	
NS 1mM VPA	25.92929	556.13945	2d	NS 1mM VPA	5.293236	unknown	
NS 1mM VPA	9.093727	575.1451545	4d	NS 1mM VPA	2.795937	Unknown	
NS 1mM VPA	9.093727	575.1451545	2d	NS 1mM VPA	4.232054	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	36.92372	575.3159222	2d	NS 1mM VPA	2.816601	Unknown	
NS 1mM VPA	36.92372	575.3159222	4d	NS 1mM VPA	3.446719	unknown	
NS 1mM VPA	80.9297	583.44194	2d	NS 1mM VPA	1.503812	Unknown	
NS 1mM VPA	80.9297	583.44194	4d	NS 1mM VPA	4.532225	Unknown	
NS 1mM VPA	4.824	594.2327	4d	NS 1mM VPA	2.212473	Unknown	
NS 1mM VPA	4.824	594.2327	2d	NS 1mM VPA	4.279664	Unknown	
NS 1mM VPA	41.278	632.232825	2d	NS 1mM VPA	3.171377	Unknown	
NS 1mM VPA	41.278	632.232825	4d	NS 1mM VPA	4.764468	Unknown	
NS 1mM VPA	14.97571	659.1506941	4d	NS 1mM VPA	3.054219	Unknown	
NS 1mM VPA	23.39707	660.1513786	2d	NS 1mM VPA	3.542683	Unknown	
NS 1mM VPA	23.39707	660.1513786	4d	NS 1mM VPA	4.100844	Unknown	
NS 1mM VPA	14.884	682.2853	2d	NS 1mM VPA	2.823221	Unknown	
NS 1mM VPA	14.884	682.2853	4d	NS 1mM VPA	5.153298	Unknown	
NS 1mM VPA	33.64471	822.2805429	2d	NS 1mM VPA	2.668941	Unknown	
NS 1mM VPA	33.64471	822.2805429	4d	NS 1mM VPA	3.816104	Unknown	
NS 1mM VPA	83.24742	907.54555	4d	NS 1mM VPA	1.507945	Unknown	
NS 1mM VPA	83.24742	907.54555	2d	NS 1mM VPA	1.586431	Unknown	

EXP	RT	roundMASS	time	trt	Fold	annotation.1	annotation.2
NS 1mM VPA	31.5316	908.22015	2d	NS 1mM VPA	1.640148	Unknown	
NS 1mM VPA	31.5316	908.22015	4d	NS 1mM VPA	1.998983	Unknown	
NS 1mM VPA	33.44333	1028.3246	2d	NS 1mM VPA	5.833998	Unknown	
NS 1mM VPA	33.44333	1028.3246	4d	NS 1mM VPA	8.654592	Unknown	
NS 1mM VPA	4.649125	1291.75965	2d	NS 1mM VPA	1.739338	beta-D-Glucosyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenol	
NS 1mM VPA	4.649125	1291.75965	4d	NS 1mM VPA	1.793695	beta-D-Glucosyl-1,4-N-acetyl-D-glucosaminyldiphosphoundecaprenol	

