State of the heart : the promise of pluripotent stem cell-derived cardiomyocytes in disease modelling, differentiation and development

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CHAPTER 6

Transcriptome of human foetal heart compared with cardiomyocytes from pluripotent stem cells

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Abstract

Differentiated derivatives of human pluripotent stem cells (hPSCs) are often considered immature because they resemble foetal cells more than adult, with hPSC-derived cardiomyocytes (hPSC-CMs) being no exception. Many functional features of these cardiomyocytes, such as their cell morphology, electrophysiological characteristics, sarcomere organization and contraction force, are underdeveloped compared with adult cardiomyocytes. However, relatively little is known about how their gene expression profiles compare with the human foetal heart, in part because of the paucity of data on the human foetal heart at different stages of development.

Here, we collected samples of matched ventricles and atria from human foetuses during the first and second trimester of development. This presented a rare opportunity to perform gene expression analysis on the individual chambers of the heart at various stages of development, allowing us to identify not only genes involved in the formation of the heart, but also specific genes upregulated in each of the four chambers and at different stages of development.

The data showed that hPSC-CMs had a gene expression profile similar to first trimester foetal heart, but after culture in conditions shown previously to induce maturation, they cluster closer to the second trimester foetal heart samples. In summary, we demonstrate how the gene expression profiles of human foetal heart samples can be used for benchmarking hPSC-CMs and also contribute to determining their equivalent stage of development.

Introduction

In the developing embryo, the heart is one of the first organs to be fully formed. It develops from a linear tube into a four-chambered organ through a complex looping process that leads to the formation of the ventricles, the atria and the outflow tract. In humans, the heart starts beating around 6 weeks of gestation and pumps blood though a closed circulatory system to provide nutrients and oxygen and remove waste products from organs as they develop. Later, the septum separates the atria into left and right halves and closes completely after birth. The left and right ventricles are separated before birth and their walls develop into strong muscles [1]. The left ventricular wall is thicker than the right because it pumps oxygenated blood from the lungs to all parts of the body via the aorta. The right side of the heart receives de-oxygenated blood and pumps it through the lungs to re-oxygenate. Although much is known about the molecular mechanisms that drive heart formation and morphogenesis in laboratory animals [2], little equivalent data is available on the human heart. This is important for understanding how specific mutations in different genes (i.e. missense mutations), rather than knockouts commonly used in experimental animals, affect human heart development and function as well as validating models of hereditary heart disease based on patient-derived human induced pluripotent stem cells (hiPSCs).

The four chambers of the mammalian heart express different genes that determine their physiological properties [3]. Most studies to date have analysed the transcriptome of adult human atria and ventricles [4], or have performed transcriptional analysis on human auricle (part of the atrium) removed during normal clinical procedures on diseased adult hearts [5,6]. Collection of healthy human ventricular tissue is more difficult and is usually only available when donor hearts are not used for transplantation. Here, we collected tissue from the chambers of human foetal hearts in isogenically matched combinations of atria and ventricles from either the first (T1) or second (T2) trimester of development and examined gene expression as a function of gestational age and region of the heart. Using the advanced microarray techniques now available, we were able to compare transcriptomes of foetal tissue using small amounts of RNA (<0.5 µg). These studies not only provided better insight into how the human heart develops, but also allowed us to compare these samples with cardiomyocytes (CMs) derived from human pluripotent stem cells (hPSCs).

hPSCs are now frequently used to investigate CM differentiation in the early embryo and also to model inherited cardiac disease [7]. Although it is widely acknowledged that hPSC-derived CMs (hPSC-CMs) are immature [8], how they compare with the individual chambers of human foetal hearts is not clear. We have examined hPSC-CMs cultured in conventional (defined) differentiation medium [9], and in medium recently reported to induce functional maturation as evidenced by increased force of contraction [10]. We found that the foetal gene expression profiles allowed us to estimate the maturity of the hPSC-CMs.

Materials and Methods

Foetal heart sample collection and ethics statement

Human foetal heart samples were collected from eight healthy individuals after elective abortions at various gestational ages (7, 10, 15, 20 and 20+ weeks of gestation) determined using obstetric ultrasonography based on crown-rump length measurements. The Medical Ethical Committee of the Leiden University Medical Center (protocol 08.087) approved the use of human foetal material and informed written consent was obtained in accordance with the World Medical Association Declaration of Helsinki guidelines.

RNA extraction

Total RNA was extracted from the samples using standard isolation techniques. The quality and integrity of the RNA samples was confirmed using Lab-on-Chip RNA 6000 Nano and RNA 6000 Pico (both Agilent) on the Agilent 2100 Bioanalyzer (Agilent Technologies) by ServiceXS B.V. (Leiden, The Netherlands).

Microarray experiments

Two reference samples (Universal Human Reference RNA, Cat #740000, Stratagene and Human Normal Heart Donor Pool Cat #R1234122-P, lot #A509251, Biochain) were co-hybridized with the experimental samples (Supplemental Table 1). These references were used for normalization between different arrays. For whole-genome microarray of foetal heart samples, biotinylated ss-cDNA was prepared using the NuGEN Ovation PicoSL WTA v2 System (NuGEN) according to the manufacturer's protocol using ~50 ng total RNA. For hESC- and hiPSC-derived CMs, biotinylated cRNA was prepared using the Illumina TotalPrep RNA Amplification Kit (Ambion) according to the manufacturer's specifications using ~200 ng total RNA. Hybridization and processing of all samples was performed on Illumina HumanHT-12 v4 microarray chips by ServiceXS B.V. (Leiden, The Netherlands). Microarray data have been deposited in Gene Expression Omnibus under accession number GSE71148.

Data analysis and statistics

The raw intensity values of the microarray data were normalized by variance stabilizing normalization using the vsn R package [11] and subsequently normalized by the common references. When a gene had more than one microarray probe, the one with the highest variance across the samples was used for subsequent analysis. The differential expression analysis was performed using the limma R package [12]. Genes were binned into 30 bins by the intensity and the *t*-test applied to each bin. The *P*-value was corrected for multiple testing by the Benjamini-Hochberg method with an adjusted *P*-value cut-off of 0.05. Genes with the mean absolute log2 fold change <1.5 were discarded. Unsupervised hierarchical clustering

of all detected genes was performed using the Euclidean distance and complete linkage method. The same parameters were used for clustering the DEGs. Fuzzy clustering of DEGs among foetal and adult heart samples was performed using the R package Mfuzz. The optimal number of clusters was detected using the C-means clustering algorithm implemented in the R package e1071. GO.BP was downloaded from http://www.geneontology.org. Fisher's exact test was performed to identify the statistical enrichment of these categories using the differentially up- or downregulated genes as the test set. All detected genes were taken as the background set. The *P*-value was corrected for multiple testing by the Benjamini-Hochberg method. Categories with an adjusted *P*-value <0.01 and odds ratio >1.0 were considered significantly enriched.

Cardiac differentiation of human ESCs and iPSCs

hPSCs were differentiated to CMs as previously described [9,13] and maintained either in LI-BPEL [13], or according to the manufacturer's protocol (Pluricyte Medium, Pluriomics). To isolate CMs from the NKX2.5-GFP reporter hESC and hiPSC lines [13] (C.W.v.d.B., C.L.M., R.P.D., unpublished), the cells were dissociated [9] and sorted based on GFP expression using a BD FACSAria III Cell Sorter (BD Biosciences).

Results and Discussion

Global gene expression analysis of foetal and adult heart samples show changes with age

We determined the transcriptional profiles of human foetal hearts collected during T1 and T2 of normal human development. We separated T1 hearts into atrium and ventricle. As T2 hearts were larger, we collected the four chambers separately. We also included a commercially available sample of pooled adult hearts as a common reference for normalization of future samples (Figure 1A; Supplemental Table 1). Human foetal heart samples were a mixed population of cardiomyocytes, fibroblasts and endothelial cells because there are no specific cell surface antibodies for cardiomyocytes that would allow them to be sorted from primary heart tissue. However, the majority of the cells in the foetal heart are cardiomyocytes, which decreases postnatally when cardiomyocyte division ceases [14].

To compare the variability between all samples, we performed gene expression cluster analysis (Figure 1B). We found that the foetal heart samples showed distinct gene expression profiles between T1 and T2. From the T1 and T2 samples, the atria and ventricles clustered separately, except for the foetal heart (FH) FH5-right atrium (RA), FH5-right ventricle (RV) and FH5-left ventricle (LV) samples, which clustered together as individual number 5. T2 ventricle samples clustered closer to the adult heart reference samples, possibly because the contribution of ventricles to the pooled adult reference sample is greater than the (smaller) atria.

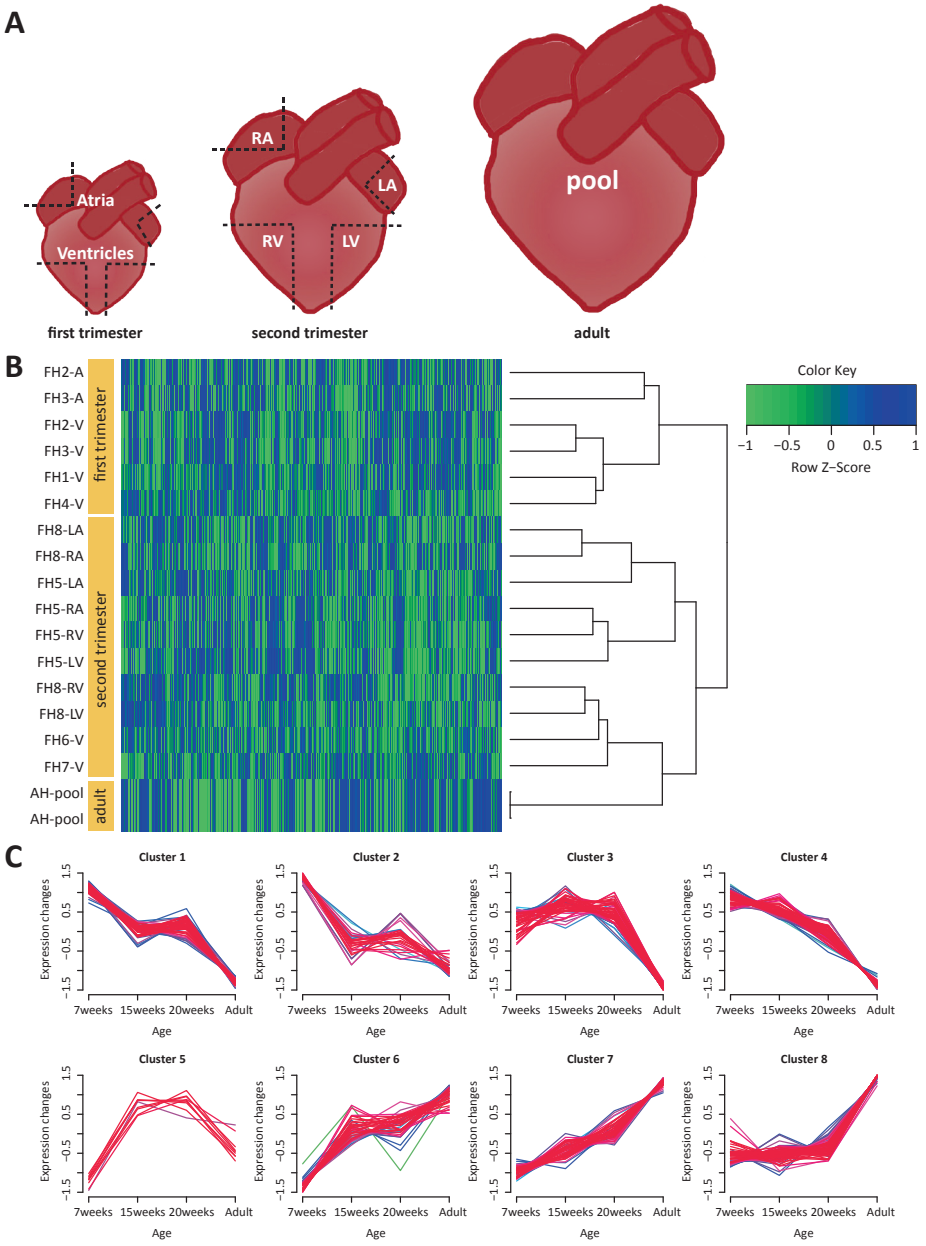

Figure 1. Hierarchical and fuzzy clustering of foetal heart samples
A, Schematic of collected samples from atria and ventricles of first and second trimester, and the commercial reference of pooled adult hearts. Dissection edges are indicated by dashed lines. **B**, Unsupervised hierarchical clustering of the global gene expression data. The dendrogram illustrates separation of the samples based on age and heart chamber. **C**, Fuzzy clustering showing all differentially expressed genes based on their expression at 7, 15 and 20 weeks of gestation and at the adult stage.

