Space microgravity increases expression of genes associated with proliferation and differentiation in human cardiac spheres

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Abstract

Efficient generation of cardiomyocytes from human induced pluripotent stem cells (hiPSCs) is important for their application in basic and translational studies. Space microgravity can significantly change cell activities and function. Previously, we reported upregulation of genes associated with cardiac proliferation in cardiac progenitors derived from hiPSCs that were exposed to space microgravity for 3 days. Here we investigated the effect of long-term exposure of hiPSC-cardiac progenitors to space microgravity on global gene expression. Cryopreserved 3D hiPSC-cardiac progenitors were sent to the International Space Station (ISS) and cultured for 3 weeks under ISS microgravity and ISS 1G conditions. RNA-sequencing analysis revealed upregulation of genes associated with cardiac differentiation, proliferation, and cardiac structure/function and downregulation of genes associated with extracellular matrix regulation in the ISS microgravity cultures compared with the ISS 1G cultures. Gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes mapping identified upregulation of biological processes, molecular function, cellular components, and pathways associated with cell cycle, cardiac differentiation, and cardiac function. Taking together, these results suggest that space microgravity has beneficial effect on differentiation and growth of cardiac progenitors.

Introduction

Human induced pluripotent stem cells (hiPSCs) may regenerate indefinitely and undergo differentiation into any cell type depending on the protocols used. In this aspect, understanding how cardiomyocytes can be obtained and grown from differentiating hiPSCs is of great importance for their applications in disease modeling, drug development and regenerative medicine[1-3]. Currently there are few limitations regarding the use of hiPSC-derived cardiomyocytes (hiPSC-CMs), including a large number of cells needed for cardiac regeneration. Understanding and improving the pathways involved in cardiomyocyte proliferation and differentiation is clearly of great interest given the potential application of these cells. Investigating cardiac progenitor cells, the intermediate stage of the cells derived from hiPSCs, could help improve the efficiency of hiPSC-CM generation.

Microgravity can significantly change cell properties such as cell metabolism and function[4-7]. Exposure of cardiac progenitors to microgravity has the potential to serve as a new method to increase cardiomyocyte differentiation efficiency. Simulated microgravity has been used in studies on animal models[8-10] and cardiomyocytes from animals[11], but relatively fewer studies focused on the effect of microgravity on cardiac progenitors or stem cell-derived cardiomyocytes[12,13]. We previously discovered that three-dimensional (3D) cardiac progenitors under a simulated microgravity environment created with a random positioning machine generated enriched cardiomyocytes at high cell yield compared with parallel cultures under standard gravity[14]. Microgravity, however, cannot be perfectly replicated on Earth, since gravitational forces are still present under simulated microgravity.

Space experimentation offers a great opportunity to explore the potential impact of microgravity on cells. This is why the International Space Station (ISS) U.S. National Laboratory and other national and space agencies are further increasing experiments and studies on space microgravity regarding biology and disease modeling[15-17]. In a recent spaceflight experiment, we found that a short-term (3 days) exposure of cardiac progenitors derived from hiPSCs to space

microgravity increased expression of genes associated with proliferation[18]. Here, we further investigated the effect of a long-term (3 weeks) exposure of cardiac progenitors to space microgravity on changes in gene expression of late-stage cardiomyocytes.