Table 7: Cellular metabolites measured in hES cells treated with alcohol

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	15.48433	99.0689	4D	1.434154	0.034571	N-Methyl-2-pyrrolidinone	
ETOH 0.1	52.01225	99.1043	4D	2.703447	0.012638	Unknown	
ETOH 0.1	13.40565	120.2112	4D	4.847027	0.029776	Unknown	
ETOH 0.1	16.73904	129.0452	24H	1.502328	0.002871	3,4-Dihydroxybutyric acid	
ETOH 0.1	88.64043	130.9541	24H	1.631614	0.046779	Unknown	
ETOH 0.1	22.22892	131.0746	24H	-1.85703	0.037466	3-Methylindole	
ETOH 0.1	14.35336	131.076	4D	3.62907	0.014778	Unknown	
ETOH 0.1	3.958833	148.0052	24H	-1.94841	0.034059	Unknown	
ETOH 0.1	52.88652	148.016	4D	-2.44409	0.047122	2-Oxo-4-methylthiobutanoic acid	
ETOH 0.1	19.18355	168.0434	4D	-1.46785	0.019426	Homogentisic acid	Vanillic acid
ETOH 0.1	26.70635	171.1244	24H	2.376107	0.008413	GABA analogue	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	22.17997	187.1343	24H	1.48782	0.045452	(+/-)-2-(4'-Isobutylphenyl)propionitrile	
ETOH 0.1	46.31086	187.1348	4D	2.329144	4.21E-05	(+/-)-2-(4'-Isobutylphenyl)propionitrile	
ETOH 0.1	5.935143	194.073	4D	-1.38924	0.003217	Phenanthrene-9,10-oxide	
ETOH 0.1	31.19917	195.124	4D	-2.22499	0.004386	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	a-[1-(ethylamino)ethyl]-p-hydroxy-Benzyl alcohol
ETOH 0.1	38.99212	195.1253	4D	2.158606	0.00953	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	a-[1-(ethylamino)ethyl]-p-hydroxy-Benzyl alcohol
ETOH 0.1	48.37093	197.1769	4D	-3.11904	0.016197	Unknown	
ETOH 0.1	9.675726	203.1138	4D	-1.70728	0.023636	Acetylcarnitine	L-Glutamic acid n-butyl ester
ETOH 0.1	6.747279	205.1304	4D	-1.2578	0.005318	Pantothenol	dimethylbutanamide
ETOH 0.1	36.18938	210.0922	4D	1.864256	0.040527	3-(2,5-Dimethoxy phenylpropionic acid	
ETOH 0.1	24.52067	229.0949	24H	-2.33044	0.008877	Malonylcarnitine	
ETOH 0.1	17.7027	243.1089	4D	-2.18071	0.016141	Unknown	
ETOH 0.1	64.66999	266.1613	24H	-1.51898	0.047652	Unknown	
ETOH 0.1	42.3656	268.2487	4D	-1.54971	0.015019	Unknown	
ETOH 0.1	4.86619	271.9364	24H	2.629339	0.04245	Unknown	
ETOH 0.1	43.99398	272.16	24H	1.929598	0.032191	Unknown	
ETOH 0.1	43.99398	272.16	4D	2.186768	0.003018	Unknown	
ETOH 0.1	63.00428	285.2285	24H	4.002774	0.018095	Unknown	
ETOH 0.1	37.97297	292.1862	4D	2.239381	0.0496	Unknown	
ETOH 0.1	4.014175	293.9773	4D	1.938848	0.036775	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	6.160071	294.0957	24H	1.269183	0.005089	Unknown	
ETOH 0.1	8.967061	295.1521	4D	1.513407	0.042025	Unknown	
ETOH 0.1	71.55535	296.2308	24H	3.545035	0.003758	Unknown	
ETOH 0.1	50.75387	298.174	4D	-3.00237	0.045113	Unknown	
ETOH 0.1	18.88505	300.1147	4D	2.686262	0.023485	Unknown	
ETOH 0.1	17.18696	300.1656	24H	1.548853	0.036582	Unknown	
ETOH 0.1	7.719471	301.1345	4D	6.648828	0.030822	Unknown	
ETOH 0.1	15.22357	312.1341	4D	-2.01503	0.041156	Unknown	
ETOH 0.1	26.51229	315.6732	4D	2.014609	0.010998	Unknown	
ETOH 0.1	18.82373	325.2711	4D	-1.75625	0.013853	Unknown	
ETOH 0.1	20.94557	325.2714	4D	-2.07426	0.00333	Unknown	
ETOH 0.1	8.542672	337.2012	24H	1.402499	0.000372	Unknown	
ETOH 0.1	3.85935	353.2765	4D	2.622059	0.002006	Unknown	
ETOH 0.1	25.16993	357.1781	4D	2.217294	0.001346	Unknown	
ETOH 0.1	24.01428	359.1532	4D	1.535704	0.033	Unknown	
ETOH 0.1	18.50245	360.1321	4D	2.11023	0.004465	Unknown	
ETOH 0.1	83.72506	362.2787	24H	2.819814	0.04795	Unknown	
ETOH 0.1	83.72506	362.2787	4D	2.916423	0.023844	Unknown	
ETOH 0.1	27.98054	368.2122	4D	1.69537	0.02565	Unknown	
ETOH 0.1	20.76168	379.1771	24H	-1.72285	0.021135	Unknown	
ETOH 0.1	20.76168	379.1771	4D	1.539861	0.019592	Unknown	
ETOH 0.1	15.20829	383.1721	4D	1.914543	0.043581	Unknown	
ETOH 0.1	23.38956	384.2127	4D	-2.55567	0.025704	Unknown	
ETOH 0.1	51.65871	386.1724	4D	5.308852	0.032774	(+)-Eudesmin	
ETOH 0.1	19.97914	387.0812	4D	-1.97698	0.024641	Unknown	
ETOH 0.1	19.97914	387.0812	24H	1.739051	0.018391	Unknown	
ETOH 0.1	17.53242	388.1815	24H	-1.44894	0.012322	Unknown	
ETOH 0.1	46.346	388.2349	4D	1.946524	0.002762	Unknown	
ETOH 0.1	15.90129	393.1889	24H	-1.43098	0.022977	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	6.259963	396.1687	24H	1.717607	0.047952	Unknown	
ETOH 0.1	51.66325	403.1978	4D	3.11601	0.048224	Unknown	
ETOH 0.1	30.70733	405.2001	4D	2.668076	0.001676	Unknown	
ETOH 0.1	16.21743	408.1636	4D	1.965641	0.028858	Unknown	
ETOH 0.1	21.14975	417.2386	4D	-1.97972	0.007183	Unknown	
ETOH 0.1	33.09057	417.2338	4D	2.032563	0.016902	Unknown	
ETOH 0.1	26.77212	420.1862	4D	3.282511	0.030236	Unknown	
ETOH 0.1	18.03482	429.2533	4D	-1.80751	0.006213	Unknown	
ETOH 0.1	30.24237	429.2535	4D	1.804876	0.044332	Unknown	
ETOH 0.1	35.58196	431.2501	4D	1.532408	0.037928	Unknown	
ETOH 0.1	32.18393	437.2042	24H	24.53212	0.001124	Unknown	
ETOH 0.1	4.808947	440.0223	4D	-1.52785	0.03034	Unknown	
ETOH 0.1	24.13915	443.2381	4D	2.985557	0.023682	Unknown	
ETOH 0.1	67.0705	443.3216	4D	1.751997	0.037074	Unknown	
ETOH 0.1	51.58546	444.2237	4D	2.130512	0.031074	Unknown	
ETOH 0.1	33.51823	460.9391	4D	-4.51805	0.005949	Unknown	
ETOH 0.1	22.95657	462.2217	24H	-1.98563	0.0437	Unknown	
ETOH 0.1	25.3287	464.225	24H	-1.63071	0.025852	Unknown	
ETOH 0.1	46.51446	467.3804	4D	2.068091	0.042613	Unknown	
ETOH 0.1	51.6158	468.2002	4D	1.875012	0.015762	Unknown	
ETOH 0.1	30.37291	471.1928	4D	2.001387	0.022662	glucuronide	
ETOH 0.1	30.72707	471.7804	4D	-2.83569	0.037476	Unknown	
ETOH 0.1	30.28867	478.2761	4D	1.698899	0.000475	Unknown	
ETOH 0.1	72.7735	482.3062	24H	-1.95925	0.042853	Unknown	
ETOH 0.1	6.676207	485.2069	24H	-2.01419	0.013732	Unknown	
ETOH 0.1	66.73744	487.3472	4D	2.931014	0.007475	Unknown	
ETOH 0.1	21.72729	489.2127	4D	-1.51037	0.0314	Unknown	
ETOH 0.1	31.22083	510.8202	4D	2.488196	0.011267	Unknown	
ETOH 0.1	34.35986	521.9924	24H	-1.44593	0.032994	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	34.57864	525.3161	4D	1.551324	0.037545	Unknown	
ETOH 0.1	51.73057	526.2773	4D	3.445707	0.006877	Unknown	
ETOH 0.1	23.87065	530.314	4D	1.964552	0.006366	L-Oleandrosyl-oleandolide	
ETOH 0.1	32.50661	531.2876	4D	2.106138	0.024689	Unknown	
ETOH 0.1	35.58454	531.3191	4D	-1.25162	0.027019	Unknown	
ETOH 0.1	66.31491	531.3736	4D	3.862674	0.01116	Unknown	
ETOH 0.1	32.24719	541.3274	4D	2.161601	0.038319	Unknown	
ETOH 0.1	17.6573	545.3029	4D	-1.39484	0.043629	Unknown	
ETOH 0.1	31.891	554.8471	4D	2.038489	0.037688	Unknown	
ETOH 0.1	15.78741	555.2406	4D	1.835025	0.014923	Unknown	
ETOH 0.1	5.742094	555.8505	4D	-1.28922	0.017625	Unknown	
ETOH 0.1	88.02533	556.3971	4D	-3.11839	0.016192	Unknown	
ETOH 0.1	31.97996	559.8329	4D	-1.7213	0.049678	Unknown	
ETOH 0.1	24.7039	574.3397	4D	1.58436	0.02116	Unknown	
ETOH 0.1	31.71105	574.3427	4D	1.750055	0.026643	Unknown	
ETOH 0.1	47.90467	576.096	4D	1.361314	0.021201	Unknown	
ETOH 0.1	16.90923	577.2825	24H	1.727637	0.047154	Unknown	
ETOH 0.1	35.26918	589.6938	4D	1.819573	0.027966	Unknown	
ETOH 0.1	31.54325	591.3789	4D	1.578222	0.000714	Unknown	
ETOH 0.1	25.14927	596.3543	4D	1.395808	0.049214	L-Urobilinogen;	
ETOH 0.1	32.67372	603.3535	4D	-2.62952	0.015253	Unknown	
ETOH 0.1	8.056862	612.1509	24H	-1.47366	0.02889	Oxidized glutathione	Oxidized glutathione; Glutathione disulfide; GSSG; Oxiglutatione
ETOH 0.1	33.68866	619.3409	4D	1.856648	0.030722	Unknown	
ETOH 0.1	32.73907	620.8861	4D	2.248558	0.00893	Unknown	
ETOH 0.1	32.08734	635.4065	4D	1.392618	0.030885	Unknown	
ETOH 0.1	5.903429	646.7084	4D	-1.27147	0.020652	Unknown	
ETOH 0.1	26.7452	661.3846	4D	1.295402	0.046573	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	33.48766	677.9101	24H	-2.22083	0.028347	Unknown	
ETOH 0.1	31.11764	693.4124	4D	2.318353	0.028562	Unknown	
ETOH 0.1	28.2045	695.4286	24H	-32.9384	0.027453	Unknown	
ETOH 0.1	33.70266	699.9225	4D	1.719036	0.039493	Unknown	
ETOH 0.1	40.84822	702.2497	4D	-2.76945	0.001031	Neu5Acalpha2-3Galbeta1-4Glcbeta-Sp	Neu5Acalpha2-6Galbeta1-4Glcbeta-Sp
ETOH 0.1	34.62439	707.3928	4D	2.136871	0.0353	Unknown	
ETOH 0.1	56.18269	707.4296	24H	1.489574	0.046762	Unknown	
ETOH 0.1	33.73428	708.9387	4D	2.159654	0.006959	Unknown	
ETOH 0.1	4.826824	711.8344	24H	-3.02053	0.013448	Unknown	
ETOH 0.1	33.89008	730.4494	4D	2.613893	0.009133	Unknown	
ETOH 0.1	47.76987	731.0954	4D	-2.77579	0.004811	Unknown	
ETOH 0.1	5.918027	732.007	4D	1.795393	0.021782	Unknown	
ETOH 0.1	35.028	751.4193	4D	1.87008	0.032616	Unknown	
ETOH 0.1	34.12668	752.4629	4D	2.066515	0.031345	Unknown	
ETOH 0.1	69.32865	765.5211	24H	1.873064	0.033127	Unknown	
ETOH 0.1	34.33545	774.4767	4D	2.103658	0.020363	Unknown	
ETOH 0.1	89.29926	774.5055	4D	-2.40077	0.006755	Unknown	
ETOH 0.1	5.886782	780.241	4D	-1.31403	0.025516	Unknown	
ETOH 0.1	34.52749	796.4891	4D	1.926524	0.049615	Unknown	
ETOH 0.1	34.60124	796.9917	4D	1.818186	0.015806	Unknown	
ETOH 0.1	4.613879	820.8181	4D	-1.47009	0.047535	Unknown	
ETOH 0.1	5.259716	888.8041	4D	-1.45771	0.021932	Unknown	
ETOH 0.1	8.502051	909.5934	24H	2.274264	0.037836	Unknown	
ETOH 0.1	5.217833	913.8074	24H	-1.86064	0.028059	Unknown	
ETOH 0.1	5.399211	921.0025	4D	1.677834	0.001526	Unknown	
ETOH 0.1	3.646902	994.0917	24H	1.441829	0.019979	Unknown	
ETOH 0.1	3.705141	1008.072	4D	1.30378	0.048393	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.1	5.177162	1038.786	4D	1.677834	0.030851	Unknown	
ETOH 0.3	85.57399	83.0372	24H	2.472036	0.010882	Unknown	
ETOH 0.3	15.48433	99.0689	4D	1.337742	0.043286	N-Methyl-2-pyrrolidinone	
ETOH 0.3	15.48433	99.0689	24H	1.467845	0.043638	N-Methyl-2-pyrrolidinone	
ETOH 0.3	52.01225	99.1043	24H	3.331103	0.000378	Unknown	
ETOH 0.3	10.21225	101.1201	24H	2.191927	0.043209	Hexylamine	
ETOH 0.3	4.032816	111.9839	4D	-8.67642	0.018374	Thiosulfate	
ETOH 0.3	3.767232	120.0436	4D	1.936297	0.034585	3,4-Dihydroxybutyric acid	
ETOH 0.3	13.40565	120.2112	4D	3.90007	0.046375	Unknown	
ETOH 0.3	16.73904	129.0452	24H	1.795891	3.32E-05	3,4-Dihydroxybutyric acid	
ETOH 0.3	88.64043	130.9541	24H	2.024969	0.006982	Unknown	
ETOH 0.3	22.22892	131.0746	4D	2.502205	0.049833	3-Methylindole	
ETOH 0.3	14.35336	131.076	4D	4.050219	0.020549	Unknown	
ETOH 0.3	3.958833	148.0052	24H	-1.72967	0.043053	Unknown	
ETOH 0.3	7.479235	149.0511	4D	-1.30477	0.014313	Amino-4methylthiobutyric acid	
ETOH 0.3	27.80141	153.0811	24H	-1.6976	0.025771	Unknown	
ETOH 0.3	5.559732	155.0681	4D	1.637846	0.022817	L-Histidine	4-propionic acid