We also grouped samples based on age (7 weeks, 15 weeks, 20 weeks and adult) and investigated all differentially expressed genes (DEGs) in the first and second trimester and adult hearts. We identified eight distinct transcriptome clusters (Figure 1C). The DEGs in Cluster 1 were upregulated at 7 weeks, showed no changes in 15- and 20-week samples and decreased in adult heart. Cluster 2 showed a similar pattern; DEGs in both clusters were involved in cell cycle regulation and chromatin organization. DEGs in Cluster 3 were upregulated during foetal development and downregulated in adult heart. This cluster included genes important in development, cell division and matrix organization that are less important in adult hearts unless the heart has been damaged, for example by myocardial infarction. As the heart develops and ages, CM proliferation, which is essential during early heart development, decreases [14]. Cluster 4 showed genes that gradually decreased over time and were involved in cell cycle regulation and chromatin organization. Cluster 5 contained DEGs involved in gaseous substance transport; these were downregulated at week 7 and in adult heart but were upregulated at intermediate stages. Clusters 6, 7 and 8 consisted of DEGs that increased over time and are important in metabolic processes, muscle organization and contraction. The foetal heart depends on carbohydrate synthesis, but it also prepares for the switch in metabolism to fatty acid oxidation shortly after birth [15]. Gene ontology terms for biological processes (GO.BP) for each cluster are listed in Supplemental Table 2.

First and second trimester atria and ventricles have distinct gene expression signatures

To investigate genes that are important for development of atria and ventricles, we divided samples into four groups according to their origin and age: atria T1 (A1), ventricles T1 (V1), atria T2 (A2) and ventricles T2 (V2). Four comparisons were made to investigate differences in gene expression between atria and ventricles, and also between T1 and T2. Gender did not appear to influence gene expression in the heart with only genes located on the sex chromosomes (i.e. *XIST*, *RPS4Y2*, *DDX3Y*, *RPS4Y1*, *EIF1AY*) being differentially expressed between age-matched male and female samples. In subsequent analyses, we therefore combined male and female samples. Using an absolute log2 fold difference ≥1.5 in combination with a significance threshold of *P*-adjusted value of 0.05, we identified a total of 156 DEGs. Two-way cluster analysis of all DEGs revealed distinct transcription profiles within all four groups (Figure 2A). In contrast to the clustering based on the global gene expression in Figure 1B, all samples now separated based on the trimester and chamber subtype. We found 24 DEGs in T1 atrium versus ventricle (A1/V1), 34 in T2 atrium versus ventricle (A2/V2), 110 in T2 versus T1 atria (A2/A1) and 39 in T2 versus T1 ventricles (V2/V1) (Supplemental Table 3). Figure 2B shows the overlap between the four comparisons, with the upper Venn diagram focusing on differences between atria and ventricles and the lower diagram on age. Overall, few genes overlapped between atria and ventricles and DEGs included genes that were reported to be expressed in a chamber-specific pattern in both mouse and human,

such as *NR2F1*, *MYL2* and *KCNA5*. Between T1 and T2, the number of overlapping genes per chamber was higher and correlated to chromatin and nucleosome structure (downregulated) and extracellular matrix and collagen organization (upregulated). The volcano plots in Figure 2C display the DEGs, with selected genes highlighted based on the results here and from earlier publications on the adult cardiac transcriptome [16,17].

In both T1 and T2 atria versus ventricle, *MYL2* was downregulated, reflecting its importance in ventricle contraction, whereas *KCNA5*, encoding the potassium channel $K_v1.5$, which conducts the ultra-rapid activating delayed rectifier K^+ current (I_{Kur}), a major repolarizing current in human atria [18], was upregulated. *MYL3* and *MYH7* were also described previously as differentially expressed in gene and protein studies comparing atria and ventricles in adult [4,17]. Among the genes expressed at higher levels in T1 and T2 atria compared with similarly aged ventricles were *NR2F1* (also known as *COUP-TFI*) and *RELN*. *NR2F1* was recently shown to be enriched in the atria of human foetal as well as adult hearts and to regulate atrial-specific ion channel genes in atrial hPSC-CMs [19]. *RELN* is involved in neuron migration and brain development [20], but has not previously been described in foetal heart development. Other studies have also detected higher expression levels of *RELN* in human adult atria [21], although its exact function in the heart is unknown.

Chromatin remodelling and histone modifications are also known to have an important role in heart development [22,23]. Genes encoding histones that influence nucleosome structure and are important in compaction of DNA (*HIST1H3I*, *HIST1H2BM*, *HIST1H2AI*) were mainly downregulated in T2 ventricles and atria, and to our knowledge have not previously been described in cardiac development. Genes important for extracellular matrix and collagen fibril organization (*COL1A2*, *COL12A1*, *COL15A1*, *DPT*) were upregulated in the T2 samples, indicating further development of the cardiac scaffold and maturation of the heart. Among the DEGs were also genes involved in cardiac development, electrical currents and sarcomere structure, such as *BMP10*, *APLNR*, *PLN*, *TNNI3K* and *MYOM2*. *BMP10* has been reported in mouse heart development [24] and previous studies have also detected *BMP10*, which is involved in the trabeculation of the heart, to be more highly expressed in atria [4,17].

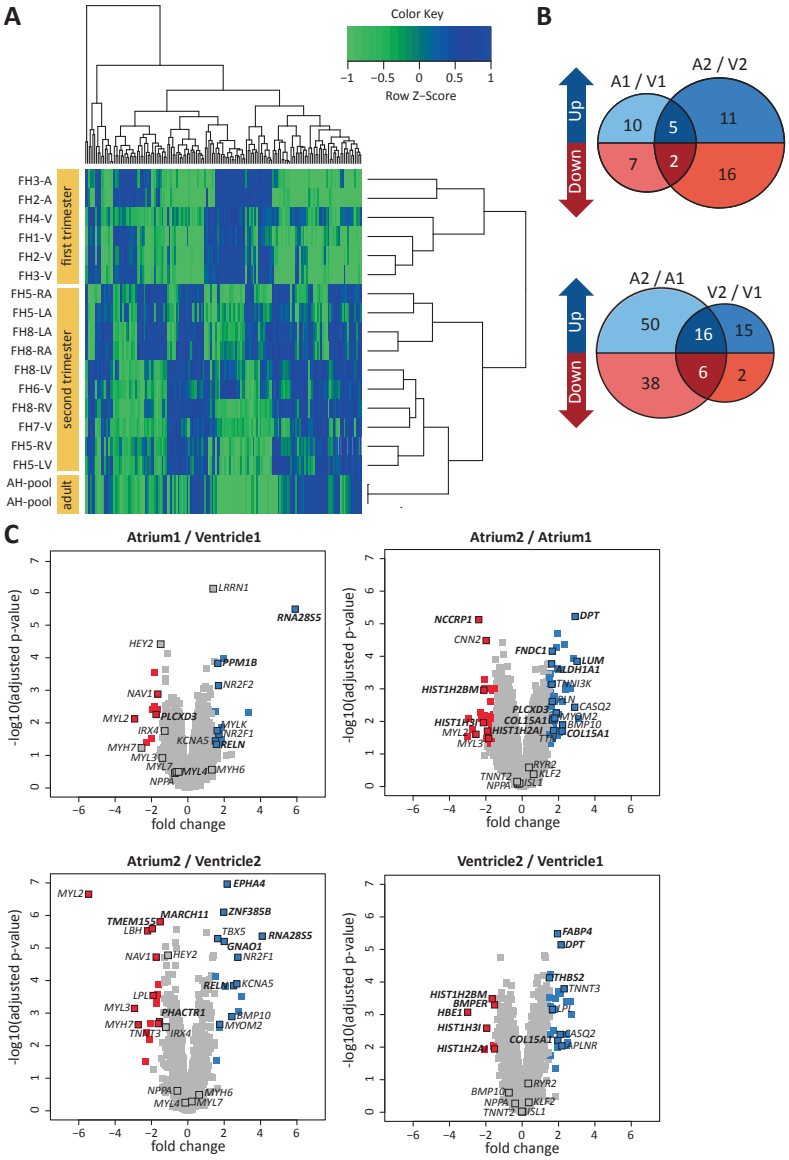

Figure 2. Comparison of differentially expressed genes (DEGs) between first and second trimester atria and ventricles

A, Unsupervised hierarchical clustering of the 156 DEGs identified. The dendrograms illustrate separation of the samples on first and second trimester and atria and ventricles. **B**, The Venn diagrams show the number of DEGs in each comparison (A1, *n*=2; A2, *n*=4; V1, *n*=4; V2, *n*=6) and the number of overlapping genes between the atria and ventricles per trimester (top) and between the first and second trimester per chamber (bottom). **C**, The Volcano plots show the total gene expression with positive (blue) and negative (red) log2 fold difference ≥1.5 (*x*-axis) against adjusted *P*-value ≤0.05 (*y*-axis). All other genes with adjusted *P*-value >0.05 are indicated in grey. Selected previously known genes and results from this study are highlighted. Genes not previously reported are indicated in bold.

Selected gene ontology terms show chamber-biased expression

To explore the functional characteristics of the DEGs, we performed GO.BP. Figure 3 and Supplemental Table 4 display GO.BP terms with adjusted *P*-value ≤0.01. As expected, among the genes expressed at lower levels in A2 versus V2 were those related to ventricle development, contraction and muscle morphogenesis and structure (Figure 3A). In A2 compared with A1, chromatin and nucleosome organizational genes were downregulated, suggesting that a process of active chromatin remodelling is slowing down at this stage of development. No GO.BP terms were enriched when V2 and V1 were compared. GO.BP terms involved in extracellular matrix organization, wound healing and blood coagulation were upregulated in T2 compared with T1 and included genes such as *VWF* and *APLNR* (Figure 3B). Foetal genes are typically upregulated during remodelling of the adult heart, for example after myocardial infarction, owing to the activation of pathways such as wound healing and stress responses [25,26]. We also found a significant over-representation of genes in atrial samples involved in neuron generation, forebrain development or neuron migration, such as *NR2F1* and *NR2F2*. This is likely to be due to innervation of the heart and the control of cardiac rhythm by the autonomous nervous system (the vagus nerve around the sinus node) at this stage of development [27], or due to genes that are expressed both in atria and the brain [28].

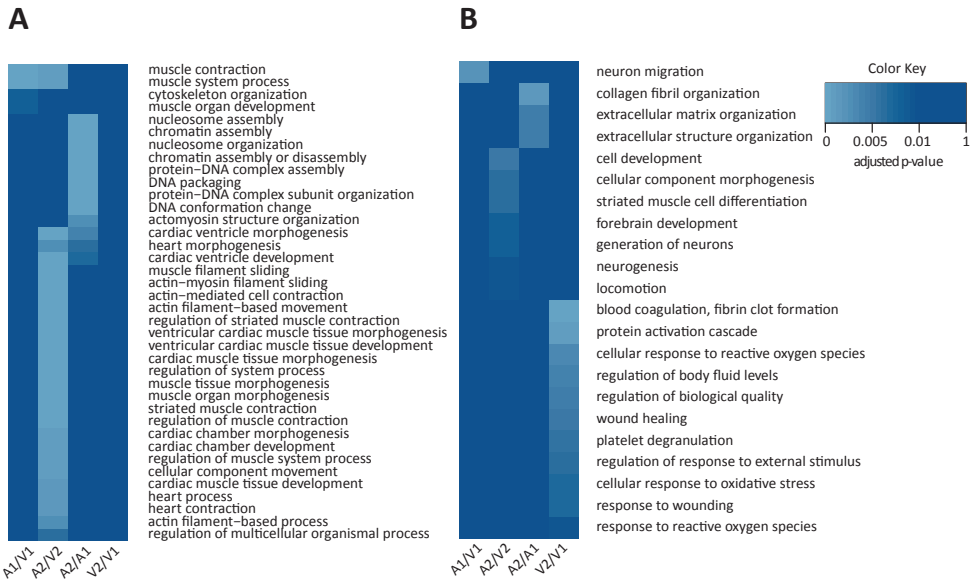

Figure 3. Enriched gene ontology terms belonging to biological processes (GO.BP)
A and **B**, Enriched GO terms of downregulated (A) and upregulated (B) differentially expressed genes for comparisons atria first trimester (A1)/ventricles first trimester (V1), atria second trimester (A2)/ventricles second trimester (V2), A2/A1 and V2/V1. Significantly enriched GO terms (Fisher's exact test; adjusted *P*-value ≤0.01) are shown in light blue.