Results

Overall experimental design

As shown in the schematic of operational procedure for our spaceflight experiment (**Figure 1**), the cryopreserved cardiac progenitor spheres were generated on the ground and sent to the ISS through the SPACEX-20 mission, a mission launched by the aerospace company SpaceX on March 6, 2020 for the delivery of cargo and supplies to the ISS (https://www.issnationallab.org/launches/spacex-crs-20/). On the ISS, the astronauts successfully thawed the cryopreserved cardiac progenitor spheres, cultured the cells using the Multi-specimen Variable-gravity Platform (MVP) from Techshot, Inc for 22 days, and then sent live cultures back to us. The MVP system has been successfully used in space experiments[19] and allows loading of multiple biological samples under both ISS μG and ISS 1G conditions (the 1G condition in the MVP system on the ISS was achieved by centrifugation). In our experiment, each condition was run in triplicates. The use of ISS 1G samples as a control in our data analysis allowed for examination of the effect of space microgravity on the cells while keeping other variables constant such as the effect of space radiation. At the end of the spaceflight experiment, we received live cardiac spheres[18] and then isolated RNA from cardiac spheres in both the ISS μG and the ISS 1G conditions for RNA sequencing (RNA-seq) to compare their global gene expression profiles and examine the effect of space microgravity on transcriptome alterations of cardiac spheres.

Long-term exposure to space microgravity increases expression of genes associated with cell proliferation and cardiac differentiation

RNA-seq analysis revealed a total of 470 differentially expressed genes (DEGs) upon applying threshold of adjusted p-value of 0.05 (**Figure 2A**). Among the 470 DEGs, 271 were significantly upregulated and 199 were significantly downregulated in the ISS μG samples compared with the ISS 1G samples. The 271 upregulated and 199 downregulated DEGs in terms of $\log_2$(fold change) value are shown in **Tables S1 and S2**. The top 10 genes that are noticeable in terms of adjusted p-value and $\log_2$(fold change) are annotated in **Figure 2A**.

Among the upregulated DEGs, several were involved in cell cycle, proliferation, survival, and cardiac differentiation, including *CCBE1, CCND2, IGFBP5* and *BDKRB2* (**Supplementary Table1**). *CCBE1* (collagen and calcium binding EGF domains 1) was the most significantly upregulated DEG in terms of adjusted p-value ($\log_2$[fold change] 3.75, adjusted p-value 7.67E-12). *CCBE1* is important for normal heart development as its downregulation disrupts differentiation of stem cells into cardiac mesoderm lineage[20]. *CCND2* was also among the top upregulated DEGs ($\log_2$[fold change] 2.01, adjusted p-value 4.41E-11). *CCND2* (cyclin D2) is a member of the cyclin family required for progression through the G1 phase of the cell cycle. *IGFBP5* (insulin like growth factor binding protein 5) was the top 5 upregulated DEGs in terms of adjusted p-value ($\log_2$[fold change] 2.19, adjusted p-value 6.94E-10). *IGFBP5* is a member of the IGF signaling pathway that regulates IGF transport and availability during embryonic development, while IGF signaling pathway plays a key role during embryonic cardiac development, regulating processes of cell proliferation and survival[21]. *BDKRB2* (bradykinin receptor B2, $\log_2$[fold change] 6.87, adjusted p-value 4.13E-03) encodes a G-protein coupled receptor for bradykinin which stimulates proliferation of smooth muscle cells[22] and endothelial cells[23].

CD24 (CD24 molecule) was identified as the most significantly downregulated DEGs ($\log_2$[fold change] -1.54, adjusted p-value 6.23E-14). *CD24* was reported as a marker for human pluripotent stem cells[24] and its downregulation may indicate enhanced cardiac differentiation in ISS μG cells. In addition, *ENC1* (ectodermal-neural cortex 1) was among the top downregulated

DEGs (log_2[fold change] -2, adjusted p-value 8.25E-03, **Table S2**), suggesting limited neural differentiation in the ISS μG cultures.

Consistently, gene ontology (GO) enrichment analysis revealed upregulation of biological process associated with muscle tissue development (GO:0060537, adjusted p-value 7.19E-07), cardiac muscle tissue development (GO:0048738, adjusted p-value 1.54E-04), and regulation of cell division (GO:0051302, adjusted p-value 1.54E-04) (**Figure 2B, Supplementary Table 3**). Upregulated DEGs including *KIF14, IGF2, KIF18B, SUSD2, RBL1, AURKB, LBH, PDGFD, and CDC42* were identified in GO term of regulation of cell division (**Figure 3, Supplementary Table 4)**. Upregulated DEGs including *MYL2, IGFBP5*, *IGF2, EDNRB*, and *NFATC2* were identified in GO term of muscle cell differentiation (**Figure 3, Supplementary Table 5**).