ETOH 0.3	14.22357	161.0805	24H	-6.43527	0.032474	Unknown	
ETOH 0.3	44.88033	162.0662	4D	1.799131	0.037703	Unknown	
ETOH 0.3	23.6763	167.0941	24H	-2.83	0.012984	3-Methoxytyramine	Phenylephrine
ETOH 0.3	19.18355	168.0434	4D	1.281914	0.028006	Homogentisic acid	Vanillic acid
ETOH 0.3	26.70635	171.1244	24H	3.755227	0.001253	GABA analogue	
ETOH 0.3	20.23014	173.084	24H	-1.43983	0.019446	1,3-Dimethyl-8-isoquinolinol	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	28.52393	178.5546	24H	-2.76389	0.001401	Unknown	
ETOH 0.3	5.935143	194.073	4D	-1.39988	0.002956	Phenanthrene-9,10-oxide	9-Hydroxyphenanthrene; 9-Phenanthrol
ETOH 0.3	22.61355	194.0836	24H	-1.70303	0.030955	Unknown	
ETOH 0.3	31.19917	195.124	4D	-1.86013	0.015911	Benzenemethanol, 2-(2-aminopropoxy)-3-methyl-	a-[1-(ethylamino)ethyl]-p-hydroxy-Benzyl alcohol
ETOH 0.3	19.48063	201.1709	4D	-2.97874	0.009458	Unknown	
ETOH 0.3	6.747279	205.1304	4D	-1.33858	0.014168	Pantothenol	dimethylbutanamide;
ETOH 0.3	36.18938	210.0922	4D	1.849968	0.042032	3-(2,5-Dimethoxy phenylpropionic acid	
ETOH 0.3	6.62669	218.0762	4D	-1.77129	0.047105	Unknown	
ETOH 0.3	27.57188	222.0401	24H	-2.11155	0.040386	Unknown	
ETOH 0.3	13.6845	223.119	24H	-2.73967	0.024694	Unknown	Unknown
ETOH 0.3	24.52067	229.0949	4D	-1.74038	0.010344	Malonylcarnitine	Malonylcarnitine
ETOH 0.3	55.50731	229.1457	4D	-1.53336	0.028395	Unknown	
ETOH 0.3	32.9941	229.2025	24H	-1.37697	0.03113	Unknown	
ETOH 0.3	47.0879	234.125	24H	-1.63184	0.029169	5-Methoxytryptophan	
ETOH 0.3	53.26863	234.1253	4D	-1.62383	0.026291	5-Methoxytryptophan	
ETOH 0.3	3.673694	237.0041	24H	2.989077	0.011016	Unknown	
ETOH 0.3	5.176232	239.9592	24H	1.640005	0.018097	Unknown	
ETOH 0.3	27.39631	243.11	4D	-1.49547	0.024259	Unknown	
ETOH 0.3	6.626769	247.1049	4D	-4.47566	0.039425	Unknown	
ETOH 0.3	9.0276	247.1408	4D	-2.75089	0.000424	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	18.84552	256.1066	4D	-2.14073	0.02288	5-Ethyl-5-(1-methyl-3-carboxypropyl)barbituric acid	
ETOH 0.3	40.75868	267.2543	4D	-1.6481	0.029485	Unknown	
ETOH 0.3	40.75868	267.2543	24H	-1.49599	0.021245	Unknown	
ETOH 0.3	42.3656	268.2487	4D	-1.51708	0.030774	Unknown	
ETOH 0.3	4.86619	271.9364	24H	4.069637	0.02742	Unknown	
ETOH 0.3	22.86183	275.1193	24H	-2.23674	0.044504	Unknown	
ETOH 0.3	5.69776	284.9798	4D	-1.2223	0.026683	Unknown	
ETOH 0.3	14.88092	286.1519	4D	-1.86167	0.033393	Unknown	
ETOH 0.3	75.76147	288.2632	24H	1.96905	0.001271	Unknown	
ETOH 0.3	66.86661	293.1952	4D	-2.15382	0.004487	Unknown	
ETOH 0.3	4.014175	293.9773	4D	-2.26718	0.013307	Unknown	
ETOH 0.3	20.67831	294.1535	4D	-1.42237	0.008416	Unknown	
ETOH 0.3	24.21651	294.1531	24H	-1.89159	0.02267	Unknown	
ETOH 0.3	8.967061	295.1521	4D	1.467032	0.021323	Unknown	
ETOH 0.3	66.35884	298.1537	24H	3.758351	0.016783	Unknown	
ETOH 0.3	19.66398	299.1929	24H	-1.57058	0.041283	Unknown	
ETOH 0.3	7.719471	301.1345	4D	2.678267	0.046654	Unknown	
ETOH 0.3	4.954547	303.8875	24H	2.069669	0.049847	Unknown	
ETOH 0.3	44.09424	313.199	24H	2.291989	0.005146	Unknown	
ETOH 0.3	26.51229	315.6732	4D	-2.86533	0.000573	Unknown	
ETOH 0.3	23.90107	322.1166	4D	-2.4215	0.010241	Unknown	
ETOH 0.3	30.19245	324.1666	24H	-1.59251	0.037632	Unknown	
ETOH 0.3	20.72194	332.1367	4D	-2.077	0.009795	Unknown	
ETOH 0.3	8.542672	337.2012	24H	1.287435	0.004465	Unknown	
ETOH 0.3	5.033976	340.9252	24H	-1.83604	0.037709	Unknown	
ETOH 0.3	5.033976	340.9252	4D	2.624968	0.049915	Unknown	
ETOH 0.3	68.02448	342.1482	24H	-1.85279	0.043066	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	3.85935	353.2765	4D	4.904139	0.000229	Unknown	
ETOH 0.3	18.50245	360.1321	24H	-2.28881	0.005116	Unknown	
ETOH 0.3	83.72506	362.2787	24H	-2.54153	0.005266	Unknown	
ETOH 0.3	20.16372	365.1606	4D	-1.92519	0.027055	Unknown	
ETOH 0.3	11.47109	375.1898	4D	2.131693	0.028486	Unknown	
ETOH 0.3	26.07722	375.1886	4D	-4.75123	0.000746	Unknown	
ETOH 0.3	41.28494	378.2956	24H	1.636485	0.027631	Unknown	
ETOH 0.3	15.20829	383.1721	4D	3.377369	0.002214	Unknown	
ETOH 0.3	51.65871	386.1724	4D	4.85106	0.031786	(+)-Eudesmin	(+)-Eudesmin
ETOH 0.3	19.97914	387.0812	4D	-1.69949	0.015257	Unknown	
ETOH 0.3	46.346	388.2349	4D	-1.53209	0.040255	Unknown	
ETOH 0.3	15.90129	393.1889	24H	-1.51089	0.011717	Unknown	
ETOH 0.3	19.69092	393.1886	4D	-2.05894	0.011258	Unknown	
ETOH 0.3	6.259963	396.1687	4D	2.422002	0.013544	Unknown	
ETOH 0.3	17.27421	403.1984	4D	-2.30266	0.00281	Unknown	
ETOH 0.3	21.14975	417.2386	4D	-1.57309	0.03368	Unknown	
ETOH 0.3	33.09057	417.2338	4D	-1.77978	0.026439	Unknown	
ETOH 0.3	13.35295	420.0513	4D	-1.90198	0.003417	Unknown	
ETOH 0.3	27.46219	421.2201	4D	-2.27332	0.012803	Unknown	
ETOH 0.3	18.03482	429.2533	4D	-2.20091	0.002807	Unknown	
ETOH 0.3	54.46495	440.0284	24H	2.51037	0.011972	Unknown	
ETOH 0.3	30.87201	443.2339	4D	2.171362	0.0186	Unknown	
ETOH 0.3	67.0705	443.3216	4D	1.688451	0.048544	Unknown	
ETOH 0.3	51.58546	444.2237	24H	2.241866	0.044444	Unknown	
ETOH 0.3	51.58546	444.2237	4D	2.030169	0.032667	Unknown	
ETOH 0.3	30.05687	444.2789	24H	-1.83503	0.021764	Unknown	
ETOH 0.3	54.8719	446.0431	24H	-2.22145	0.049006	Unknown	
ETOH 0.3	54.8719	446.0431	4D	1.933882	0.047398	Unknown	
ETOH 0.3	29.86415	447.2509	4D	-1.85819	0.025964	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	29.86415	447.2509	24H	2.10322	0.036254	Unknown	
ETOH 0.3	30.45343	449.2653	4D	-2.41429	0.016795	Unknown	
ETOH 0.3	44.98056	449.2611	24H	-3.47136	0.038457	Unknown	
ETOH 0.3	28.27806	455.2052	24H	-2.50898	0.005338	Unknown	
ETOH 0.3	33.51823	460.9391	4D	-3.60151	0.010558	Unknown	
ETOH 0.3	22.95657	462.2217	4D	1.935626	0.03474	Unknown	
ETOH 0.3	33.5733	463.2914	24H	-1.66521	0.034862	Unknown	
ETOH 0.3	25.3287	464.225	24H	-1.71665	0.033532	Unknown	
ETOH 0.3	30.46172	466.2921	24H	-1.95938	0.012601	Unknown	
ETOH 0.3	33.68894	466.615	24H	-3.27865	0.008212	Unknown	
ETOH 0.3	46.51446	467.3804	24H	-2.13465	0.013009	Unknown	
ETOH 0.3	51.6158	468.2002	4D	1.919859	0.003122	Unknown	
ETOH 0.3	30.37291	471.1928	4D	2.725649	0.008164	glucuronide	
ETOH 0.3	30.72707	471.7804	4D	-3.44069	0.010281	Unknown	
ETOH 0.3	30.28867	478.2761	4D	1.509949	0.018484	Unknown	
ETOH 0.3	10.82859	482.1942	24H	-1.52795	0.033711	Unknown	
ETOH 0.3	72.7735	482.3062	24H	-2.61806	0.0161	Unknown	
ETOH 0.3	10.8217	485.204	24H	2.038065	0.038466	Unknown	
ETOH 0.3	66.73744	487.3472	4D	2.877867	0.020235	Unknown	
ETOH 0.3	30.83472	488.305	24H	-2.18525	0.009407	Unknown	
ETOH 0.3	30.88032	488.8071	24H	-1.91959	0.040672	Unknown	
ETOH 0.3	21.72729	489.2127	4D	2.372158	0.000369	Unknown	
ETOH 0.3	13.78553	502.2258	4D	1.866325	0.010364	Unknown	
ETOH 0.3	18.36887	505.2616	4D	2.008892	0.036368	Unknown	
ETOH 0.3	5.891069	509.6704	4D	1.467845	0.0228	Unknown	
ETOH 0.3	31.20706	510.3182	24H	-2.26373	0.006715	Unknown	
ETOH 0.3	31.22083	510.8202	24H	-2.04514	0.041219	Unknown	
ETOH 0.3	31.22083	510.8202	4D	2.502378	0.010461	Unknown	
ETOH 0.3	46.57666	518.3914	4D	2.288814	0.002533	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	46.57666	518.3914	24H	-2.70682	0.017765	Unknown	
ETOH 0.3	34.35986	521.9924	24H	-1.58403	0.024454	Unknown	
ETOH 0.3	31.53434	523.8187	24H	-2.17272	0.02888	Unknown	
ETOH 0.3	51.73057	526.2773	4D	2.714525	0.010415	Unknown	
ETOH 0.3	71.36012	528.3631	4D	2.361003	0.026297	Unknown	
ETOH 0.3	23.87065	530.314	4D	2.211921	0.001298	L-Oleandrosyl-oleandolide	
ETOH 0.3	32.50661	531.2876	4D	2.36084	0.014947	Unknown	
ETOH 0.3	35.58454	531.3191	4D	-3.0409	0.000281	Unknown	
ETOH 0.3	66.31491	531.3736	4D	2.87388	0.031879	Unknown	
ETOH 0.3	31.58889	532.8335	24H	-2.16926	0.015971	Unknown	
ETOH 0.3	51.52268	539.4374	24H	-1.92987	0.031525	Unknown	
ETOH 0.3	32.24719	541.3274	24H	-2.12255	0.01445	Unknown	
ETOH 0.3	31.8519	554.3444	24H	-2.36953	0.00655	Unknown	
ETOH 0.3	31.891	554.8471	24H	-2.29708	0.010734	Unknown	
ETOH 0.3	15.78741	555.2406	4D	2.470837	0.001092	Unknown	
ETOH 0.3	5.742094	555.8505	4D	-1.3396	0.023948	Unknown	
ETOH 0.3	5.742094	555.8505	24H	-1.51803	0.031838	Unknown	
ETOH 0.3	88.02533	556.3971	4D	-2.38386	0.035829	Unknown	
ETOH 0.3	31.97996	559.8329	4D	-2.56596	0.002155	Unknown	
ETOH 0.3	14.9927	566.2265	4D	1.624054	0.006806	Unknown	
ETOH 0.3	24.7039	574.3397	4D	1.523934	0.011558	Unknown	
ETOH 0.3	47.90467	576.096	4D	1.557573	0.020904	Unknown	
ETOH 0.3	32.15777	576.3582	24H	-2.20886	0.012421	Unknown	
ETOH 0.3	16.90923	577.2825	4D	-8.91726	0.02781	Unknown	
ETOH 0.3	5.903169	579.2519	4D	-2.11184	0.001075	Ethanesulfonic acid	
ETOH 0.3	5.903169	579.2519	24H	-1.48021	0.01083	Ethanesulfonic acid	
ETOH 0.3	31.54325	591.3789	24H	-1.98165	0.010883	Unknown	
ETOH 0.3	25.14927	596.3543	4D	1.545421	0.015277	L-Urobilinogen;	
ETOH 0.3	25.14927	596.3543	24H	1.834898	0.021555	L-Urobilinogen;	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	32.67372	603.3535	4D	-2.93997	0.007904	Unknown	
ETOH 0.3	56.3513	611.4952	24H	-1.9811	0.007557	Unknown	
ETOH 0.3	4.936704	611.8727	4D	2.009588	0.018619	Unknown	
ETOH 0.3	32.70236	611.871	24H	-2.19208	0.044039	Unknown	
ETOH 0.3	8.056862	612.1509	24H	-1.59538	0.010611	Oxidized glutathione	
ETOH 0.3	33.68866	619.3409	4D	2.125939	0.015211	Unknown	
ETOH 0.3	32.73907	620.8861	24H	-2.32076	0.039661	Unknown	
ETOH 0.3	32.08734	635.4065	4D	1.394744	0.031645	Unknown	
ETOH 0.3	32.95522	642.397	24H	-2.21146	0.035834	Unknown	
ETOH 0.3	5.903429	646.7084	4D	2.946495	2.66E-05	Unknown	
ETOH 0.3	8.787827	658.2544	4D	-3.20961	0.030138	Unknown	
ETOH 0.3	26.7452	661.3846	4D	1.546707	0.010714	Unknown	
ETOH 0.3	16.3338	666.3836	24H	-1.37135	0.037278	Unknown	
ETOH 0.3	33.48766	677.9101	24H	-2.64653	0.009907	Unknown	
ETOH 0.3	40.84822	702.2497	24H	1.283337	0.019733	Neu5Acalpha2-3Galbeta1-4Glcbeta-Sp	
ETOH 0.3	40.84822	702.2497	4D	-2.21591	0.004389	Neu5Acalpha2-3Galbeta1-4Glcbeta-Sp	
ETOH 0.3	30.43889	707.3933	24H	-1.79166	0.048232	Unknown	
ETOH 0.3	56.18269	707.4296	24H	-2.43361	0.009442	Unknown	
ETOH 0.3	4.826824	711.8344	24H	-2.25605	0.017007	Unknown	
ETOH 0.3	47.76987	731.0954	4D	-3.25036	0.027339	Unknown	
ETOH 0.3	5.918027	732.007	4D	1.76125	0.038454	Unknown	
ETOH 0.3	35.028	751.4193	24H	-1.70279	0.045682	Unknown	
ETOH 0.3	35.028	751.4193	4D	1.845101	0.0375	Unknown	
ETOH 0.3	69.32865	765.5211	24H	1.884393	0.027105	Unknown	
ETOH 0.3	34.33545	774.4767	24H	-2.34161	0.036388	Unknown	
ETOH 0.3	34.60124	796.9917	4D	1.688919	0.047852	Unknown	