The foetal heart transcriptome can indicate the maturation state of hPSC-CMs

To demonstrate the utility of this dataset, we compared human embryonic stem cell (hESC)- and hiPSC-derived CMs with the foetal heart samples with the expectation that this would provide insight into how their gene expression profiles relate to primary cardiac tissue (Figure 4A). An earlier study described the similarities and differences between foetal heart samples and CMs but the hESC-derived CMs included were derived from mixed population clusters and foetal heart samples were only obtained from third trimester donors [29]. Here, we had the opportunity to compare the hPSC-CMs with primary tissue at earlier stages of foetal development and from different chambers of the heart.

hPSC-CMs were initially purified from mixed populations of differentiated cells maintained in LI-BPEL medium on the basis of the reporter gene eGFP, which had been inserted into the cardiac transcription factor NKX2-5 (NKX2.5-GFP) genomic locus of hESCs [13] and hiPSCs (C.W.v.d.B., C.L.M. and R.P.D., unpublished). The differentiation protocols used here yield primarily ventricular-like cardiomyocytes on the basis of action potentials in patch clamp electrophysiology [30,31] and by excluding the NKX2.5-GFP$^-$ cardiomyocytes, we also excluded pacemaker-like cells [32]. Furthermore, we also examined hESC-derived CMs that were cultured in commercially available maturation medium (MM) containing T3 hormone as a principal component [10]. To examine similarities and differences between in vitro hPSC-CMs and foetal heart, we compared transcriptional profiles of the samples with the foetal heart from each trimester. Initially, we performed hierarchical cluster analysis of all PSC-derived cardiomyocytes together with all foetal heart samples (Supplemental Figure 1). As expected, all the foetal heart samples clustered closer to each other than to the hPSC-CMs. However, we did observe that hPSC-CMs cultured in MM clustered closer to the foetal heart samples than to the hPSC-CMs maintained in standard culture medium, suggesting that the cardiomyocytes had indeed developed further. To investigate which foetal heart age group the hPSC-CMs in LI-BPEL or MM most closely matched, we performed cluster analysis of the hPSC-CMs with the foetal heart samples from T1 and T2 separately (Figure 4B and 4C). The cells maintained in the regular LI-BPEL culture medium were more closely related to T1 foetal heart samples than were hPSC-CMs cultured in MM. When comparing the in vitro CMs to T2 samples, the CMs that had been maintained in MM more closely resembled the T2 heart. The partial maturation of the hESC-CMs in MM that we observed correlated well with another recent study that also showed that individual hPSC-CMs cultured in MM developed functional features closer to that of T2 foetal cardiomyocytes, with most strikingly a greater than twofold increase in contraction stress compared with hPSC-CMs cultured in regular differentiation conditions [10].

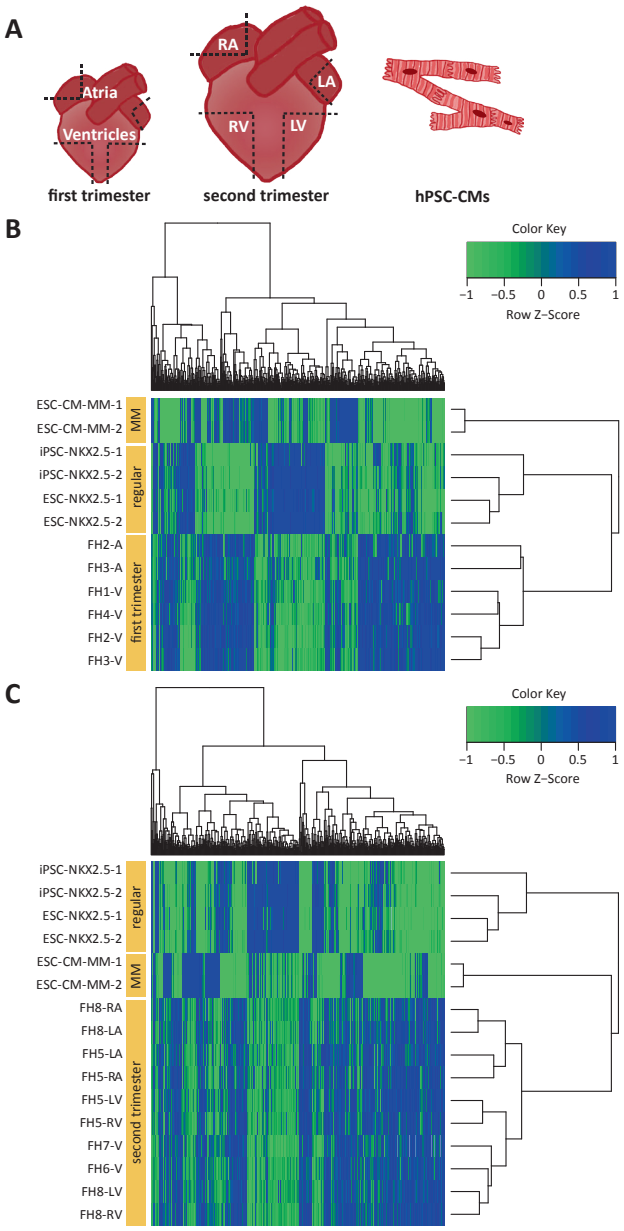

Figure 4. Hierarchical clustering of differentially expressed genes between foetal heart samples and human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs)

A, Schematic of compared samples from atria and ventricles of first and second trimester, and the hPSC-CMs. Dissection edges of the foetal heart samples are indicated by dashed lines. **B** and **C**, First trimester (B) and second trimester (C) foetal heart samples are compared with hPSC-CMs in LI-BPEL differentiation medium (regular) and maturation medium (MM). The dendrograms illustrate clustering of the hPSC-CMs with the first and second trimester foetal heart samples.

Conclusions

We have analysed here gene expression profiles in a rare and complete set of isogenic foetal heart samples and described differences in genes expressed between different chambers of the heart during the first and second trimester of development. We showed that microarray analysis could be performed on RNA samples as small as 50 ng, a technical advance that allowed inclusion of hearts at the very earliest stages of development. Our results revealed a group of nucleosome- and histone-related genes expressed in human foetal hearts that to our knowledge have not been described before in cardiac development in mice. Furthermore, we demonstrated how the foetal heart dataset can be used to benchmark hPSC-CMs in terms of their maturation state. The question of how mature hPSC-CMs are frequently arises and our dataset provides a means of answering this by global gene expression rather than on the basis of specific markers. We have included commercially available reference RNA sets in the analysis in order to characterize future sets of hPSC-CMs cultured in conditions that further induce maturation improvements. These reference sets can be used by other laboratories, as well as with other microarray platforms, for normalization and benchmarking of future datasets to the human foetal heart dataset. The foetal heart dataset is provided here as a resource to the community.

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Disclosures

S.R.B., R.P. and C.L.M. are co-founders and S.R.B. CSO of Pluriomics B.V.

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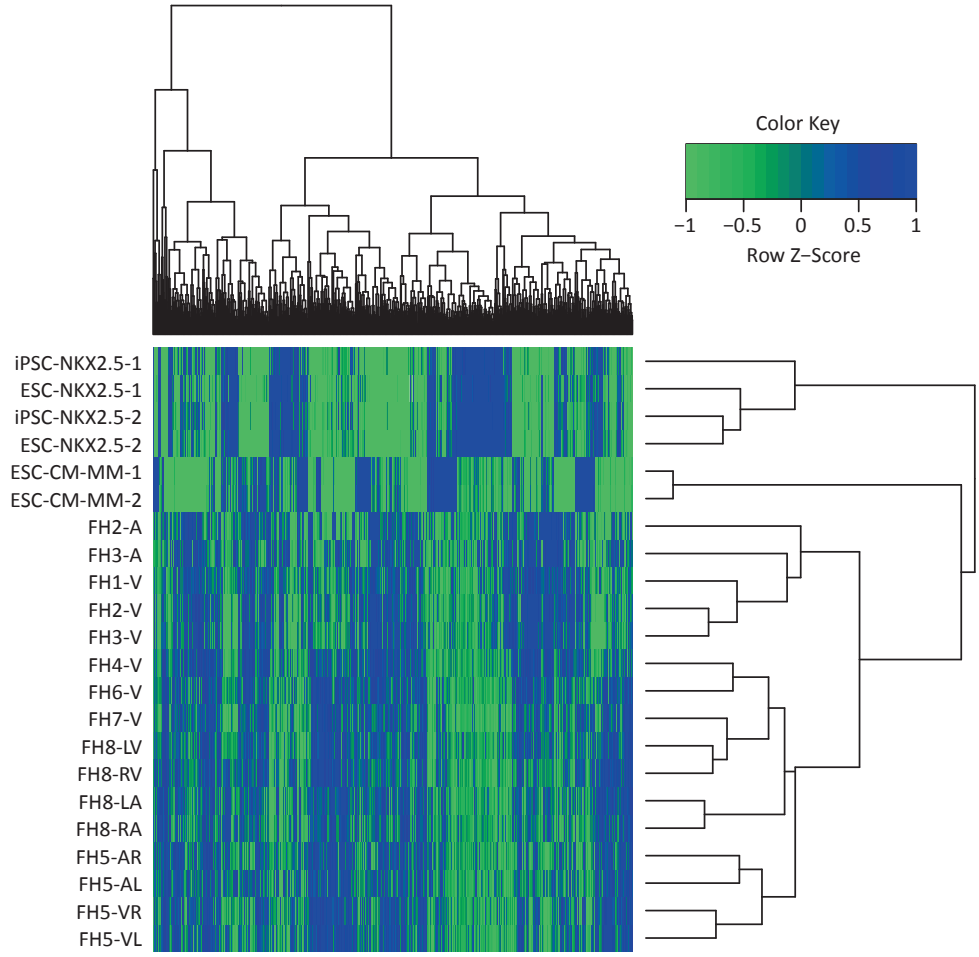

Supplemental Figure 1: Hierarchical clustering of differentially expressed genes between foetal heart samples and human pluripotent stem cell derived cardiomyocytes (PSC-CMs).
First trimester and second trimester foetal heart samples are compared with hPSC-CMs in LI-BPEL differentiation medium and maturation medium (MM). The dendograms illustrate closer clustering of the hPSC-CMs in MM with the foetal heart samples than the hiPSC-CMs in LI-BPEL (Fisher's exact test and Benjamini-Hochberg method).