Furthermore, the top downregulated biological processes (**Figure 3, Supplementary Table 6**) include axon development (GO:0061564, adjusted p-value 6.22E-03), regulation of neuron projection development (GO:0010975, adjusted p-value 8.87E-03) and endothelial cell migration (GO:0043542, adjusted p-value 7.05E-03), suggesting limited neuronal and endothelial differentiation in the ISS μG cultures, which was consistent with efficient cardiac differentiation.

In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway mapping showed upregulated genes in the TGF-beta signaling pathway (hsa04350) and adrenergic signaling pathway (hsa04261) (**Supplementary Figure 1**). The TGF-beta protein superfamily plays key roles during heart development, and has an important role also in cardiac cell proliferation, differentiation and heart regeneration[25]. The adrenergic signaling pathway is tightly connected to calcium signaling, cell contraction and can affect cardiomyocyte differentiation and maturation[26].

Long-term exposure to space microgravity increases expression of genes associated with cardiac function

Several upregulated genes were involved in increasing cardiac function, including *THBD* (thrombomodulin, $\log_2$[fold change] 5.76, adjusted p-value 1.31E-04), and *GADL1* (glutamate decarboxylase like 1, $\log_2$[fold change] 6.20, adjusted p-value 3.81E-02) (**Table S1**). Upregulation of *THBD* in hypertrophic cardiomyocytes was shown to decrease cell apoptosis and help maintain a normal contractile function[27]. *GADL1* is involved in the production of carnosine and taurine, while carnosine and taurine are predominantly expressed in muscle tissue, where they regulate calcium signaling and indirectly regulate oxidative stress with their antioxidant effects[28,29].

GO terms analysis showed that space microgravity upregulated genes associated with biological processes related to muscle system process (GO:0003012, adjusted p-value 4.91E-09), muscle contractions (GO:0006936, adjusted p-value 7.77E-09), heart process (GO:0003015, adjusted p-value 8.21E-05) and heart contraction (GO:0060047, adjusted p-value 1.54E-04) (**Figure 2B, Supplementary Table 3**). Upregulated DEGs like *MYL2*, *ATP2B4*, *EDNRB*, and *IGF2* were identified in enriched GO terms associated with muscle system process and muscle contraction (**Figure 3**).

In addition, upregulation of genes known to be involved with cardiac development and contraction were observed, including *MYL2, TNNI3*, *RYR2, TCAP*, and *SCN5A* (**Supplementary Table 7)** Genes relevant for cardiac conduction were also observed, including *ATP2B4, PRKACA* and *ANK2* (**Supplementary Table 7**).

Similar results were observed in GO terms of cellular components and molecular functions associated with upregulated genes involved in muscle structure. These included sarcomere (GO:0030017, adjusted p-value 1.76E-03), myofibril (GO:0030016, adjusted p-value 1.76E-03), cation channel complex (GO:0034703, adjusted p-value 1.04E-02), contractile fiber

(GO:0043292, adjusted p-value 1.76E-03), z disc (GO:0030018, adjusted p-value 9.17E-03) and I band (GO:0031674, adjusted p-value 1.05E-02) (**Figure 4, Supplementary Table 3**).

GO terms of membrane raft (GO:0045121, adjusted p-value 1.86E-03) and membrane microdomain (GO:0098857, adjusted p-value 1.86E-03) were among the top upregulated cellular components (**Figure 4, Supplementary Table 3**). Membrane/lipid rafts and microdomains play an important role in targeting and controlling the interaction of proteins including cardiac channel subunits localized in lipid rafts[30].