Experiment	Retention time	Mass	Time	Fold	p-value	Compound 1	Compound 2
ETOH 0.3	4.613879	820.8181	24H	-1.85112	0.038895	Unknown	
ETOH 0.3	4.613879	820.8181	4D	-1.57113	0.014605	Unknown	
ETOH 0.3	5.259716	888.8041	24H	1.221624	0.043348	Unknown	
ETOH 0.3	5.217833	913.8074	24H	-1.88465	0.026148	Unknown	
ETOH 0.3	5.399211	921.0025	4D	1.944905	2.78E-05	Unknown	
ETOH 0.3	5.387775	922.0048	24H	-2.75471	0.015691	Unknown	
ETOH 0.3	3.680188	980.075	4D	1.480926	0.012893	Unknown	
ETOH 0.3	3.646902	994.0917	4D	1.604696	0.000395	Unknown	
ETOH 0.3	3.705141	1008.072	4D	1.415783	0.021503	Unknown	
ETOH 0.3	5.177162	1038.786	4D	1.588208	0.030971	Unknown	
ETOH 0.3	5.8905	1040.323	4D	-1.47887	0.009656	Unknown	

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All references cited herein are incorporated by reference. In addition, the invention is not intended to be limited to the disclosed embodiments of the invention. It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A method for identifying one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with a test compound, the method comprising the steps of:

a) culturing hESCs or hESC-derived lineage-specific cells in the presence or absence of a test compound;

b) assaying one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons in hESCs or hESC-derived lineage-specific cells cultured in the presence and absence of the test compound; and

c) identifying at least one metabolite product having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound.

2. A method according to claim 1, wherein at least one of the cellular metabolites is produced in greater amounts in the presence of the test compound than in the absence of the test compound.

3. A method according to claim 1, wherein at least one of the cellular metabolites is produced in greater amounts in the absence of the test compound than in the presence of the test compound.

4. A method according to claim 1, wherein the cellular metabolites are secreted or excreted from the hESCs or hESC-derived lineage-specific cells.

5. A method according to claim 1, wherein the cellular metabolite is a small molecule product having a molecular weight of from about 100 to about 1000 Daltons.

6. A method according to claim 1, wherein the test compound is a toxic or teratogenic compound.

7. A method according to claim 1, wherein one or a plurality of cellular metabolites is assayed using a physical separation method.

8. A method according to claim 7, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry.

9. A method according to claim 1, wherein the cellular metabolites are tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

10. A method according to claim 1, wherein the candidate cellular metabolites are kynurenine, 8-methoxykynurenate , N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate, 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, 3,4-dihydroxybutyric acid, serotonin, or gamma-aminobutyric acid (GABA)..

11. A method according to claim 1, wherein a plurality of cellular metabolites are identified.

12. A method according to claim 10, wherein the plurality of identified cellular metabolites comprise a biomarker profile.

13. A method according to claim 11, wherein one of the cellular metabolites comprising a biomarker profile is kynurenine.

14. A method according to claim 11, wherein the test compound is a toxic or teratogenic compound.

15. A method according to claim 14, wherein the plurality of identified cellular metabolites comprise a biomarker profile characteristic of hESC or hESC-derived lineage-specific cell response to a toxic or teratogenic compound.

16. A method for screening a test compound to identify an effect of the compound on human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with the test compound, the method comprising the steps of:

a) culturing hESCs or hESC-derived lineage-specific cells in the presence or absence of a test compound

b) assaying the hESCs or hESC-derived lineage-specific cells cultured in the presence and absence of the test compound for production of one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons; and

c) identifying test compounds that produce at least one cellular metabolite having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound.

17. A method according to claim 16, wherein at least one of the cellular metabolites is produced in greater amounts in the presence of the test compound than in the absence of the test compound.

18. A method according to claim 16, wherein at least one of the cellular metabolites is produced in greater amounts in the absence of the test compound than in the presence of the test compound.

19. A method according to claim 16, wherein the cellular metabolites are secreted or excreted from the hESCs or hESC-derived lineage-specific cells.

20. A method according to claim 16, wherein at least one of the cellular metabolites is a small molecule metabolite having a molecular weight of from about 100 to about 1000 Daltons.

21. A method according to claim 16, wherein the test compound is a toxic or teratogenic compound.

22. A method according to claim 16, wherein the plurality of cellular metabolites are assayed using a physical separation method.

23. A method according to claim 22, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry.

24. A method according to claim 16, wherein the candidate cellular metabolites are tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

25. A method according to claim 16, wherein the candidate cellular metabolites are kynurenine, 8-methoxykynurenate , N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate, 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, serotonin, gamma-aminobutyric acid (GABA) or other butyric acid species.

26. A method according to claim 16 wherein a plurality of cellular metabolites are identified.

27. A method according to claim 26, wherein the plurality of identified cellular metabolites comprise a biomarker profile.

28. A method according to claim 27, wherein one of the cellular metabolites comprising a biomarker profile is kynurenine.

29. A method according to claim 27, wherein the test compound is a toxic or teratogenic compound.

30. A method according to claim 29, wherein the plurality of identified cellular metabolites comprise a biomarker profile characteristic of hESC response to a toxic or teratogenic compound.

31. A method for assaying a test compound for toxicity or teratogenicity to human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with the test compound, the method comprising the steps of:

a) culturing hESCs or hESC-derived lineage-specific cells in the presence or absence of a test compound

b) assaying hESCs or hESC-derived lineage-specific cells cultured in the presence and absence of the test compound to identify one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons produced by hESCs contacted with a toxic or teratogenic compound; and

c) identifying test compounds wherein hESCs or hESC-derived lineage-specific cells produce one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons in the presence or absence of the test compound wherein said cellular metabolites are produced by hESCs or hESC-derived lineage-specific cells contacted with a toxic or teratogenic compound.

32. A method according to claim 31, wherein at least one of the cellular metabolites is produced in greater amounts in the presence of the test compound than in the absence of the test compound.

33. A method according to claim 31, wherein at least one of the cellular metabolites is produced in greater amounts in the absence of the test compound than in the presence of the test compound.

34. A method according to claim 31, wherein the cellular metabolites are secreted or excreted from the hESCs or hESC-derived lineage-specific cells.

35. A method according to claim 31, wherein the cellular metabolite is a small molecule metabolite having a molecular weight of from about 100 to about 1000 Daltons.

36. A method according to claim 31, wherein the test compound is a toxic or teratogenic compound.

37. A method according to claim 31, wherein cellular metabolites are assayed using a physical separation method.

38. A method according to claim 37, wherein the physical separation method is liquid chromatography/electrospray ionization time of flight mass spectrometry.

39. A method according to claim 31, wherein the candidate cellular metabolites are tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

40 . A method according to claim 31, wherein the candidate cellular metabolites are kynurenine, 8-methoxykynurenate, N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, 3,4-dihydroxybutyric acid, serotonin, gamma-aminobutyric acid (GABA) or other butyric acid species..

41. A method according to claim 31, wherein a plurality of cellular metabolites are identified.

42. A method according to claim 4`, wherein the plurality of identified cellular metabolites comprise a biomarker profile.

43. A method according to claim 42, wherein one of the cellular metabolites comprising a biomarker profile is kynurenine.

44. A method according to claim 43, wherein the test compound is a toxic or teratogenic compound.

45. A method according to claim 31, wherein the plurality of identified cellular metabolites comprise a biomarker profile characteristic of hESC response to a toxic or teratogenic compound.

46. A biomarker profile for identifying a test compound as a toxic compound, wherein the biomarker profile comprises one or a plurality of cellular metabolites

having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with a toxic compound or compounds.

47. A biomarker profile of claim 46, comprising tetrahydrofolate, dihydrofolate or other metabolites in the folate metabolic pathway, glutathione, or oxidized glutathione.

48. A biomarker profile of claim 46, comprising kynurenine, 8-methoxykynurenate, N'-formylkynurenine 7,8-dihydro-7,8-dihydroxykynurenate 5-Hydroxytryptophan, N-acetyl-D-tryptophan, glutamate, pyroglutamic acid or other metabolites in the tryptophan or glutamate metabolic pathways, histamine, dopamine, 3,4-dihydroxybutyric acid, serotonin, gamma-aminobutyric acid (GABA) or other butyric acid species.

49. A method of claim 1, further comprising the step of comparing at least one metabolite product having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound with a biomarker profile comprising one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with a toxic compound or compounds.

50. A method of claim 16, further comprising the step of comparing at least one metabolite product having a molecular weight of from about 10 to about 1500 Daltons

that is differentially produced in the cells in the presence or absence of the test compound with a biomarker profile comprising one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with a toxic compound or compounds.

51. A method of claim 31, further comprising the step of comparing at least one metabolite product having a molecular weight of from about 10 to about 1500 Daltons that is differentially produced in the cells in the presence or absence of the test compound with a biomarker profile comprising one or a plurality of cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in human embryonic stem cells (hESCs) or hESC-derived lineage-specific cells contacted with a toxic compound or compounds.

52. The method of claim 1, wherein at least two cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in the cells in the presence or absence of the test compound are detected.

53 The method of claim 16, wherein at least two cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in the cells in the presence or absence of the test compound are detected.

54. The method of claim 31, wherein at least two cellular metabolites having a molecular weight of from about 10 to about 1500 Daltons that are differentially produced in the cells in the presence or absence of the test compound are detected.