Supplemental Table 1. Description of fetal heart and commercial reference samples

First trimester			
Code	**Name**	**Age of gestation (weeks.days)**	**Gender**
FH1-V	heart ventricle	7.2	female
FH2-V	heart ventricle	7.4	female
FH2-A	heart atrium	7.4	female
FH3-V	heart ventricle	7.4	male
FH3-A	heart atrium	7.4	male
FH4-V	heart ventricle	10.5	female
Second trimester			
Code	**Name**	**Age of gestation (weeks.days)**	**Gender**
FH5-LV	heart left ventricle	15	male
FH5-RV	heart right ventricle	15	male
FH5-LA	heart left atrium	15	male
FH5-RA	heart right atrium	15	male
FH6-V	heart ventricle	15.3	male
FH7-V	heart ventricle	20	male
FH8-LV	heart left ventricle	20+	female
FH8-RV	heart right ventricle	20+	female
FH8-LA	heart left atrium	20+	female
FH8-RA	heart right atrium	20+	female
Reference samples			
Code	**Name**	**Age (years)**	**Gender**
AH-pool	Human Normal Heart Donor Pool	21, 24, 27, 29, 44	male
Ref-pool	Universal Human Reference RNA		

Supplemental Table 2. GO.BP terms 7, 15, 20 weeks and adult

Cluster 1

ID	oddsRatio	padj	Name
GO:0006334	84,69	0,0000	nucleosome assembly
GO:0031497	78,48	0,0000	chromatin assembly
GO:0034728	74,23	0,0000	nucleosome organization
GO:0006333	67,80	0,0000	chromatin assembly or disassembly
GO:0065004	63,74	0,0000	protein-DNA complex assembly
GO:0006323	60,31	0,0000	DNA packaging
GO:0071824	60,31	0,0000	protein-DNA complex subunit organization
GO:0071103	48,49	0,0000	DNA conformation change
GO:0006325	17,07	0,0000	chromatin organization
GO:0034622	15,19	0,0000	cellular macromolecular complex assembly
GO:0051276	12,71	0,0000	chromosome organization
GO:0071844	10,43	0,0000	cellular component assembly at cellular level
GO:0034621	11,77	0,0000	cellular macromolecular complex subunit organization
GO:0022607	8,47	0,0000	cellular component assembly
GO:0065003	9,86	0,0000	macromolecular complex assembly
GO:0044085	7,43	0,0000	cellular component biogenesis
GO:0071842	6,09	0,0000	cellular component organization at cellular level
GO:0043933	8,30	0,0000	macromolecular complex subunit organization
GO:0016043	5,87	0,0000	cellular component organization
GO:0006259	8,89	0,0000	DNA metabolic process
GO:0045653	277,47	0,0000	negative regulation of megakaryocyte differentiation
GO:0071841	5,80	0,0000	cellular component organization or biogenesis at cellular level
GO:0071840	5,62	0,0000	cellular component organization or biogenesis
GO:0006996	6,09	0,0000	organelle organization
GO:0045652	123,78	0,0000	regulation of megakaryocyte differentiation
GO:0030219	79,66	0,0001	megakaryocyte differentiation
GO:0048015	25,17	0,0003	phosphatidylinositol-mediated signaling
GO:0048017	25,17	0,0003	inositol lipid-mediated signaling
GO:0006336	53,15	0,0003	DNA replication-independent nucleosome assembly
GO:0034080	53,15	0,0003	CenH3-containing nucleosome assembly at centromere
GO:0034724	53,15	0,0003	DNA replication-independent nucleosome organization
GO:0031055	48,54	0,0004	chromatin remodeling at centromere
GO:0043486	48,54	0,0004	histone exchange
GO:0045638	44,68	0,0005	negative regulation of myeloid cell differentiation
GO:0051093	9,97	0,0005	negative regulation of developmental process
GO:0043044	43,02	0,0005	ATP-dependent chromatin remodeling
GO:0051047	18,71	0,0007	positive regulation of secretion
GO:0060341	8,57	0,0011	regulation of cellular localization
GO:0051046	10,52	0,0014	regulation of secretion
GO:0044260	3,73	0,0014	cellular macromolecule metabolic process
GO:0045596	10,03	0,0016	negative regulation of cell differentiation
GO:0090304	3,71	0,0017	nucleic acid metabolic process
GO:0030154	3,91	0,0027	cell differentiation

GO:0000723	21,88	0,0029	telomere maintenance
GO:0032879	5,13	0,0029	regulation of localization
GO:0048856	3,48	0,0030	anatomical structure development
GO:0032200	21,46	0,0030	telomere organization
GO:0048731	3,55	0,0031	system development
GO:0043170	3,37	0,0034	macromolecule metabolic process
GO:0048869	3,71	0,0038	cellular developmental process
GO:0044281	3,21	0,0043	small molecule metabolic process
GO:0007275	3,31	0,0043	multicellular organismal development
GO:0006139	3,21	0,0043	nucleobase-containing compound metabolic process
GO:0051239	4,32	0,0044	regulation of multicellular organismal process
GO:0006338	17,16	0,0050	chromatin remodeling
GO:0030182	5,15	0,0050	neuron differentiation
GO:0030326	16,90	0,0050	embryonic limb morphogenesis
GO:0035113	16,90	0,0050	embryonic appendage morphogenesis
GO:0032502	3,26	0,0051	developmental process
GO:0045637	16,64	0,0051	regulation of myeloid cell differentiation
GO:0050796	16,64	0,0051	regulation of insulin secretion
GO:0048519	3,38	0,0051	negative regulation of biological process
GO:0090276	16,16	0,0054	regulation of peptide hormone secretion
GO:0046903	5,78	0,0056	secretion
GO:0002791	15,70	0,0058	regulation of peptide secretion
GO:0090087	15,70	0,0058	regulation of peptide transport
GO:0009987	5,28	0,0068	cellular process
GO:0048699	4,73	0,0071	generation of neurons
GO:0019932	8,86	0,0071	second-messenger-mediated signaling
GO:0030073	14,29	0,0071	insulin secretion
GO:0035107	14,29	0,0071	appendage morphogenesis
GO:0035108	14,29	0,0071	limb morphogenesis
GO:0048736	13,59	0,0079	appendage development
GO:0060173	13,59	0,0079	limb development
GO:0048523	3,26	0,0079	negative regulation of cellular process
GO:0030072	13,26	0,0083	peptide hormone secretion
GO:0046883	13,10	0,0085	regulation of hormone secretion
GO:0051049	5,15	0,0085	regulation of transport
GO:0032501	2,97	0,0088	multicellular organismal process
GO:0050794	2,94	0,0090	regulation of cellular process
GO:0034641	2,90	0,0090	cellular nitrogen compound metabolic process
GO:0002790	12,65	0,0090	peptide secretion
GO:0022008	4,42	0,0090	neurogenesis
GO:0006807	2,84	0,0099	nitrogen compound metabolic process
GO:0008284	5,89	0,0100	positive regulation of cell proliferation
GO:0051050	7,68	0,0101	positive regulation of transport
GO:0006935	5,85	0,0101	chemotaxis
GO:0042330	5,85	0,0101	taxis
GO:0045595	4,85	0,0104	regulation of cell differentiation
GO:0015833	11,59	0,0107	peptide transport

GO:0048522	3,06	0,0108	positive regulation of cellular process
GO:0008150	6,05	0,0110	biological_process
GO:0006352	11,13	0,0115	transcription initiation, DNA-dependent
GO:0048513	3,29	0,0115	organ development
GO:0009605	4,08	0,0123	response to external stimulus
GO:0001763	10,69	0,0125	morphogenesis of a branching structure
GO:0046879	10,69	0,0125	hormone secretion
GO:0032940	5,40	0,0129	secretion by cell
GO:0050793	4,01	0,0129	regulation of developmental process
GO:0009914	10,20	0,0138	hormone transport
GO:0007411	6,68	0,0144	axon guidance
GO:0065007	2,82	0,0153	biological regulation
GO:2000026	4,32	0,0157	regulation of multicellular organismal development
GO:0050789	2,70	0,0157	regulation of biological process
GO:0060249	9,58	0,0158	anatomical structure homeostasis
GO:0044238	2,75	0,0174	primary metabolic process
GO:0044237	2,74	0,0176	cellular metabolic process
GO:0000904	4,81	0,0185	cell morphogenesis involved in differentiation
GO:0007399	3,28	0,0211	nervous system development
GO:0031175	4,62	0,0213	neuron projection development
GO:0030099	8,16	0,0223	myeloid cell differentiation
GO:0001934	8,04	0,0230	positive regulation of protein phosphorylation
GO:0048518	2,79	0,0233	positive regulation of biological process
GO:0060429	5,35	0,0260	epithelium development
GO:0010564	5,29	0,0267	regulation of cell cycle process
GO:0042127	3,69	0,0270	regulation of cell proliferation
GO:0048732	7,39	0,0270	gland development
GO:0008219	3,08	0,0270	cell death
GO:0016265	3,08	0,0271	death
GO:0048666	4,19	0,0284	neuron development
GO:0040011	3,63	0,0286	locomotion
GO:0008283	3,24	0,0295	cell proliferation
GO:0042327	7,01	0,0295	positive regulation of phosphorylation
GO:0001932	4,05	0,0309	regulation of protein phosphorylation
GO:0051128	3,53	0,0309	regulation of cellular component organization
GO:0003001	6,79	0,0314	generation of a signal involved in cell-cell signaling
GO:0023061	6,79	0,0314	signal release
GO:0010562	6,71	0,0318	positive regulation of phosphorus metabolic process
GO:0045937	6,71	0,0318	positive regulation of phosphate metabolic process
GO:0035239	6,67	0,0320	tube morphogenesis
GO:0035556	2,92	0,0320	intracellular signal transduction
GO:0065008	2,76	0,0320	regulation of biological quality
GO:0016568	4,79	0,0325	chromatin modification
GO:0007409	4,76	0,0331	axonogenesis
GO:0045664	6,47	0,0336	regulation of neuron differentiation
GO:0032880	6,32	0,0354	regulation of protein localization
GO:0009653	2,83	0,0361	anatomical structure morphogenesis
GO:0010817	6,14	0,0376	regulation of hormone levels

GO:0042325	3,74	0,0377	regulation of phosphorylation
GO:0000902	3,73	0,0379	cell morphogenesis
GO:0009628	4,44	0,0392	response to abiotic stimulus
GO:0048667	4,40	0,0402	cell morphogenesis involved in neuron differentiation
GO:0030030	3,64	0,0407	cell projection organization
GO:0006915	2,93	0,0407	apoptosis
GO:0002009	5,78	0,0420	morphogenesis of an epithelium
GO:0019220	3,59	0,0420	regulation of phosphate metabolic process
GO:0051174	3,59	0,0420	regulation of phosphorus metabolic process
GO:0012501	2,90	0,0420	programmed cell death
GO:0048812	4,29	0,0422	neuron projection morphogenesis
GO:0010646	3,15	0,0429	regulation of cell communication
GO:0009967	4,20	0,0441	positive regulation of signal transduction
GO:0032989	3,48	0,0456	cellular component morphogenesis
GO:0045893	3,45	0,0468	positive regulation of transcription, DNA-dependent
GO:0050767	5,43	0,0468	regulation of neurogenesis
GO:0048585	4,09	0,0469	negative regulation of response to stimulus
GO:0010941	3,05	0,0471	regulation of cell death
GO:0010647	4,04	0,0483	positive regulation of cell communication
GO:0023056	4,00	0,0492	positive regulation of signaling

Cluster 2

ID	oddsRatio	padj	Name
GO:0006334	76,94	0,0000	nucleosome assembly
GO:0031497	71,36	0,0000	chromatin assembly
GO:0034728	67,44	0,0000	nucleosome organization
GO:0006333	61,48	0,0000	chromatin assembly or disassembly
GO:0065004	57,94	0,0000	protein-DNA complex assembly
GO:0006323	54,74	0,0000	DNA packaging
GO:0071824	54,74	0,0000	protein-DNA complex subunit organization
GO:0071103	43,84	0,0000	DNA conformation change
GO:0034622	17,14	0,0000	cellular macromolecular complex assembly
GO:0071844	11,25	0,0000	cellular component assembly at cellular level
GO:0034621	13,27	0,0000	cellular macromolecular complex subunit organization
GO:0006325	13,45	0,0001	chromatin organization
GO:0006996	7,72	0,0001	organelle organization
GO:0006259	10,03	0,0002	DNA metabolic process
GO:0065003	9,75	0,0002	macromolecular complex assembly
GO:0022607	8,08	0,0003	cellular component assembly
GO:0051276	10,01	0,0005	chromosome organization
GO:0043933	8,21	0,0005	macromolecular complex subunit organization
GO:0071842	5,88	0,0006	cellular component organization at cellular level
GO:0044085	7,08	0,0006	cellular component biogenesis
GO:0071841	5,59	0,0008	cellular component organization or biogenesis at cellular level
GO:0009611	7,86	0,0014	response to wounding
GO:0042060	9,60	0,0015	wound healing
GO:0006950	4,94	0,0029	response to stress

GO:0032501	4,46	0,0032	multicellular organismal process
GO:0016043	4,44	0,0034	cellular component organization
GO:0071840	4,25	0,0045	cellular component organization or biogenesis
GO:0007596	8,84	0,0058	blood coagulation
GO:0050817	8,75	0,0059	coagulation
GO:0007599	8,73	0,0059	hemostasis
GO:0050878	7,67	0,0087	regulation of body fluid levels
GO:0009987	9,70	0,0104	cellular process
GO:0043170	3,76	0,0129	macromolecule metabolic process
GO:0007010	6,08	0,0182	cytoskeleton organization
GO:0048610	7,73	0,0244	cellular process involved in reproduction
GO:0048731	3,38	0,0262	system development
GO:0008152	3,93	0,0288	metabolic process
GO:0006139	3,10	0,0293	nucleobase-containing compound metabolic process
GO:0090304	3,30	0,0314	nucleic acid metabolic process
GO:0071496	9,96	0,0459	cellular response to external stimulus
GO:0048856	2,94	0,0459	anatomical structure development
GO:0022414	4,30	0,0476	reproductive process
GO:0048608	9,58	0,0476	reproductive structure development
GO:0000003	4,28	0,0476	reproduction
GO:0008150	7,19	0,0476	biological_process