GO terms related to protein kinase A were also upregulated (**Figure 4, Supplementary Table 3**). These included protein kinase A binding (GO:0051018, adjusted p-value 7.32E-03) and protein kinase A regulatory subunit binding (GO:0034237, adjusted p-value 2.94E-03). Protein kinase A plays a central role in cardiac function by enhancing Ca^{2+} cycling and increasing cardiac muscle contractility[31]. Protein kinase A also regulates fuel supply and energy-generating pathways, moving cardiomyocytes from glucose toward lipid metabolism as energy source[32].

Long-term exposure to space microgravity reduces expression of genes associated with extracellular matrix (ECM) regulation

Our transcriptomics analyses also revealed downregulation of extracellular structures associated with 3D hiPSC-CMs under space microgravity. For example, downregulated DEGs including *ITIH5*, *COL4A4*, *PTPR21*, COL26A1, and *HPX* were associated with ECM regulation (**Supplementary Table 8**). Downregulated DEGs including *ITGA11*, *PROCR*, *ANXA1*, and *LIMA1* were associated with focal adhesion (**Supplementary Table 9**).

GO term analysis performed on downregulated genes revealed downregulation of biological process terms like actin filament organization (GO:0007015, adjusted p-value 1.75E-06), wound healing (GO:0042060, adjusted p-value 0.00317314) and endothelial cell migration (GO:0043542, adjusted p-value 0.00704613) (**Figure 2B, Supplementary Table 6**).

Downregulated molecular process terms included actin binding (GO:0003779, adjusted p-value 1.34E-03), cadherin binding (GO:0045296, adjusted p-value 5.84E-03), and collagen binding (GO:0005518, adjusted p-value 3.96E-02) (**Figure 4, Supplementary Table 6**). Downregulated cellular component terms included collagen-containing extracellular matrix (GO:0062023, adjusted p-value 1.45E-06), focal adhesion (GO:0005925, adjusted p-value 2.59E-04), primary lysosome (GO:0005766, adjusted p-value 1.76E-02) and myofibril (GO:0030016, adjusted p-value 3.19E-02) (**Figure 4, Supplementary Table 6**). Consistently, KEGG pathway mapping revealed downregulated genes in pathways of ECM-receptor interaction (HSA04512) and focal adhesion (HSA04510) (**Supplementary Figure 2**).

Long-term exposure to space microgravity alters expression of long non-coding RNAs (lncRNAs)

Our transcriptomics analyses revealed altered expression in a limited number of lncRNAs associated with 3D hiPSC-CMs under long-term exposure to space microgravity (**Supplementary Tables 1 and 2**). Among the 14 lncRNA showing altered expression, 9 were upregulated and 5 downregulated. Two among the 9 upregulated lncRNA were recently found to have important roles in cardiomyocytes development and functions. *LINC00881* (long intergenic non-protein coding RNA 881*,* adjusted p-value 9.41E-03) was identified as an essential regulator of sarcomere and calcium channel gene expression including *MYH6, CACNA1C*, and *RYR2,* and *LINC00881* knockdown was shown to significantly reduce peak calcium amplitude in beating cells[33]. *BANCR* (BRAF-activated non-protein coding RNA, adjusted p-value 1.85E-05) was shown to be expressed in fetal heart and pluripotent stem-cell derived cardiomyocytes, and functional studies also revealed that *BANCR* promoted cardiomyocytes migration *in vitro* and ventricular enlargement *in vivo*[34]. Many of the altered lncRNA are transcripts without a defined function; further studies are needed to evaluate their possible role in cardiac development and function. In addition, proper methods for lncRNA sequencing such

as library preparation and sequencing depth setting are needed for future projects related to microgravity in order to confirm these initial findings and perform a network-based analysis to gain a deeper insight.