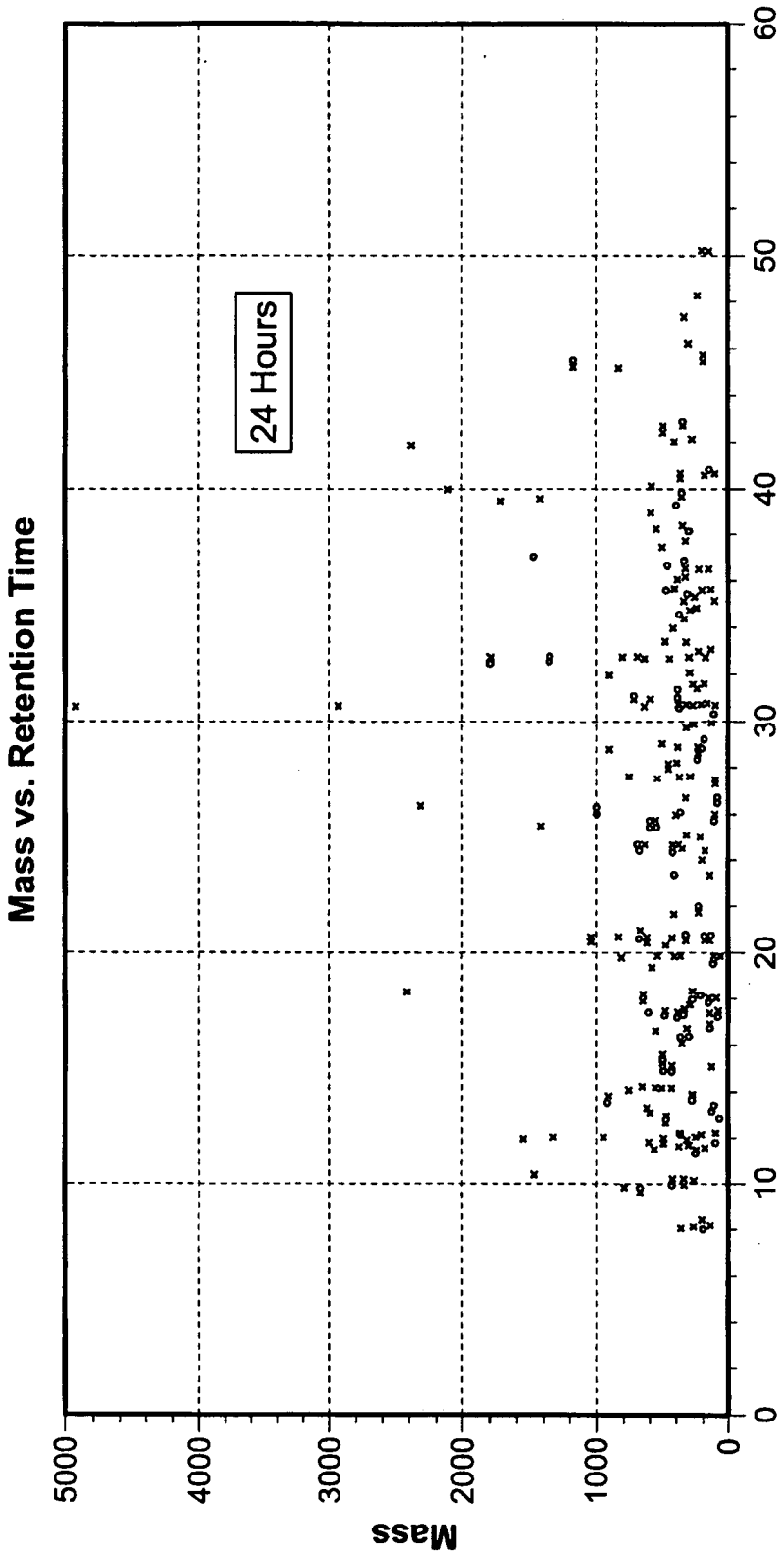
Mass vs. Retention Time
24 Hours
Mass
5000
4000
3000
2000
1000
0
Retention Time (min)
0
10
20
30
40
50
60

FIG. 1A

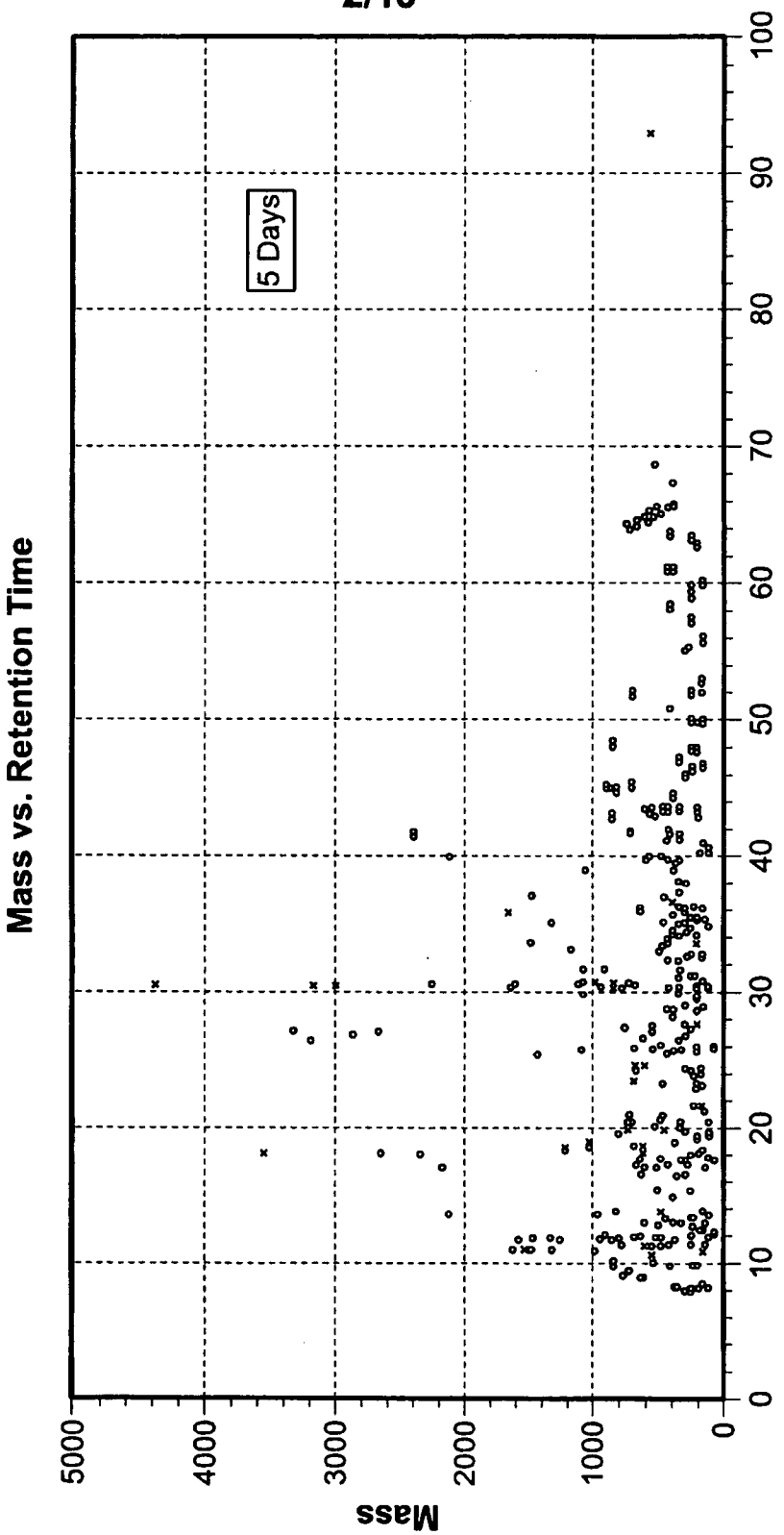
Mass vs. Retention Time
5 Days
Mass
5000
4000
3000
2000
1000
0
Retention Time (min)
0
10
20
30
40
50
60
70
80
90
100

FIG. 1B

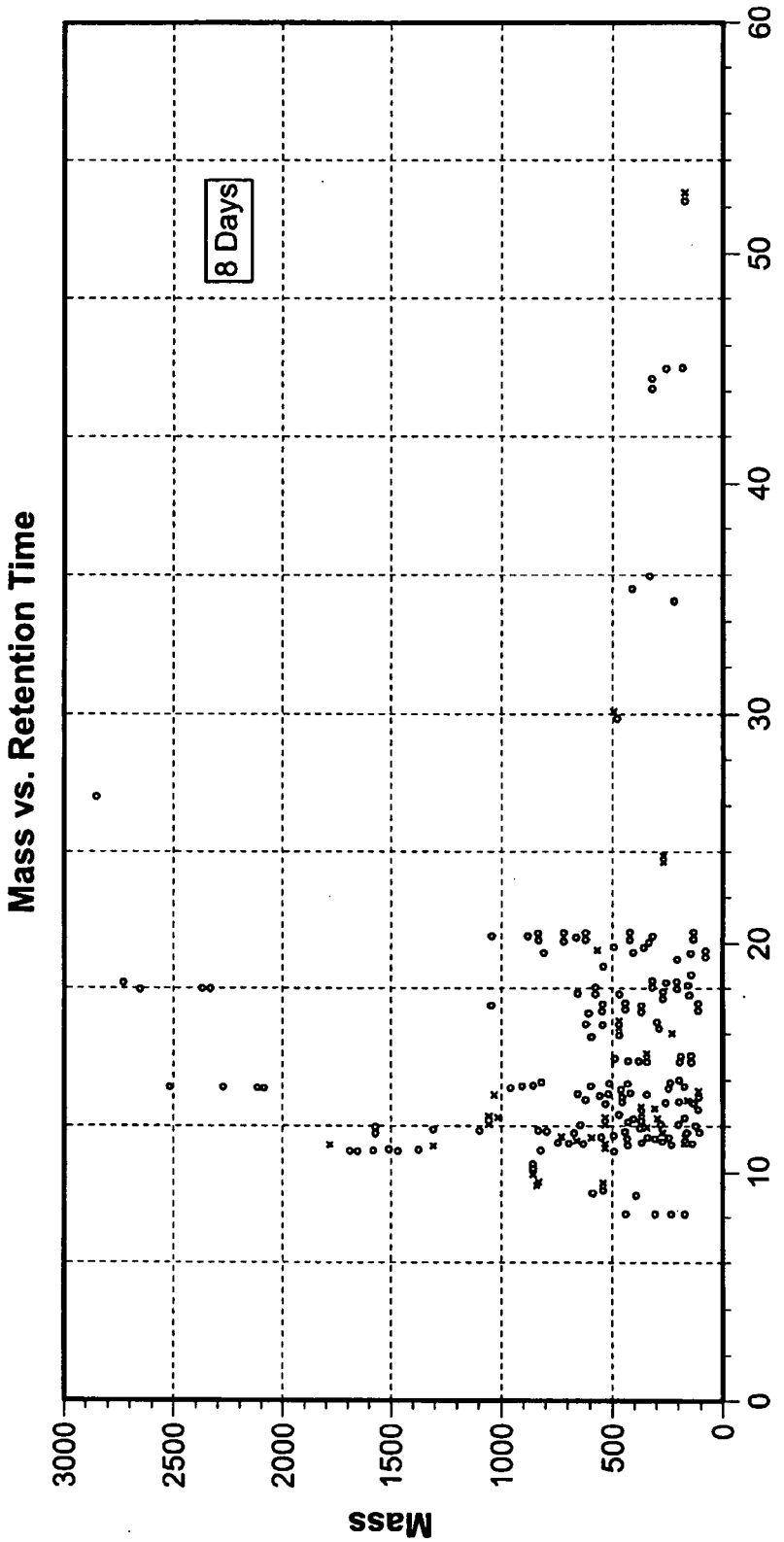
Mass vs. Retention Time
8 Days
Mass
3000
2500
2000
1500
1000
500
0
Retention Time (min)
0
10
20
30
40
50
60