Cluster 3

ID	oddsRatio	padj	Name
GO:0001501	16,49	0,0000	skeletal system development
GO:0009888	8,30	0,0000	tissue development
GO:0030198	24,56	0,0000	extracellular matrix organization
GO:0043062	24,56	0,0000	extracellular structure organization
GO:0030199	56,29	0,0000	collagen fibril organization
GO:0043588	54,08	0,0000	skin development
GO:0071230	46,87	0,0000	cellular response to amino acid stimulus
GO:0071229	42,64	0,0000	cellular response to acid
GO:0071418	41,38	0,0000	cellular response to amine stimulus
GO:0071417	39,07	0,0000	cellular response to organic nitrogen
GO:0007179	20,30	0,0000	transforming growth factor beta receptor signaling pathway
GO:0032501	4,23	0,0000	multicellular organismal process
GO:0048468	5,82	0,0000	cell development
GO:0043200	27,58	0,0000	response to amino acid stimulus
GO:0050896	4,14	0,0000	response to stimulus
GO:0000904	7,76	0,0000	cell morphogenesis involved in differentiation
GO:0048731	4,26	0,0000	system development
GO:0032502	4,00	0,0000	developmental process
GO:0048513	4,64	0,0001	organ development
GO:0048856	4,04	0,0001	anatomical structure development
GO:0030182	6,24	0,0001	neuron differentiation
GO:0001101	20,07	0,0001	response to acid
GO:0014075	19,78	0,0001	response to amine stimulus

GO:0007275	3,84	0,0001	multicellular organismal development
GO:0030154	4,21	0,0001	cell differentiation
GO:0007178	12,73	0,0001	transmembrane receptor protein serine/threonine kinase signaling pathway
GO:0048699	5,73	0,0002	generation of neurons
GO:0042060	7,03	0,0002	wound healing
GO:0000902	6,01	0,0002	cell morphogenesis
GO:0071842	3,70	0,0002	cellular component organization at cellular level
GO:0048869	3,99	0,0002	cellular developmental process
GO:0032964	61,95	0,0002	collagen biosynthetic process
GO:0007411	8,99	0,0002	axon guidance
GO:0007409	7,52	0,0002	axonogenesis
GO:0022008	5,35	0,0002	neurogenesis
GO:0031175	6,52	0,0002	neuron projection development
GO:0007596	7,47	0,0002	blood coagulation
GO:0040011	5,32	0,0002	locomotion
GO:0050817	7,39	0,0002	coagulation
GO:0007599	7,37	0,0003	hemostasis
GO:0006935	7,14	0,0003	chemotaxis
GO:0042330	7,14	0,0003	taxis
GO:0017015	22,87	0,0003	regulation of transforming growth factor beta receptor signaling pathway
GO:0032989	5,60	0,0003	cellular component morphogenesis
GO:0010243	14,31	0,0003	response to organic nitrogen
GO:0071841	3,52	0,0003	cellular component organization or biogenesis at cellular level
GO:0048667	6,95	0,0003	cell morphogenesis involved in neuron differentiation
GO:0048812	6,77	0,0004	neuron projection morphogenesis
GO:0008544	13,21	0,0004	epidermis development
GO:0048666	5,90	0,0004	neuron development
GO:0016043	3,33	0,0005	cellular component organization
GO:0009653	4,02	0,0005	anatomical structure morphogenesis
GO:0050878	6,48	0,0005	regulation of body fluid levels
GO:0007399	4,20	0,0005	nervous system development
GO:0000279	6,41	0,0005	M phase
GO:0009611	5,10	0,0005	response to wounding
GO:0006950	3,54	0,0006	response to stress
GO:0065008	3,71	0,0007	regulation of biological quality
GO:0071840	3,19	0,0007	cellular component organization or biogenesis
GO:0007155	5,41	0,0007	cell adhesion
GO:0022610	5,41	0,0007	biological adhesion
GO:0001568	6,97	0,0007	blood vessel development
GO:0048858	6,00	0,0007	cell projection morphogenesis
GO:0032963	33,58	0,0007	collagen metabolic process
GO:0032990	5,89	0,0008	cell part morphogenesis
GO:0030030	5,14	0,0009	cell projection organization
GO:0007346	8,06	0,0009	regulation of mitotic cell cycle
GO:0044259	29,85	0,0010	multicellular organismal macromolecule metabolic process

GO:0001944	6,56	0,0010	vasculature development
GO:0009887	5,58	0,0011	organ morphogenesis
GO:0044236	26,01	0,0014	multicellular organismal metabolic process
GO:0009605	4,37	0,0015	response to external stimulus
GO:0051301	6,03	0,0015	cell division
GO:0010720	12,89	0,0017	positive regulation of cell development
GO:0030168	8,88	0,0018	platelet activation
GO:0045597	6,95	0,0018	positive regulation of cell differentiation
GO:0090092	12,59	0,0018	regulation of transmembrane receptor protein serine/ threonine kinase signaling pathway
GO:0072358	4,86	0,0023	cardiovascular system development
GO:0072359	4,86	0,0023	circulatory system development
GO:0045595	4,78	0,0024	regulation of cell differentiation
GO:0008150	5,42	0,0026	biological_process
GO:0051726	4,69	0,0027	regulation of cell cycle
GO:0007059	10,83	0,0028	chromosome segregation
GO:0000280	6,13	0,0029	nuclear division
GO:0007067	6,13	0,0029	mitosis
GO:0009719	4,58	0,0030	response to endogenous stimulus
GO:0065007	2,79	0,0030	biological regulation
GO:0000087	6,02	0,0031	M phase of mitotic cell cycle
GO:0010564	5,95	0,0032	regulation of cell cycle process
GO:0001775	5,06	0,0033	cell activation
GO:0048285	5,87	0,0034	organelle fission
GO:0071495	5,73	0,0038	cellular response to endogenous stimulus
GO:0007166	3,27	0,0041	cell surface receptor linked signaling pathway
GO:0007049	3,43	0,0042	cell cycle
GO:0016477	4,75	0,0044	cell migration
GO:0050900	9,18	0,0045	leukocyte migration
GO:0051716	2,67	0,0047	cellular response to stimulus
GO:0042221	2,96	0,0048	response to chemical stimulus
GO:0000278	4,13	0,0050	mitotic cell cycle
GO:0050793	3,78	0,0050	regulation of developmental process
GO:0048870	4,54	0,0054	cell motility
GO:0051674	4,54	0,0054	localization of cell
GO:0048015	13,64	0,0057	phosphatidylinositol-mediated signaling
GO:0048017	13,64	0,0057	inositol lipid-mediated signaling
GO:0022402	3,62	0,0064	cell cycle process
GO:0051094	5,01	0,0066	positive regulation of developmental process
GO:0006928	3,89	0,0067	cellular component movement
GO:0022403	3,85	0,0071	cell cycle phase
GO:0048592	12,37	0,0071	eye morphogenesis
GO:0010001	12,00	0,0076	glial cell differentiation
GO:0014031	12,00	0,0076	mesenchymal cell development
GO:0048762	11,16	0,0090	mesenchymal cell differentiation
GO:0048729	5,51	0,0091	tissue morphogenesis
GO:0001503	7,08	0,0095	ossification

GO:0051216	10,72	0,0099	cartilage development
GO:0060284	5,36	0,0100	regulation of cell development
GO:0035107	10,30	0,0108	appendage morphogenesis
GO:0035108	10,30	0,0108	limb morphogenesis
GO:0001649	10,04	0,0114	osteoblast differentiation
GO:0016331	10,04	0,0114	morphogenesis of embryonic epithelium
GO:0042063	9,92	0,0117	gliogenesis
GO:0048705	9,92	0,0117	skeletal system morphogenesis
GO:0071310	3,81	0,0117	cellular response to organic substance
GO:0007165	2,46	0,0118	signal transduction
GO:0048736	9,79	0,0119	appendage development
GO:0060173	9,79	0,0119	limb development
GO:0060485	9,67	0,0121	mesenchyme development
GO:0007126	9,34	0,0131	meiosis
GO:0051327	9,34	0,0131	M phase of meiotic cell cycle
GO:0060429	4,89	0,0133	epithelium development
GO:0007167	3,68	0,0133	enzyme linked receptor protein signaling pathway
GO:0016055	6,14	0,0138	Wnt receptor signaling pathway
GO:2000026	3,63	0,0139	regulation of multicellular organismal development
GO:0051321	9,02	0,0139	meiotic cell cycle
GO:0002376	3,04	0,0149	immune system process
GO:0000075	5,81	0,0158	cell cycle checkpoint
GO:0070887	3,17	0,0167	cellular response to chemical stimulus
GO:0008285	4,47	0,0175	negative regulation of cell proliferation
GO:0071156	5,60	0,0175	regulation of cell cycle arrest
GO:0048646	3,73	0,0196	anatomical structure formation involved in morphogenesis
GO:0010033	2,86	0,0200	response to organic substance
GO:0007093	7,56	0,0205	mitotic cell cycle checkpoint
GO:0003007	6,96	0,0247	heart morphogenesis
GO:0031589	6,30	0,0314	cell-substrate adhesion
GO:0051128	2,96	0,0330	regulation of cellular component organization
GO:0010647	3,69	0,0335	positive regulation of cell communication
GO:0048598	4,43	0,0342	embryonic morphogenesis
GO:0023056	3,66	0,0342	positive regulation of signaling
GO:0023052	2,18	0,0353	signaling
GO:0008283	2,63	0,0385	cell proliferation
GO:0007154	2,10	0,0385	cell communication
GO:0001654	5,59	0,0397	eye development
GO:0022604	5,55	0,0401	regulation of cell morphogenesis
GO:0051239	2,53	0,0446	regulation of multicellular organismal process
GO:0007507	3,97	0,0446	heart development

Cluster 4

ID	oddsRatio	padj	Name
GO:0022402	9,63	0,0000	cell cycle process
GO:0022403	10,16	0,0000	cell cycle phase
GO:0000278	10,08	0,0000	mitotic cell cycle
GO:0007049	7,06	0,0000	cell cycle
GO:0000279	10,14	0,0000	M phase
GO:0000280	9,99	0,0000	nuclear division
GO:0007067	9,99	0,0000	mitosis
GO:0000087	9,81	0,0000	M phase of mitotic cell cycle
GO:0048285	9,56	0,0000	organelle fission
GO:0007017	9,51	0,0000	microtubule-based process
GO:0051301	8,22	0,0000	cell division
GO:0071103	14,78	0,0000	DNA conformation change
GO:0000070	29,02	0,0000	mitotic sister chromatid segregation
GO:0000819	26,95	0,0000	sister chromatid segregation
GO:0006259	5,64	0,0001	DNA metabolic process
GO:0006323	15,51	0,0001	DNA packaging
GO:0007051	20,54	0,0001	spindle organization
GO:0051329	7,42	0,0001	interphase of mitotic cell cycle
GO:0048015	19,16	0,0002	phosphatidylinositol-mediated signaling
GO:0048017	19,16	0,0002	inositol lipid-mediated signaling
GO:0071841	3,44	0,0002	cellular component organization or biogenesis at cellular level
GO:0051325	7,26	0,0002	interphase
GO:0071842	3,35	0,0003	cellular component organization at cellular level
GO:0006996	3,66	0,0003	organelle organization
GO:0071840	3,02	0,0007	cellular component organization or biogenesis
GO:0010564	6,62	0,0007	regulation of cell cycle process
GO:0030154	3,40	0,0008	cell differentiation
GO:0030261	38,46	0,0010	chromosome condensation
GO:0016043	2,93	0,0010	cellular component organization
GO:0048869	3,22	0,0012	cellular developmental process
GO:0007059	11,16	0,0012	chromosome segregation
GO:0007052	28,45	0,0018	mitotic spindle organization
GO:2000026	4,40	0,0019	regulation of multicellular organismal development
GO:0000226	7,49	0,0019	microtubule cytoskeleton organization
GO:0007088	13,86	0,0020	regulation of mitosis
GO:0051783	13,86	0,0020	regulation of nuclear division
GO:0000075	7,32	0,0020	cell cycle checkpoint
GO:0007010	4,66	0,0022	cytoskeleton organization
GO:0071156	7,05	0,0023	regulation of cell cycle arrest
GO:0071174	24,24	0,0024	mitotic cell cycle spindle checkpoint
GO:0006271	23,35	0,0026	DNA strand elongation involved in DNA replication
GO:0031145	12,48	0,0027	anaphase-promoting complex-dependent proteasomal ubiquitin-dependent protein catabolic process
GO:0051276	4,49	0,0027	chromosome organization
GO:0022616	21,80	0,0030	DNA strand elongation