RNA-seq analysis comparison between short-term and long-term exposure to space microgravity

We previously described the changes in gene expression during a short-term (3 days) exposure of cardiac progenitors to space microgravity[18] and in this study we examined the effect of a long-term (3 weeks) exposure of space microgravity. By comparing the RNA-seq data from the long-term exposure of space microgravity with those from the short-term exposure, we observed a number of common upregulated and downregulated genes, including 11 upregulated genes and 9 downregulated (**Supplementary Tables 10 and 11**). Among the common upregulated genes, we found the aforementioned *CCND2,* which has roles in cell cycle, proliferation and cardiac differentiation, and FGFR2 (FGF receptor 2) which by binding with protein FGF10 mediates differentiation of stem cells into cardiomyocytes[35]. Most of the other upregulated genes have roles related to cardiac function, such as *ATP2B4* which is involved cardiac contraction and conduction, *MYO18B* (Myosin-18B) which binds actin thin filaments and is incorporated in sarcomeres of cardiomyocytes, and *TFRC* (Transferrin receptor) which promotes iron uptake through the transferrin cycle essential for cardiac function[36]. The common downregulated genes show that microgravity affected the extracellular matrix regulation, including the aforementioned downregulated gene *LIMA1* which is involved in focal adhesion while *EFEMP1* (EGF containing fibulin extracellular matrix protein 1) encodes Fibulin-3, an extracellular matrix glycoprotein. A deficiency of Fibulin-3 was recently shown to protect cardiac spheroids against cardiac fibrosis and improving their vascular network[37]. Other downregulated genes are involved in cell senescence and apoptosis, like *TKT* (Transketolase) which promotes cardiomyocyte apoptosis via the cleaved Parp1/Aif pathway[38] and *DDIT3*

(DNA-damage inducible transcript 3) which is involved in endoplasmic reticulum protein processing and plays a role in cardiomyocyte senescence[39]. These results suggest that even a short-term exposure to microgravity improved cardiomyocyte differentiation, cell survivability and cardiac functions and that some of those changes were not temporary but maintained in the long-term ISS cultures.

Discussion

Following transcriptomics analyses of live cell samples, we found that 3D cardiac spheres that were under long-term space microgravity (3-weeks on the ISS) were in a state of increased cell growth and cardiac differentiation compared with the ISS 1G control samples. We also discovered that long-term exposure to space microgravity resulted in gene expression profiles of enhanced cardiac development and function. Additionally, downregulation of genes associated with extracellular matrix was observed in the ISS μG samples compared with the ISS 1G samples. Our conclusions are based on the results of DEGs identified by RNA-seq together with both the GO term analysis and the KEGG pathway mapping.

These effects of long-term exposure to space microgravity on hiPSC-CMs were also consistent with the previous findings on 3-day (short-term) exposure to space microgravity[18]. In our previous study, we also examined by quantitative RT-PCR analysis of the expression of genes related to cell cycle and cell proliferation (such as *CCND1* and *CCND2*), cardiac structure (such as *MYL2 and TNNI3*) and Ca^{2+} handling (such as *ATP2B4*) in both short-term and long-term ISS cultures. We confirmed that the changes in the expression of these genes observed in short-term exposure to microgravity were also present in long-term exposure samples[18], which was consistent with gene expression changes detected by RNA-seq in this study.

Beneficial effects of microgravity such as increased proliferation have also been observed in other cell types. Compared with ground-based control, neonatal cardiovascular progenitors cultured aboard the ISS for 30 days had enhanced cell proliferation[40]. Under

simulated microgravity, bone marrow-derived human mesenchymal stem cells[41] and adipose-derived stem cells[42] had increased cell proliferation. In addition, culture of human mesenchymal stem cells on the ISS for 7 and 14 days increased immunosuppressive capacity of the cells, which could facilitate the use of these cells in therapy[43]. Together, our study adds to the currently limited body of research on human cardiac cells in space and on changes in proliferation and differentiation of cells in microgravity.