FIG. 1C

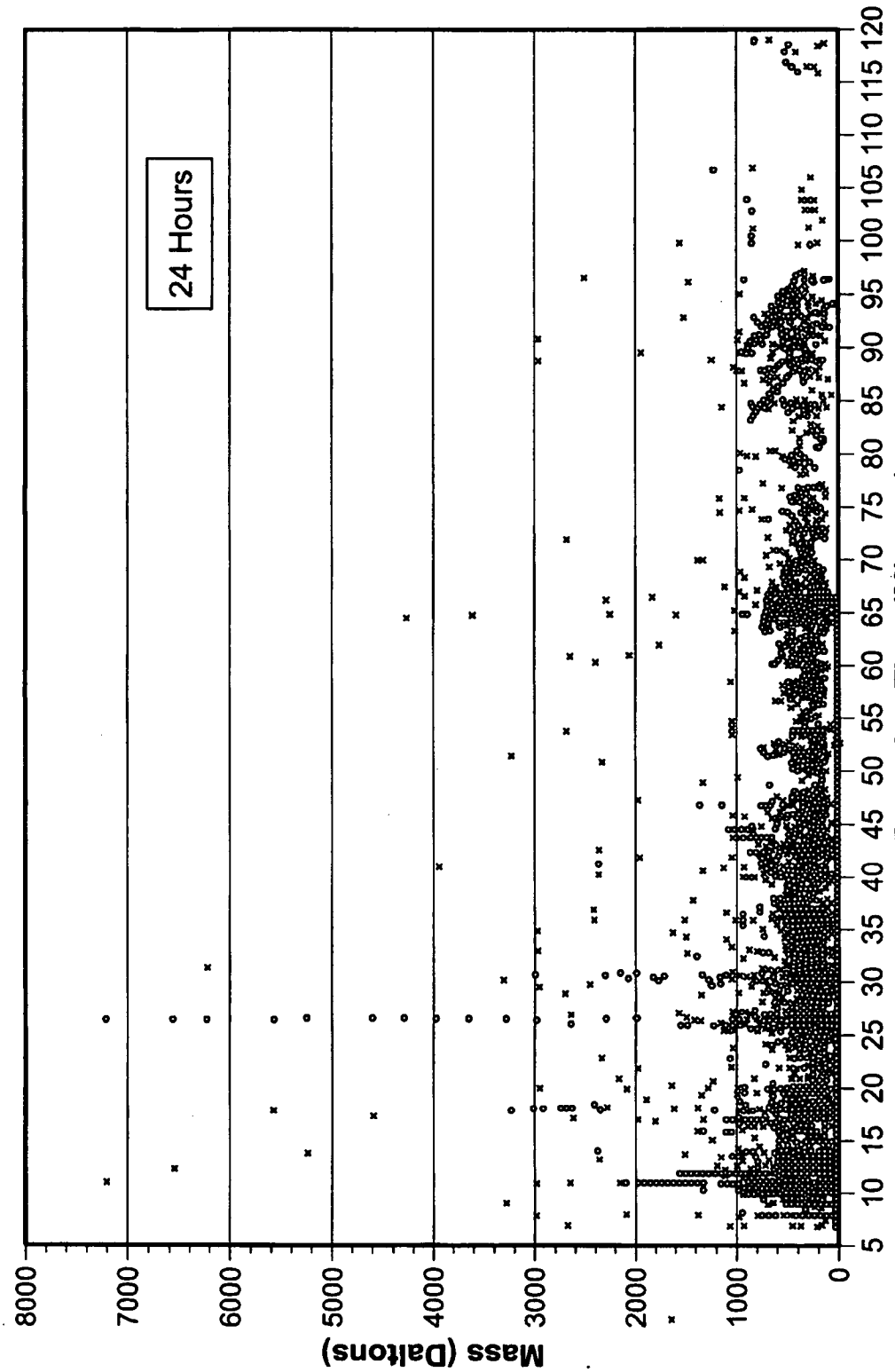
24 Hours
Mass (Daltons)
8000
7000
6000
5000
4000
3000
2000
1000
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
Retention Time (Minutes)

FIG. 2A

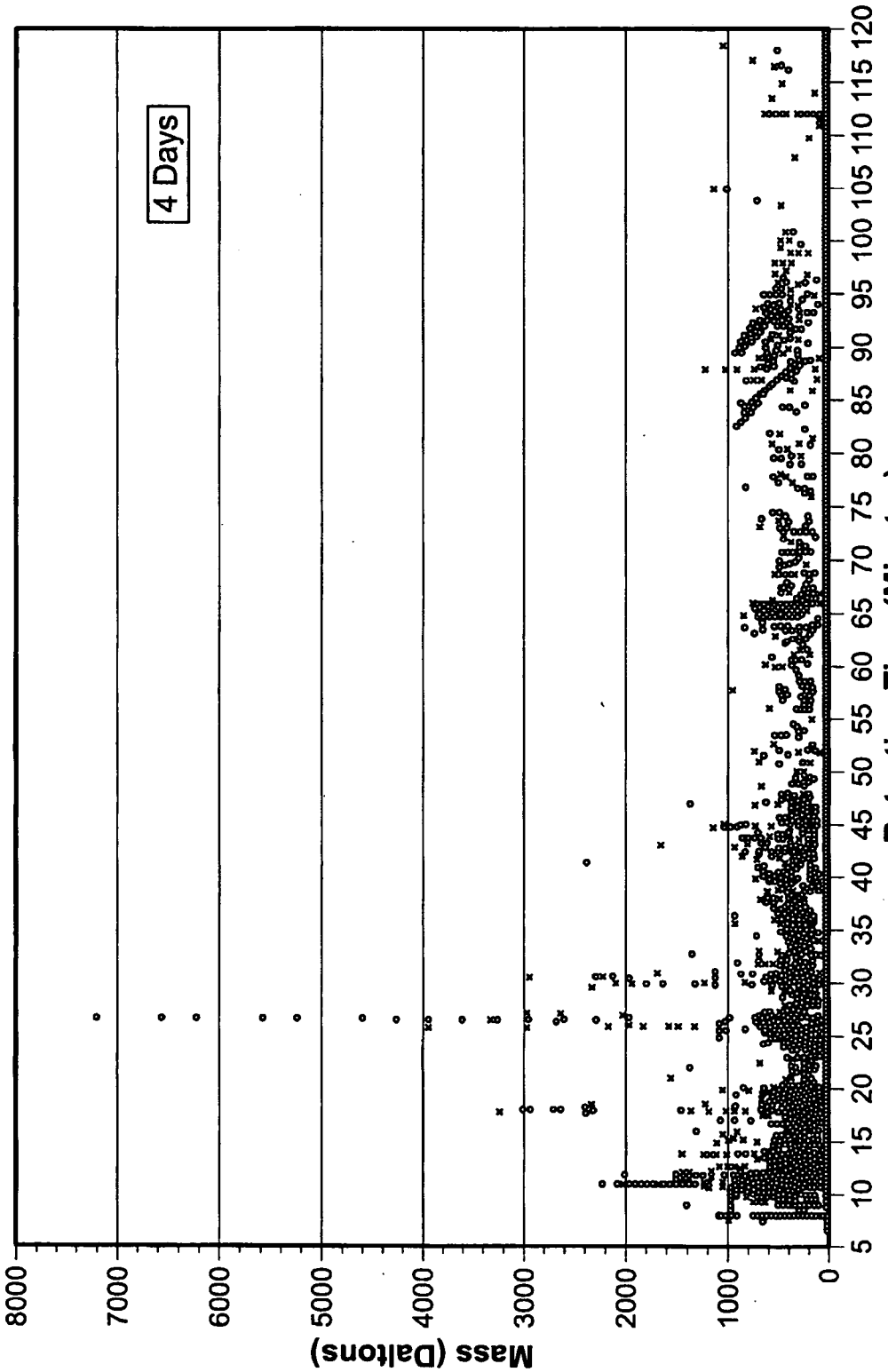
4 Days
Mass (Daltons)
8000
7000
6000
5000
4000
3000
2000
1000
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
Retention Time (Minutes)

FIG. 2B

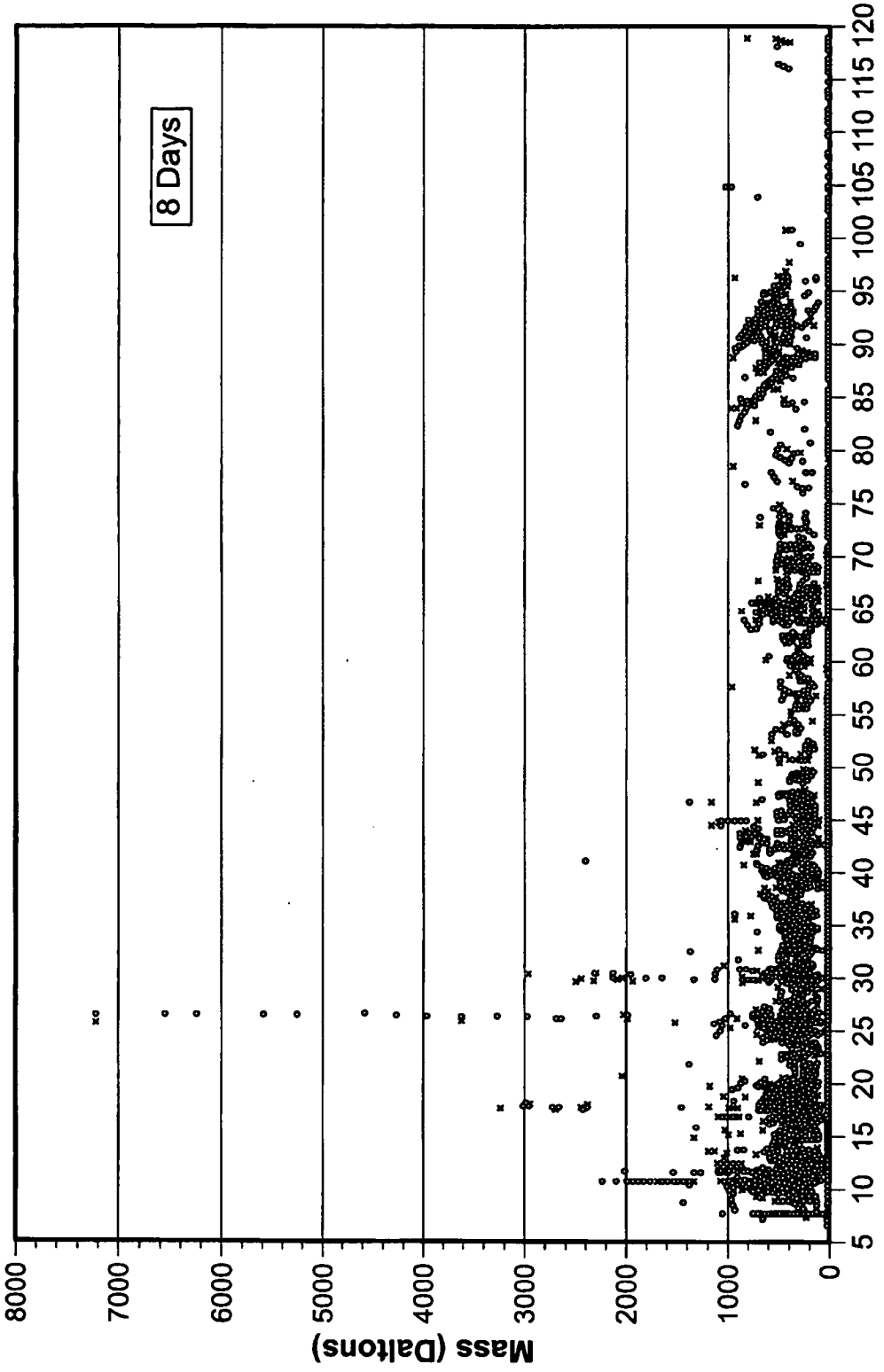
8 Days
Mass (Daltons)
8000
7000
6000
5000
4000
3000
2000
1000
0
Retention Time (Minutes)
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120