GO:0031577	21,80	0,0030	spindle checkpoint
GO:0030071	21,10	0,0032	regulation of mitotic metaphase/anaphase transition
GO:0007346	6,44	0,0033	regulation of mitotic cell cycle
GO:0000236	11,51	0,0033	mitotic prometaphase
GO:0000082	8,08	0,0034	G1/S transition of mitotic cell cycle
GO:0051726	4,27	0,0034	regulation of cell cycle
GO:0048856	2,65	0,0042	anatomical structure development
GO:0051656	10,06	0,0050	establishment of organelle localization
GO:0007091	17,21	0,0050	mitotic metaphase/anaphase transition
GO:0072527	16,76	0,0053	pyrimidine-containing compound metabolic process
GO:0031398	9,52	0,0059	positive regulation of protein ubiquitination
GO:0043161	6,88	0,0061	proteasomal ubiquitin-dependent protein catabolic process
GO:0010498	6,67	0,0069	proteasomal protein catabolic process
GO:0019932	6,59	0,0071	second-messenger-mediated signaling
GO:0009987	3,07	0,0075	cellular process
GO:0090090	13,91	0,0082	negative regulation of canonical Wnt receptor signaling pathway
GO:0007050	5,13	0,0084	cell cycle arrest
GO:0050793	3,38	0,0085	regulation of developmental process
GO:0007093	8,34	0,0085	mitotic cell cycle checkpoint
GO:0060070	8,26	0,0087	canonical Wnt receptor signaling pathway
GO:0010975	7,96	0,0098	regulation of neuron projection development
GO:0048699	3,49	0,0103	generation of neurons
GO:0000084	7,75	0,0106	S phase of mitotic cell cycle
GO:0006260	5,70	0,0114	DNA replication
GO:0009790	3,65	0,0122	embryo development
GO:0051320	7,24	0,0124	S phase
GO:0051640	7,24	0,0124	organelle localization
GO:0031396	7,12	0,0129	regulation of protein ubiquitination
GO:0051239	2,94	0,0129	regulation of multicellular organismal process
GO:0000910	10,88	0,0130	cytokinesis
GO:0048731	2,40	0,0132	system development
GO:0007275	2,33	0,0132	multicellular organismal development
GO:0022008	3,26	0,0134	neurogenesis
GO:0031344	6,89	0,0135	regulation of cell projection organization
GO:0051437	10,53	0,0136	positive regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle
GO:0006334	10,36	0,0142	nucleosome assembly
GO:0045786	4,31	0,0150	negative regulation of cell cycle
GO:0030178	10,04	0,0151	negative regulation of Wnt receptor signaling pathway
GO:0043009	4,30	0,0151	chordate embryonic development
GO:0009792	4,23	0,0157	embryo development ending in birth or egg hatching
GO:0000079	9,74	0,0157	regulation of cyclin-dependent protein kinase activity
GO:0045787	9,74	0,0157	positive regulation of cell cycle
GO:0051439	9,74	0,0157	regulation of ubiquitin-protein ligase activity involved in mitotic cell cycle
GO:0051443	9,74	0,0157	positive regulation of ubiquitin-protein ligase activity
GO:0031497	9,59	0,0162	chromatin assembly

GO:0006261	9,32	0,0173	DNA-dependent DNA replication
GO:0030182	3,31	0,0175	neuron differentiation
GO:0051351	9,19	0,0178	positive regulation of ligase activity
GO:0034728	9,06	0,0181	nucleosome organization
GO:0050789	2,11	0,0197	regulation of biological process
GO:0048666	3,47	0,0210	neuron development
GO:0021915	8,47	0,0210	neural tube development
GO:0006333	8,25	0,0223	chromatin assembly or disassembly
GO:0051438	8,25	0,0223	regulation of ubiquitin-protein ligase activity
GO:0060828	8,15	0,0229	regulation of canonical Wnt receptor signaling pathway
GO:0051130	4,45	0,0230	positive regulation of cellular component organization
GO:0007399	2,61	0,0231	nervous system development
GO:0008283	2,72	0,0238	cell proliferation
GO:0032502	2,20	0,0242	developmental process
GO:0051340	7,85	0,0243	regulation of ligase activity
GO:0033043	4,33	0,0247	regulation of organelle organization
GO:0007018	7,76	0,0248	microtubule-based movement
GO:0065004	7,76	0,0248	protein-DNA complex assembly
GO:0045595	3,25	0,0262	regulation of cell differentiation
GO:0000072	207,99	0,0271	M phase specific microtubule process
GO:0071824	7,32	0,0275	protein-DNA complex subunit organization
GO:0045664	5,14	0,0276	regulation of neuron differentiation
GO:0008150	2,65	0,0286	biological_process
GO:0090068	7,16	0,0286	positive regulation of cell cycle process
GO:0021700	7,00	0,0301	developmental maturation
GO:0016055	4,96	0,0302	Wnt receptor signaling pathway
GO:0006511	3,99	0,0309	ubiquitin-dependent protein catabolic process
GO:0019941	3,91	0,0332	modification-dependent protein catabolic process
GO:0050794	1,99	0,0336	regulation of cellular process
GO:0043632	3,86	0,0343	modification-dependent macromolecule catabolic process
GO:0023052	2,06	0,0345	signaling
GO:0030030	3,02	0,0347	cell projection organization
GO:0051129	4,64	0,0357	negative regulation of cellular component organization
GO:0051603	3,77	0,0365	proteolysis involved in cellular protein catabolic process
GO:0051128	2,75	0,0366	regulation of cellular component organization
GO:0065007	1,94	0,0375	biological regulation
GO:0010639	6,20	0,0381	negative regulation of organelle organization
GO:0044257	3,69	0,0384	cellular protein catabolic process
GO:0048468	2,55	0,0386	cell development
GO:0031175	3,22	0,0386	neuron projection development
GO:0010638	5,97	0,0408	positive regulation of organelle organization
GO:0030111	5,91	0,0416	regulation of Wnt receptor signaling pathway
GO:0032269	4,32	0,0421	negative regulation of cellular protein metabolic process
GO:0050767	4,32	0,0421	regulation of neurogenesis
GO:0032501	1,93	0,0477	multicellular organismal process
GO:0044265	3,02	0,0478	cellular macromolecule catabolic process

Cluster 5

ID	oddsRatio	padj	Name
GO:0030185	737,90	0,0214	nitric oxide transport
GO:0015671	454,83	0,0267	oxygen transport
GO:0015669	283,64	0,0327	gas transport
GO:0003008	13,68	0,0351	system process
GO:0042744	190,99	0,0402	hydrogen peroxide catabolic process

Cluster 6

ID	oddsRatio	padj	Name
GO:0072376	49,70	0,0015	protein activation cascade
GO:0006067	96,07	0,0047	ethanol metabolic process
GO:0006069	96,07	0,0047	ethanol oxidation
GO:0034308	96,07	0,0047	primary alcohol metabolic process
GO:0050896	3,23	0,0142	response to stimulus
GO:0032501	2,98	0,0238	multicellular organismal process
GO:0009611	4,24	0,0343	response to wounding
GO:0042221	3,07	0,0343	response to chemical stimulus
GO:0006952	4,34	0,0441	defense response
GO:0006936	7,40	0,0441	muscle contraction
GO:0009605	3,63	0,0441	response to external stimulus
GO:0007155	4,12	0,0441	cell adhesion
GO:0022610	4,12	0,0441	biological adhesion
GO:0003012	6,75	0,0457	muscle system process
GO:0006805	11,64	0,0496	xenobiotic metabolic process
GO:0071466	11,64	0,0496	cellular response to xenobiotic stimulus

Cluster 7

ID	oddsRatio	padj	Name
GO:0055098	227,55	0,0003	response to low-density lipoprotein particle stimulus
GO:0055094	113,84	0,0007	response to lipoprotein stimulus
GO:0000302	19,72	0,0008	response to reactive oxygen species
GO:0034614	26,54	0,0018	cellular response to reactive oxygen species
GO:0042542	21,86	0,0020	response to hydrogen peroxide
GO:0034599	18,59	0,0033	cellular response to oxidative stress
GO:0070301	31,44	0,0050	cellular response to hydrogen peroxide
GO:0006979	9,74	0,0056	response to oxidative stress
GO:0050921	24,00	0,0057	positive regulation of chemotaxis
GO:0003012	9,06	0,0057	muscle system process
GO:0019752	5,11	0,0061	carboxylic acid metabolic process
GO:0043436	5,11	0,0061	oxoacid metabolic process
GO:0006082	5,04	0,0063	organic acid metabolic process
GO:0006084	21,69	0,0063	acetyl-CoA metabolic process
GO:0048520	21,69	0,0063	positive regulation of behavior
GO:0042180	4,92	0,0067	cellular ketone metabolic process
GO:0050920	18,22	0,0087	regulation of chemotaxis
GO:0050795	15,70	0,0122	regulation of behavior

GO:0032787	6,72	0,0130	monocarboxylic acid metabolic process
GO:0010035	6,60	0,0137	response to inorganic substance
GO:0010952	13,79	0,0145	positive regulation of peptidase activity
GO:0032103	13,58	0,0149	positive regulation of response to external stimulus
GO:0009719	4,48	0,0150	response to endogenous stimulus
GO:0032101	8,19	0,0156	regulation of response to external stimulus
GO:0006936	7,73	0,0181	muscle contraction
GO:0006633	12,12	0,0181	fatty acid biosynthetic process
GO:0016053	7,63	0,0181	organic acid biosynthetic process
GO:0046394	7,63	0,0181	carboxylic acid biosynthetic process
GO:0006575	11,51	0,0191	cellular modified amino acid metabolic process
GO:0043408	7,05	0,0220	regulation of MAPKKK cascade
GO:0010627	5,09	0,0265	regulation of intracellular protein kinase cascade
GO:0010565	8,56	0,0350	regulation of cellular ketone metabolic process
GO:0009987	2,98	0,0404	cellular process
GO:0006139	0,27	0,0405	nucleobase-containing compound metabolic process
GO:0008150	3,50	0,0405	biological_process
GO:0030335	7,49	0,0438	positive regulation of cell migration
GO:2000147	7,36	0,0453	positive regulation of cell motility
GO:0052547	7,31	0,0458	regulation of peptidase activity
GO:0007610	5,09	0,0468	behavior
GO:0040017	7,13	0,0468	positive regulation of locomotion
GO:0010876	7,07	0,0468	lipid localization
GO:0051272	6,96	0,0482	positive regulation of cellular component movement
GO:0080135	6,75	0,0500	regulation of cellular response to stress

Cluster 8

ID	oddsRatio	padj	Name
GO:0006629	4,47	0,0024	lipid metabolic process
GO:0032787	6,64	0,0056	monocarboxylic acid metabolic process
GO:0006631	7,28	0,0083	fatty acid metabolic process
GO:0006805	12,93	0,0089	xenobiotic metabolic process
GO:0071466	12,93	0,0089	cellular response to xenobiotic stimulus
GO:0009410	12,74	0,0091	response to xenobiotic stimulus
GO:0016042	8,52	0,0099	lipid catabolic process
GO:0031032	18,53	0,0137	actomyosin structure organization
GO:0051591	16,15	0,0166	response to cAMP
GO:0019752	3,87	0,0166	carboxylic acid metabolic process
GO:0043436	3,87	0,0166	oxoacid metabolic process
GO:0006082	3,82	0,0175	organic acid metabolic process
GO:0009056	2,80	0,0176	catabolic process
GO:0042180	3,73	0,0182	cellular ketone metabolic process
GO:0006956	25,83	0,0323	complement activation
GO:0019395	9,98	0,0366	fatty acid oxidation
GO:0034440	9,83	0,0366	lipid oxidation

Supplemental Table 3. Differentially expressed genes

A1-V1

Upregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
RNA28S5	0,00	5,91	RNA, 28S ribosomal 5	ENSG00000266658	100008589
RNA18S5	0,00	3,32	RNA, 18S ribosomal 5	ENSG00000272060	100008588
NCCRP1	0,00	1,95	non-specific cytotoxic cell receptor protein 1 homolog (zebrafish)	ENSG00000188505	342897
RAB27A	0,01	1,87	RAB27A, member RAS oncogene family	ENSG00000069974	5873
NFE2	0,03	1,82	nuclear factor, erythroid 2	ENSG00000123405	4778
KIF1B	0,04	1,77	kinesin family member 1B	ENSG00000054523	23095
NR2F1	0,02	1,71	nuclear receptor subfamily 2, group F, member 1	ENSG00000175745	7025
NR2F2	0,00	1,69	nuclear receptor subfamily 2, group F, member 2	ENSG00000185551	7026
LRRC45	0,02	1,66	leucine rich repeat containing 45	ENSG00000169683	201255
PPM1B	0,00	1,65	protein phosphatase, Mg2+/Mn2+ dependent, 1B	ENSG00000138032	5495
MYLK	0,02	1,61	myosin light chain kinase	ENSG00000065534	4638
RELN	0,05	1,57	reelin	ENSG00000189056	5649
LOC595101	0,04	1,54	smg-1 homolog, phosphatidylinositol 3-kinase-related kinase (C. elegans) pseudogene	ENSG00000183604	595101
KCNA5	0,04	1,52	potassium voltage-gated channel, shaker-related subfamily, member 5	ENSG00000130037	3741
PLCG2	0,00	1,50	phospholipase C, gamma 2 (phosphatidylinositol-specific)	ENSG00000197943	5336
Downregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
MYL2	0,01	-2,93	myosin, light chain 2, regulatory, cardiac, slow	ENSG00000111245	4633
SORBS1	0,04	-2,27	sorbin and SH3 domain containing 1	ENSG00000095637	10580

SNORD46	0,03	-2,01	small nucleolar RNA, C/D box 46	ENSG00000200913	94161
FGF12	0,00	-1,97	fibroblast growth factor 12	ENSG00000114279	2257
GYG2	0,00	-1,85	glycogenin 2	ENSG00000056998	8908
GJA1	0,00	-1,84	gap junction protein, alpha 1, 43kDa	ENSG00000152661	2697
PLCXD3	0,01	-1,75	phosphatidylinositol-specific phospholipase C, X domain containing 3	ENSG00000182836	345557
CRYAB	0,00	-1,67	crystallin, alpha B	ENSG00000109846	1410
NAV1	0,00	-1,65	neuron navigator 1	ENSG00000134369	89796

A2-V2

Upregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
RNA28S5	0,00	4,09	RNA, 28S ribosomal 5	ENSG00000266658	100008589
C19orf59	0,00	2,95	chromosome 19 open reading frame 59	ENSG00000183019	199675
RNA18S5	0,00	2,79	RNA, 18S ribosomal 5	ENSG00000272060	100008588
NR2F1	0,00	2,76	nuclear receptor subfamily 2, group F, member 1	ENSG00000175745	7025
KCNA5	0,00	2,70	potassium voltage-gated channel, shaker-related subfamily, member 5	ENSG00000130037	3741
RELN	0,00	2,51	reelin	ENSG00000189056	5649
BMP10	0,00	2,42	bone morphogenetic protein 10	ENSG00000163217	27302
EPHA4	0,00	2,16	EPH receptor A4	ENSG00000116106	2043
CLDN18	0,00	2,09	claudin 18	ENSG00000066405	51208
GNAO1	0,00	2,00	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide O	ENSG00000087258	2775
ZNF385B	0,00	1,97	zinc finger protein 385B	ENSG00000144331	151126
MYOM2	0,00	1,75	myomesin 2	ENSG00000036448	9172
ADAMTS8	0,00	1,74	ADAM metallopeptidase with thrombospondin type 1 motif, 8	ENSG00000134917	11095
TBX5	0,00	1,64	T-box 5	ENSG00000089225	6910
TNC	0,03	1,59	tenascin C	ENSG00000041982	3371
TSPAN7	0,00	1,51	tetraspanin 7	ENSG00000156298	7102

Downregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
MYL2	0,00	-5,46	myosin, light chain 2, regulatory, cardiac, slow	ENSG00000111245	4633
MYL3	0,00	-2,94	myosin, light chain 3, alkali; ventricular, skeletal, slow	ENSG00000160808	4634
MYH7	0,00	-2,73	myosin, heavy chain 7, cardiac muscle, beta	ENSG00000092054	4625
FAM69C	0,03	-2,36	family with sequence similarity 69, member C	ENSG00000187773	125704
CPVL	0,00	-2,30	carboxypeptidase, vitellogenic-like	ENSG00000106066	54504
LBH	0,00	-2,22	limb bud and heart development	ENSG00000213626	81606
PRND	0,01	-2,10	prion protein 2 (dublet)	ENSG00000171864	23627
PRDM8	0,00	-2,07	PR domain containing 8	ENSG00000152784	56978
TMEM155	0,00	-1,96	transmembrane protein 155	ENSG00000164112	132332
LPL	0,00	-1,91	lipoprotein lipase	ENSG00000175445	4023
NAV1	0,00	-1,74	neuron navigator 1	ENSG00000134369	89796
XPO4	0,00	-1,73	exportin 4	ENSG00000132953	64328
F3	0,00	-1,72	coagulation factor III (thromboplastin, tissue factor)	ENSG00000117525	2152
SLC1A3	0,00	-1,67	solute carrier family 1 (glial high affinity glutamate transporter), member 3	ENSG00000079215	6507
TNNT3	0,00	-1,63	troponin T type 3 (skeletal, fast)	ENSG00000130595	7140
PHLDB2	0,00	-1,59	pleckstrin homology-like domain, family B, member 2	ENSG00000144824	90102
PHACTR1	0,00	-1,56	phosphatase and actin regulator 1	ENSG00000112137	221692
MARCH11	0,00	-1,53	membrane-associated ring finger (C3HC4) 11	ENSG00000183654	441061

A2-A1

Upregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
HBB	0,01	3,11	hemoglobin, beta	ENSG00000244734	3043
LUM	0,00	3,02	lumican	ENSG00000139329	4060
DPT	0,00	2,91	dermatopontin	ENSG00000143196	1805
CASQ2	0,00	2,89	calsequestrin 2 (cardiac muscle)	ENSG00000118729	845
OGN	0,00	2,82	osteoglycin	ENSG00000106809	4969
DCN	0,00	2,65	decorin	ENSG00000011465	1634
RNY3	0,00	2,45	RNA, Ro-associated Y3	ENSG00000202354	6085
PODN	0,00	2,37	podocan	ENSG00000174348	127435
THBS2	0,00	2,30	thrombospondin 2	ENSG00000186340	7058
BMP10	0,01	2,22	bone morphogenetic protein 10	ENSG00000163217	27302
FGF12	0,00	2,21	fibroblast growth factor 12	ENSG00000114279	2257
COL15A1	0,02	2,19	collagen, type XV, alpha 1	ENSG00000204291	1306
SPARCL1	0,00	2,11	SPARC-like 1 (hevin)	ENSG00000152583	8404
POSTN	0,02	2,08	periostin, osteoblast specific factor	ENSG00000133110	10631
SNORD3C	0,02	2,03	small nucleolar RNA, C/D box 3C	ENSG00000264940	780853
DHRS7C	0,01	1,97	dehydrogenase/reductase (SDR family) member 7C	ENSG00000184544	201140
EPHA4	0,00	1,94	EPH receptor A4	ENSG00000116106	2043
SNORD3A	0,03	1,93	small nucleolar RNA, C/D box 3A	ENSG00000263934	780851
PLCB1	0,00	1,92	phospholipase C, beta 1 (phosphoinositide-specific)	ENSG00000182621	23236
CCDC102B	0,00	1,88	coiled-coil domain containing 102B	ENSG00000150636	79839
PLCXD3	0,01	1,88	phosphatidylinositol-specific phospholipase C, X domain containing 3	ENSG00000182836	345557
SNORD3D	0,02	1,84	small nucleolar RNA, C/D box 3D	ENSG00000262202	780854
MOB2	0,00	1,80	MOB kinase activator 2	ENSG00000182208	81532
MYOM2	0,01	1,80	myomesin 2	ENSG00000036448	9172
ZNF706	0,01	1,78	zinc finger protein 706	ENSG00000120963	51123
SLC9A4	0,00	1,77	solute carrier family 9, subfamily A (NHE4, cation proton antiporter 4), member 4	ENSG00000180251	389015
INMT	0,00	1,73	indolethylamine N-methyltransferase	ENSG00000241644	11185

GJA1	0,00	1,73	gap junction protein, alpha 1, 43kDa	ENSG00000152661	2697
TTN	0,02	1,73	titin	ENSG00000155657	7273
TPR	0,01	1,71	translocated promoter region, nuclear basket protein	ENSG00000047410	7175
ALDOC	0,02	1,70	aldolase C, fructose-bisphosphate	ENSG00000109107	230
CDR1	0,02	1,70	cerebellar degeneration-related protein 1, 34kDa	ENSG00000184258	1038
C19orf59	0,03	1,68	chromosome 19 open reading frame 59	ENSG00000183019	199675
SERPINE2	0,03	1,68	serpin peptidase inhi-bitor, clade E (nexin, plasmi-nogen activator inhibitor type 1), member 2	ENSG00000135919	5270
TMPRSS11BNL	0,01	1,68	TMPRSS11B N-terminal like	ENSG00000226894	401136
COL1A2	0,01	1,67	collagen, type I, alpha 2	ENSG00000164692	1278
FNDC1	0,00	1,65	fibronectin type III domain containing 1	ENSG00000164694	84624
PLN	0,00	1,65	phospholamban	ENSG00000198523	5350
SYNE1	0,00	1,65	spectrin repeat containing, nuclear envelope 1	ENSG00000131018	23345
CLDN18	0,01	1,65	claudin 18	ENSG00000066405	51208
HHATL	0,01	1,64	hedgehog acyltransferase-like	ENSG00000010282	57467
RBP7	0,02	1,64	retinol binding protein 7, cellular	ENSG00000162444	116362
TNNI3K	0,00	1,63	TNNI3 interacting kinase	ENSG00000116783	51086
MIR1299	0,02	1,63	microRNA 1299	ENSG00000239070	100302167
C7	0,01	1,63	complement component 7	ENSG00000112936	730
ALDH1A1	0,00	1,62	aldehyde dehydrogenase 1 family, member A1	ENSG00000165092	216
EPHA3	0,01	1,62	EPH receptor A3	ENSG00000044524	2042
TRIM4	0,00	1,61	tripartite motif containing 4	ENSG00000146833	89122
EIF4E	0,01	1,61	eukaryotic translation initiation factor 4E	ENSG00000151247	1977
ZDHHC2	0,01	1,60	zinc finger, DHHC-type containing 2	ENSG00000104219	51201
IPW	0,02	1,59	imprinted in Prader-Willi syndrome (non-protein coding)	ENSG00000224078	3653
SGSM1	0,01	1,59	small G protein signaling modulator 1	ENSG00000167037	129049
ABLIM3	0,00	1,58	actin binding LIM protein family, member 3	ENSG00000173210	22885

A2M	0,02	1,58	alpha-2-macroglobulin	ENSG00000175899	2
COL12A1	0,00	1,58	collagen, type XII, alpha 1	ENSG00000111799	1303
LRR1	0,01	1,58	leucine rich repeat protein 1	ENSG00000165501	122769
CYLC2	0,01	1,56	cylicin, basic protein of sperm head cytoskeleton 2	ENSG00000155833	1539
MB	0,05	1,56	myoglobin	ENSG00000198125	4151
CMYA5	0,01	1,55	cardiomyopathy associated 5	ENSG00000164309	202333
COX7A1	0,02	1,55	cytochrome c oxidase subunit VIIa polypeptide 1 (muscle)	ENSG00000161281	1346
CYP2B6	0,03	1,54	cytochrome P450, family 2, subfamily B, polypeptide 6	ENSG00000197408	1555
SNORD116-4	0,02	1,53	small nucleolar RNA, C/D box 116-4	ENSG00000224078	100033416
MSTO2P	0,01	1,52	misato family member 2, pseudogene	NA	100129405
TXNIP	0,00	1,52	thioredoxin interacting protein	ENSG00000117289	10628
ZNHIT3	0,02	1,51	zinc finger, HIT-type containing 3	ENSG00000108278	9326
SDPR	0,00	1,51	serum deprivation response	ENSG00000168497	8436

Downregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
MT1H	0,03	-3,03	metallothionein 1H	ENSG00000205358	4496
HBE1	0,01	-2,96	hemoglobin, epsilon 1	ENSG00000213931	3046
HBZ	0,02	-2,75	hemoglobin, zeta	ENSG00000130656	3050
MYL2	0,02	-2,54	myosin, light chain 2, regulatory, cardiac, slow	ENSG00000111245	4633
NCCRP1	0,00	-2,39	non-specific cytotoxic cell receptor protein 1 homolog (zebrafish)	ENSG00000188505	342897
HIST2H2AA3	0,01	-2,30	histone cluster 2, H2aa3	ENSG00000183558	8337
HIST1H4B	0,01	-2,25	histone cluster 1, H4b	ENSG00000124529	8366
HIST1H3I	0,01	-2,14	histone cluster 1, H3i	ENSG00000182572	8354
HAPLN1	0,01	-2,12	hyaluronan and proteoglycan link protein 1	ENSG00000145681	1404
HIST1H2BM	0,00	-2,10	histone cluster 1, H2bm	ENSG00000196374	8342
COL2A1	0,01	-2,07	collagen, type II, alpha 1	ENSG00000139219	1280
DOK4	0,00	-2,06	docking protein 4	ENSG00000125170	55715
SFRP2	0,00	-2,06	secreted frizzled-related protein 2	ENSG00000145423	6423

HIST2H3C	0,01	-2,02	histone cluster 2, H3c	ENSG00000183598	126961
CNN2	0,00	-1,99	calponin 2	ENSG00000064666	1265
MT1G	0,03	-1,98	metallothionein 1G	ENSG00000125144	4495
HIST1H2AI	0,02	-1,91	histone cluster 1, H2ai	ENSG00000196747	8329
HIST1H2BK	0,00	-1,91	histone cluster 1, H2bk	ENSG00000197903	85236
MAGED2	0,00	-1,87	melanoma antigen family D, 2	ENSG00000102316	10916
HIST1H4H	0,02	-1,85	histone cluster 1, H4h	ENSG00000158406	8365
HIST2H2AB	0,04	-1,85	histone cluster 2, H2ab	ENSG00000184270	317772
MYL3	0,03	-1,84	myosin, light chain 3, alkali; ventricular, skeletal, slow	ENSG00000160808	4634
HIST1H2BF	0,01	-1,79	histone cluster 1, H2bf	ENSG00000180596	8343
KRT19	0,01	-1,77	keratin 19	ENSG00000171345	3880
FBN3	0,00	-1,75	fibrillin 3	ENSG00000142449	84467
RNA28S5	0,05	-1,74	RNA, 28S ribosomal 5	ENSG00000266658	100008589
HIST1H4L	0,01	-1,74	histone cluster 1, H4l	ENSG00000198558	8368
ELOF1	0,04	-1,74	elongation factor 1 homolog (S. cerevisiae)	ENSG00000130165	84337
HIST1H3F	0,02	-1,71	histone cluster 1, H3f	ENSG00000256316	8968
HIST1H2AE	0,01	-1,71	histone cluster 1, H2ae	ENSG00000168274	3012
HIST1H4C	0,01	-1,70	histone cluster 1, H4c	ENSG00000183941	8364
HIST1H4E	0,04	-1,65	histone cluster 1, H4e	ENSG00000183941	8367
LOC595101	0,02	-1,65	smg-1 homolog, phosphatidylinositol 3-kinase-related kinase (C. elegans) pseudogene	ENSG00000183604	595101
HIST1H1A	0,00	-1,63	histone cluster 1, H1a	ENSG00000124610	3024
KRT8	0,01	-1,60	keratin 8	ENSG00000170421	3856
HIST1H4I	0,02	-1,59	histone cluster 1, H4i	ENSG00000183941	8294
LRRC45	0,02	-1,59	leucine rich repeat containing 45	ENSG00000169683	201255
RAB27A	0,02	-1,57	RAB27A, member RAS oncogene family	ENSG00000069974	5873
GLIPR2	0,01	-1,56	GLI pathogenesis-related 2	ENSG00000122694	152007
HIST1H2AJ	0,01	-1,56	histone cluster 1, H2aj	ENSG00000182611	8331
CTSV	0,01	-1,52	cathepsin V	ENSG00000136943	1515
PRDX2	0,02	-1,52	peroxiredoxin 2	ENSG00000167815	7001
CRABP2	0,01	-1,52	cellular retinoic acid binding protein 2	ENSG00000143320	1382
SMTNL2	0,00	-1,50	smoothelin-like 2	ENSG00000188176	342527

V2-V1

Upregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
SNORD3C	0,00	2,71	small nucleolar RNA, C/D box 3C	ENSG00000264940	780853
SNORD3A	0,00	2,67	small nucleolar RNA, C/D box 3A	ENSG00000263934	780851
FAM69C	0,01	2,49	family with sequence similarity 69, member C	ENSG00000187773	125704
GPNMB	0,01	2,45	glycoprotein (transmembrane) nmb	ENSG00000136235	10457
HBB	0,00	2,43	hemoglobin, beta	ENSG00000244734	3043
SNORD3D	0,00	2,43	small nucleolar RNA, C/D box 3D	ENSG00000262202	780854
TNNT3	0,00	2,30	troponin T type 3 (skeletal, fast)	ENSG00000130595	7140
APLNR	0,01	2,18	apelin receptor	ENSG00000134817	187
DPT	0,00	2,14	dermatopontin	ENSG00000143196	1805
CASQ2	0,00	2,09	calsequestrin 2 (cardiac muscle)	ENSG00000118729	845
RBP7	0,00	2,08	retinol binding protein 7, cellular	ENSG00000162444	116362
LUM	0,00	1,99	lumican	ENSG00000139329	4060
COL15A1	0,01	1,96	collagen, type XV, alpha 1	ENSG00000204291	1306
FABP4	0,00	1,94	fatty acid binding protein 4, adipocyte	ENSG00000170323	2167
MX1	0,01	1,93	myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)	ENSG00000157601	4599
FAM129A	0,05	1,85	family with sequence similarity 129, member A	ENSG00000135842	116496
F3	0,00	1,84	coagulation factor III (thromboplastin, tissue factor)	ENSG00000117525	2152
A2M	0,00	1,80	alpha-2-macroglobulin	ENSG00000175899	2
HHATL	0,00	1,79	hedgehog acyltransferase-like	ENSG00000010282	57467
MXRA5	0,00	1,73	matrix-remodelling associated 5	ENSG00000101825	25878
LPL	0,00	1,66	lipoprotein lipase	ENSG00000175445	4023
CD36	0,00	1,65	CD36 molecule (thrombospondin receptor)	ENSG00000135218	948
PODN	0,00	1,63	podocan	ENSG00000174348	127435
OGN	0,00	1,59	osteoglycin	ENSG00000106809	4969
VWF	0,00	1,58	von Willebrand factor	ENSG00000110799	7450
SULT1E1	0,00	1,54	sulfotransferase family 1E, estrogen-preferring, member 1	ENSG00000109193	6783
TMEM155	0,00	1,53	transmembrane protein 155	ENSG00000164112	132332
PRND	0,02	1,53	prion protein 2 (dublet)	ENSG00000171864	23627

DCN	0,00	1,51	decorin	ENSG00000011465	1634
SPARCL1	0,00	1,50	SPARC-like 1 (hevin)	ENSG00000152583	8404
THBS2	0,00	1,50	thrombospondin 2	ENSG00000186340	7058

Downregulated genes					
Gene.Symbol	**adjusted p-value**	**log2 fold difference**	**Gene.Name**	**Ensembl**	**Entrez**
HBE1	0,00	-3,00	hemoglobin, epsilon 1	ENSG00000213931	3046
HBZ	0,01	-2,10	hemoglobin, zeta	ENSG00000130656	3050
HIST1H3I	0,00	-1,95	histone cluster 1, H3i	ENSG00000182572	8354
HIST1H2BM	0,00	-1,65	histone cluster 1, H2bm	ENSG00000196374	8342
TNC	0,01	-1,61	tenascin C	ENSG00000041982	3371
HAPLN1	0,01	-1,55	hyaluronan and proteoglycan link protein 1	ENSG00000145681	1404
HIST1H2AI	0,01	-1,53	histone cluster 1, H2ai	ENSG00000196747	8329
BMPER	0,00	-1,52	BMP binding endothelial regulator	ENSG00000164619	168667

Supplemental Table 4. GO.BP terms Differentially expressed genes

A1-V1

Downregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0006936	57,14	0,00030	muscle contraction
GO:0003012	52,19	0,00030	muscle system process
GO:0007010	19,45	0,00824	cytoskeleton organization
GO:0007517	26,81	0,00869	muscle organ development

Upregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0001764	66,92	0,00201	neuron migration

A2-A1

Downregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0006334	28,87	0,00001	nucleosome assembly
GO:0031497	26,72	0,00001	chromatin assembly
GO:0034728	25,23	0,00001	nucleosome organization
GO:0006333	22,99	0,00002	chromatin assembly or disassembly
GO:0065004	21,63	0,00002	protein-DNA complex assembly
GO:0006323	20,40	0,00002	DNA packaging
GO:0071824	20,40	0,00002	protein-DNA complex subunit organization
GO:0071103	16,33	0,00006	DNA conformation change
GO:0031032	24,87	0,00259	actomyosin structure organization
GO:0003208	21,12	0,00392	cardiac ventricle morphogenesis
GO:0003007	9,98	0,00686	heart morphogenesis
GO:0003231	16,23	0,00746	cardiac ventricle development
Upregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0030199	30,31	0,00127	collagen fibril organization
GO:0030198	11,42	0,00482	extracellular matrix organization
GO:0043062	11,42	0,00482	extracellular structure organization

A2-V2

Downregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0030049	121,62	0,00001	muscle filament sliding
GO:0033275	121,62	0,00001	actin-myosin filament sliding
GO:0070252	117,07	0,00001	actin-mediated cell contraction
GO:0030048	86,36	0,00002	actin filament-based movement
GO:0006942	99,83	0,00022	regulation of striated muscle contraction
GO:0055010	88,55	0,00023	ventricular cardiac muscle tissue morphogenesis
GO:0003229	79,34	0,00023	ventricular cardiac muscle tissue development
GO:0055008	69,81	0,00023	cardiac muscle tissue morphogenesis
GO:0044057	18,42	0,00023	regulation of system process

GO:0060415	63,99	0,00023	muscle tissue morphogenesis
GO:0048644	59,08	0,00027	muscle organ morphogenesis
GO:0003208	57,60	0,00027	cardiac ventricle morphogenesis
GO:0003231	44,30	0,00055	cardiac ventricle development
GO:0006941	43,46	0,00056	striated muscle contraction
GO:0006937	41,11	0,00062	regulation of muscle contraction
GO:0006936	20,41	0,00063	muscle contraction
GO:0003206	38,37	0,00071	cardiac chamber morphogenesis
GO:0003205	35,98	0,00077	cardiac chamber development
GO:0003012	18,63	0,00077	muscle system process
GO:0090257	34,88	0,00081	regulation of muscle system process
GO:0006928	9,25	0,00101	cellular component movement
GO:0048738	27,35	0,00144	cardiac muscle tissue development
GO:0003015	27,03	0,00144	heart process
GO:0060047	27,03	0,00144	heart contraction
GO:0030029	12,31	0,00260	actin filament-based process
GO:0003007	19,95	0,00293	heart morphogenesis
GO:0051239	6,01	0,00661	regulation of multicellular organismal process

Upregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0048468	7,98	0,00522	cell development
GO:0032989	9,16	0,00642	cellular component morphogenesis
GO:0051146	20,19	0,00675	striated muscle cell differentiation
GO:0030900	18,09	0,00836	forebrain development
GO:0048699	8,29	0,00836	generation of neurons
GO:0022008	7,75	0,00949	neurogenesis
GO:0040011	7,69	0,00949	locomotion

V2-V1

Downregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
-	-	-	-

Upregulated			
ID	**oddsRatio**	**adjusted p-value**	**Name**
GO:0072378	123,29	0,00020	blood coagulation, fibrin clot formation
GO:0072376	51,47	0,00102	protein activation cascade
GO:0034614	26,30	0,00356	cellular response to reactive oxygen species
GO:0050878	7,38	0,00416	regulation of body fluid levels
GO:0065008	4,22	0,00479	regulation of biological quality
GO:0042060	6,93	0,00517	wound healing
GO:0002576	20,59	0,00567	platelet degranulation
GO:0032101	11,22	0,00639	regulation of response to external stimulus
GO:0034599	18,42	0,00688	cellular response to oxidative stress
GO:0009611	5,36	0,00716	response to wounding
GO:0000302	15,22	0,00995	response to reactive oxygen species