FIG. 2C

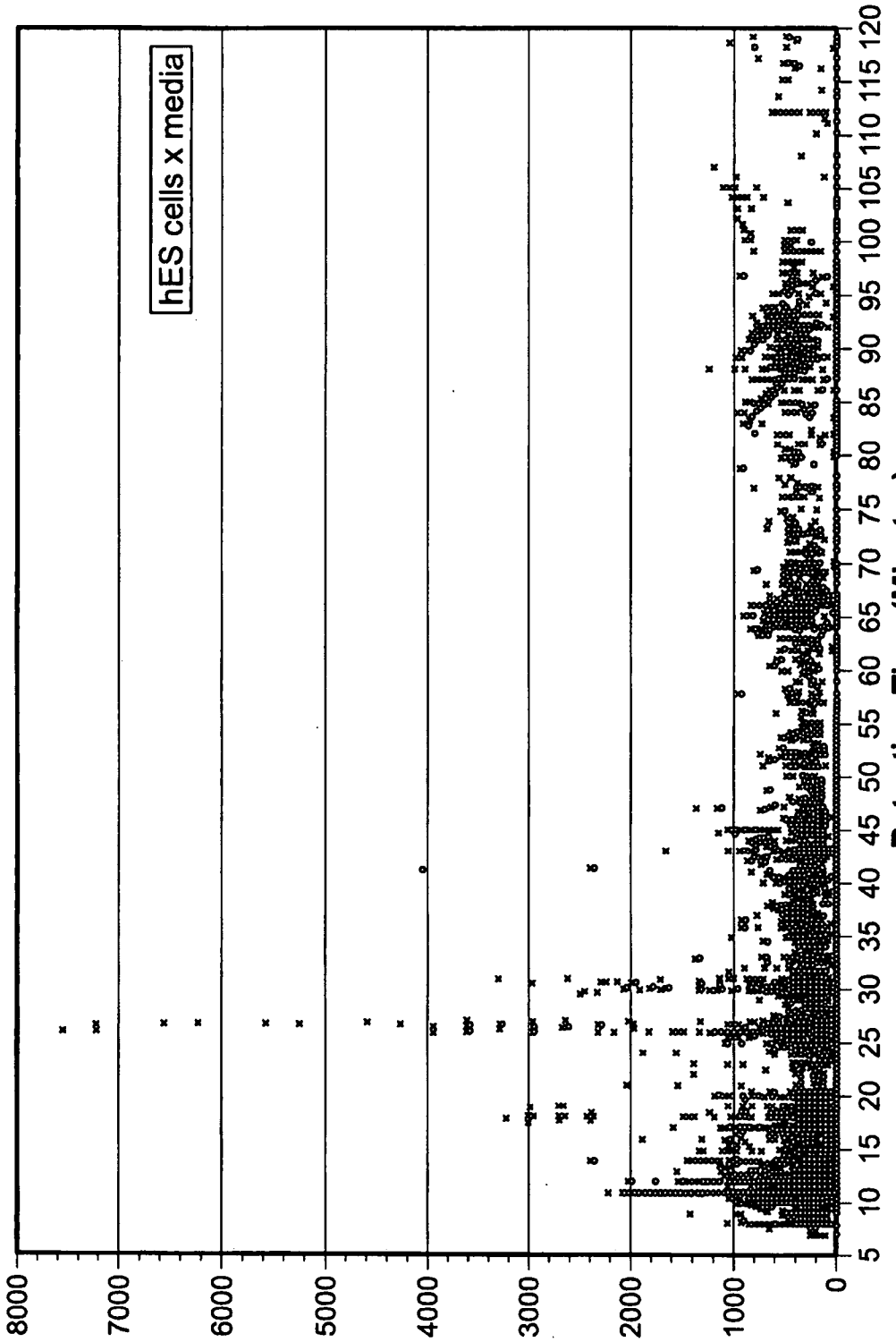
hES cells x media
8000
7000
6000
5000
4000
3000
2000
1000
0
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120
Retention Time (Minutes)

FIG. 2D

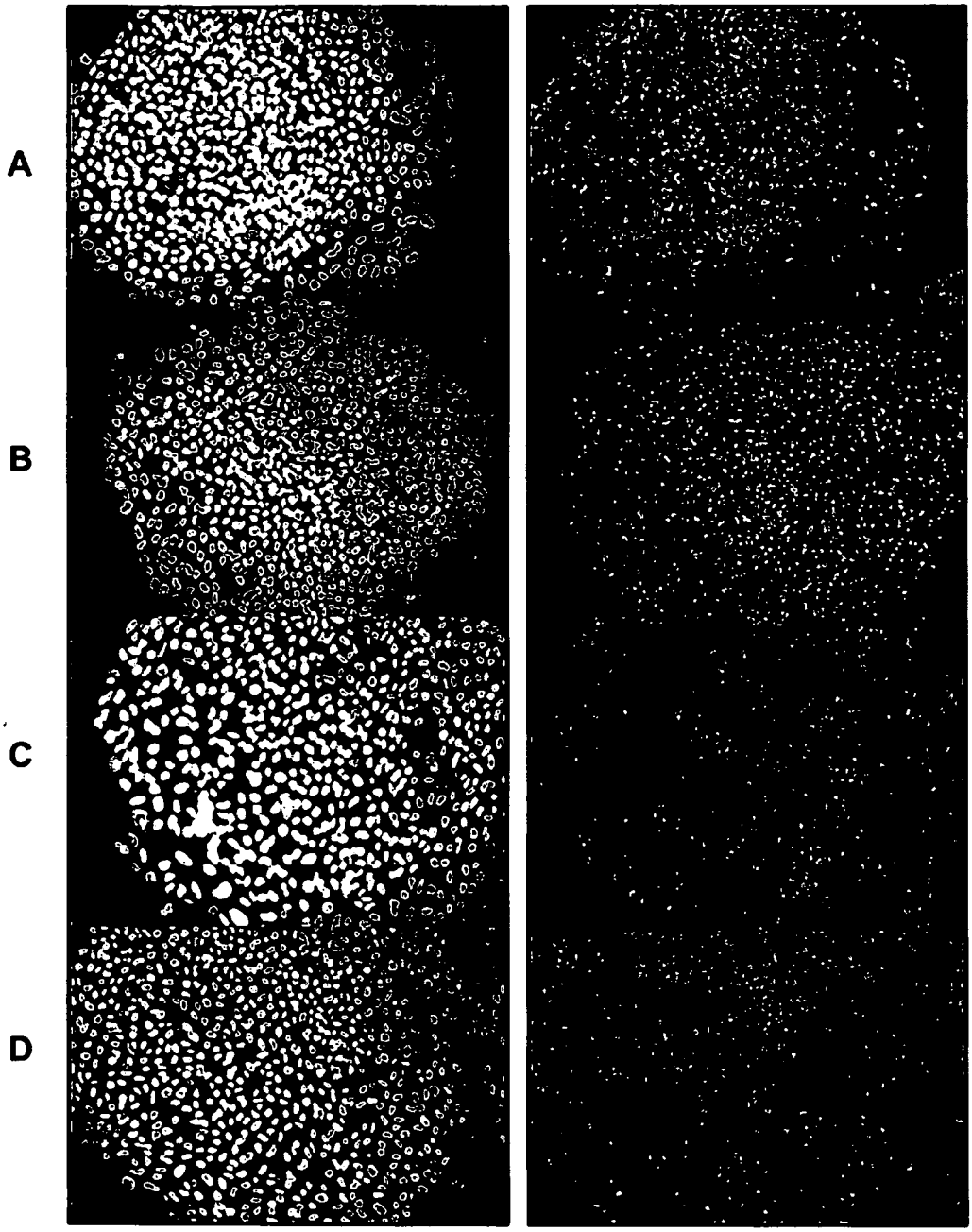

FIG. 3

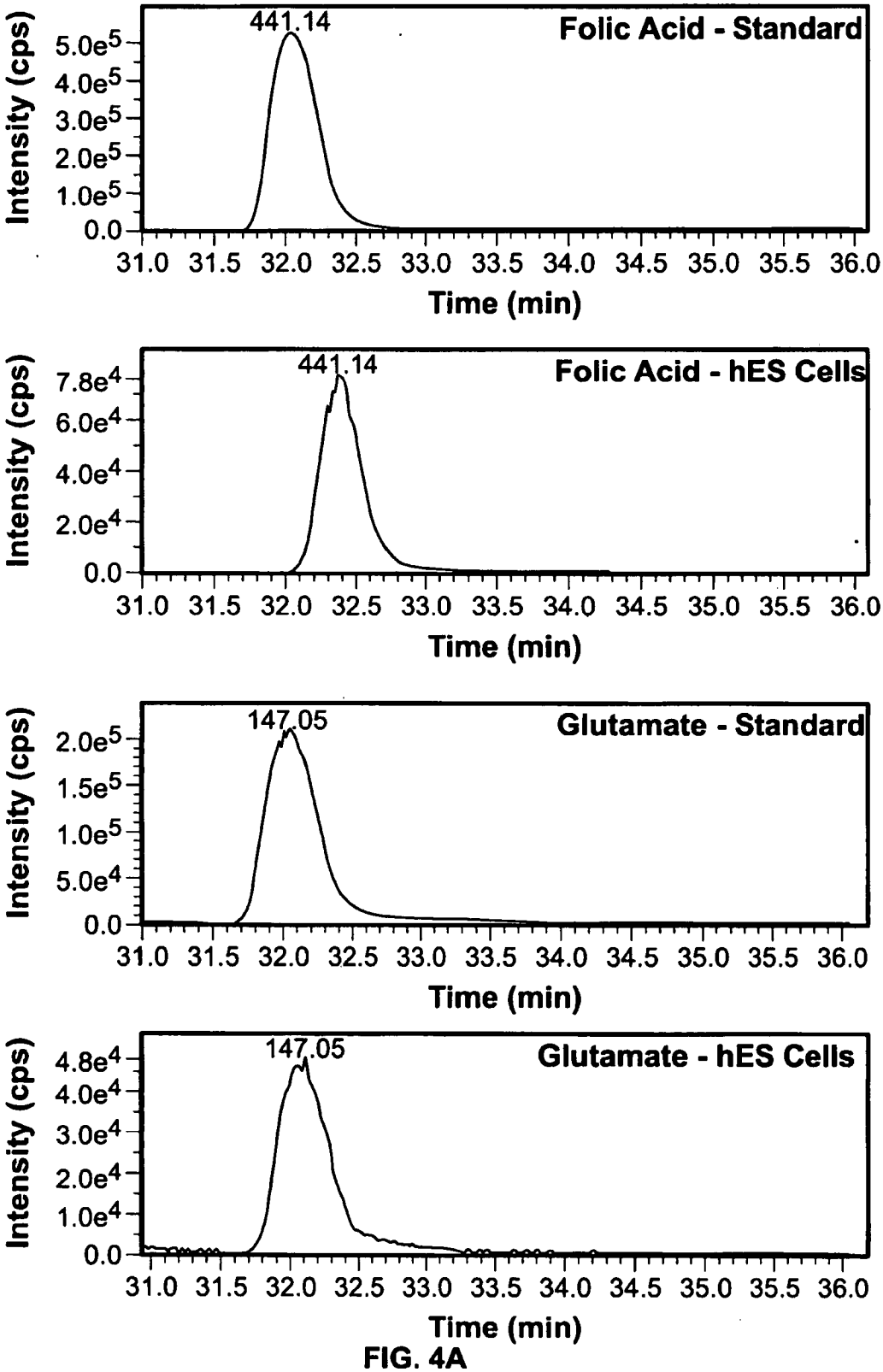
441.14
Folic Acid - Standard
Intensity (cps)
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)
441.14
Folic Acid - hES Cells
Intensity (cps)
7.8e4
6.0e4
4.0e4
2.0e4
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)
147.05
Glutamate - Standard
Intensity (cps)
2.0e5
1.5e5
1.0e5
5.0e4
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)
147.05
Glutamate - hES Cells
Intensity (cps)
4.8e4
4.0e4
3.0e4
2.0e4
1.0e4
0.0
31.0 31.5 32.0 32.5 33.0 33.5 34.0 34.5 35.0 35.5 36.0
Time (min)

FIG. 4A

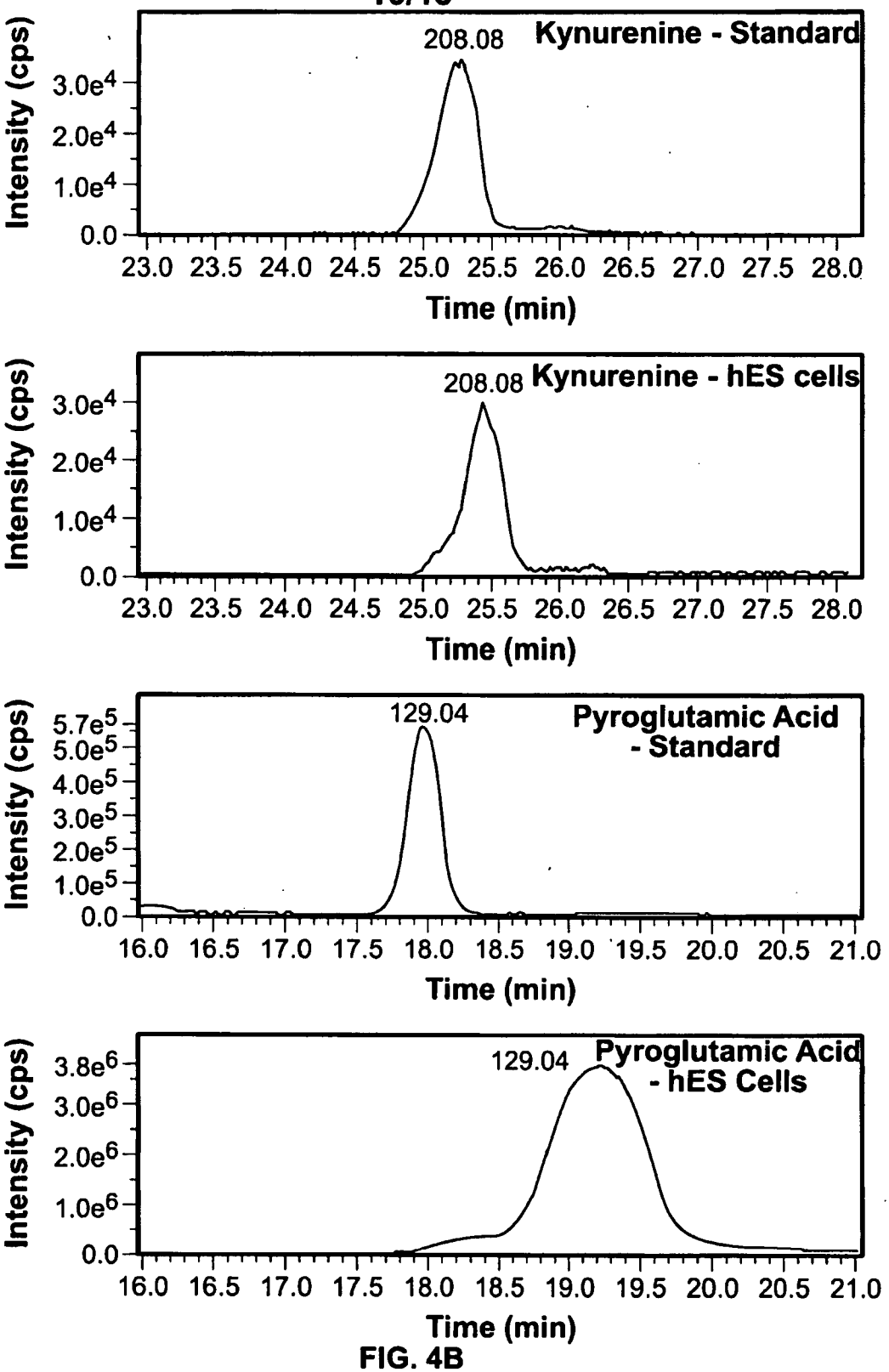
208.08
Kynurenine - Standard
Intensity (cps)
3.0e4
2.0e4
1.0e4
0.0
23.0 23.5 24.0 24.5 25.0 25.5 26.0 26.5 27.0 27.5 28.0
Time (min)
208.08
Kynurenine - hES cells
Intensity (cps)
3.0e4
2.0e4
1.0e4
0.0
23.0 23.5 24.0 24.5 25.0 25.5 26.0 26.5 27.0 27.5 28.0
Time (min)
129.04
Pyroglutamic Acid
- Standard
Intensity (cps)
5.7e5
5.0e5
4.0e5
3.0e5
2.0e5
1.0e5
0.0
16.0 16.5 17.0 17.5 18.0 18.5 19.0 19.5 20.0 20.5 21.0
Time (min)
129.04
Pyroglutamic Acid
- hES Cells
Intensity (cps)
3.8e6
3.0e6
2.0e6
1.0e6
0.0
16.0 16.5 17.0 17.5 18.0 18.5 19.0 19.5 20.0 20.5 21.0
Time (min)

FIG. 4B

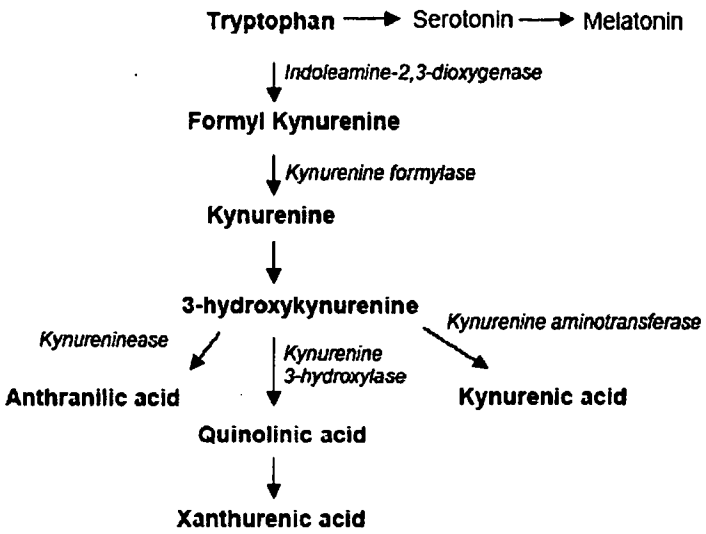
Tryptophan
Serotonin
Melatonin
Indoleamine-2,3-dioxygenase
Formyl Kynurenine
Kynurenine formylase
Kynurenine
3-hydroxykynurenine
Kynurenine aminotransferase
Kynureninease
Kynurenine 3-hydroxylase
Anthranilic acid
Kynurenic acid
Quinolinic acid
Xanthurenic acid

FIG. 5

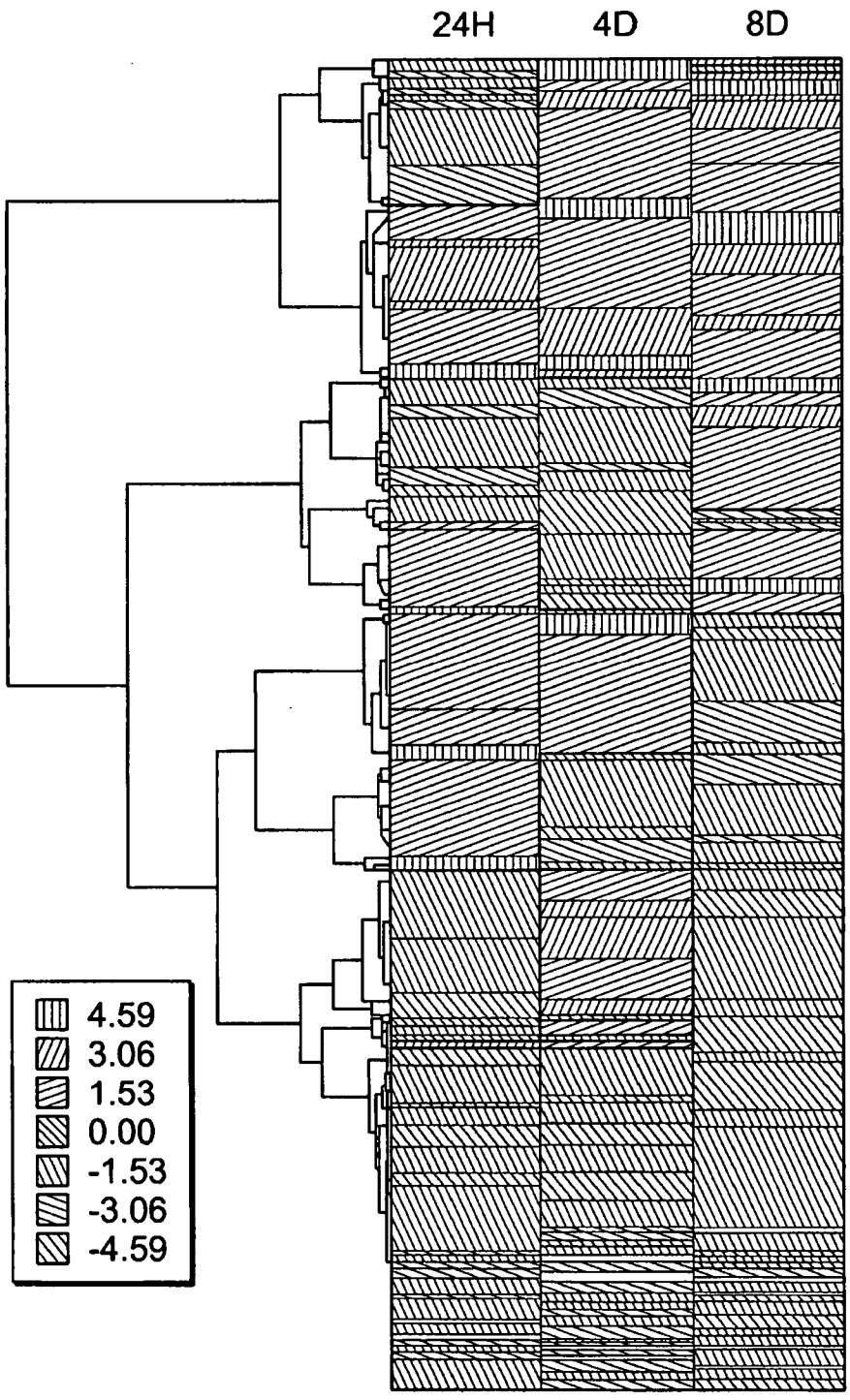

FIG. 6

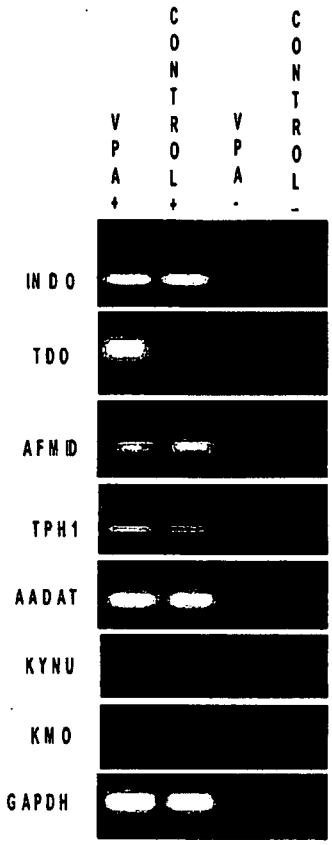
VPA+
CONTROL+
VPA-
CONTROL-
INDO
TDO
AFMID
TPH1
AADAT
KYNU
KMO
GAPDH

FIG. 7