An innovative aspect of our project is that cells were cultured at 37°C using the MVP system on the ISS. The MVP is configured to load multiple cultures under both ISS μG and ISS 1G conditions; the 1G condition on the ISS was achieved by centrifugation of one carousel within the same MVP system. Consequently, it allowed for better characterization of the impact of space microgravity alone on gene expression of stem-cell derived cardiomyocytes. Unlike a ground control, the 1G condition obtained through the MVP module allowed us to focus only on the effect of space microgravity without any background noise related to the space environment, such as space radiation, that could potentially alter or mask the effect of microgravity on gene expression. Therefore, the effects of space microgravity on the growth and development of cardiac progenitors we observed were independent of other potential external factors that might also affect cultures on the ISS.

Our RNA-seq results indicate enhanced cardiac differentiation. For instance, we detected upregulation of genes known to be involved with cardiac development, contraction, and conduction such as *MYL2, TNNI3, SCN5A, ATP2B4,* and *RYR2*. Consistently, KEGG pathway map of “adrenergic signaling in cardiomyocytes” shows similar pattern of gene expression necessary for proper cardiomyocyte electrophysiology and contraction. These results are consistent with our observation that space microgravity improved intracellular Ca^{2+} handling in hiPSC-CMs[18]. In addition, increased expression of Ca^{2+} handling and signaling genes has been observed in human cardiovascular progenitors following spaceflight[13,44]. Increased expression of genes associated with mitochondrial metabolism and cardiac structural

proteins has also been observed in 2D cultures of late-stage hiPSC-CMs following spaceflight[12]. Together, these results indicate that space microgravity can potentially affect the function of cardiac cells, although additional functional studies are needed for the quantification of the effects of microgravity.

Our results also suggest that exposure to space microgravity could lead to enhanced cell proliferation. For example, we detected upregulation of genes involved with regulation of cell division such as *KIF14, IGF2, KIF18B, SUSD2, RBL1,* and *AURKB*. While not included in any specific GO terms associated with proliferation, upregulation of cell cycle regulators *CCND1, CCND2, IGF2*, and *TBX3* were also detected. These genes play a role in cell cycle, cell proliferation, and heart regeneration[45-48]. For example, in a swine model of myocardial infarction, *CCND2* overexpression in hiPSC-CMs enhances myocardial repair[49]. In addition, we observed that the expression of *CCND1, CCND2, IGF2*,and *TBX3* was highly upregulated in cardiac progenitor cells subjected to both a short-term exposure (3 days) and a long-term (3 weeks) to space microgravity as detected by quantitative RT-PCR[18], further confirming their role in cardiac differentiation and proliferation. *CCND2* together with other cell cycle regulator genes identified in this study could be interesting targets for future investigation, since factors that stimulate proliferation of cardiomyocytes or cardiac progenitors could help to enhance generation of cardiomyocytes from hiPSCs and facilitate their application in basic and translational studies. Therefore, the ISS experiments are valuable for understanding the mechanisms to generate functional cardiomyocytes under 1G conditions in a more efficient way for different applications. Future studies are needed to focus more on mechanistic aspects of the cardiomyogenic effects of microgravity, since elucidation of the mechanisms may contribute to generating functional cardiomyocytes under 1G conditions.

In addition to GO analysis, KEGG pathway maps were useful for visualizing underlying processes that may contribute to the overall transcriptomic profile. Adrenergic signaling pathway mapping, for instance, was helpful in confirming one of the underlying mechanisms by which the

ISS μG hiPSC-CMs were able to show enhanced cardiac function. Likewise, downregulated genes in KEGG pathway of ECM-receptor interaction were also consistent with what was observed from GO analysis in terms of ECM-related terms and focal adhesion. Whether downregulation of genes in ECM-term and focal adhesion is relevant to cell proliferation and cardiac development in the context of *in vitro* 3D hiPSC-CM system remains an interesting question, since ECM downregulation has been previously reported to promote cardiac dedifferentiation and proliferation by regulating mechanical stiffness of its microenvironment[50].

In this study, we investigated the impact of long-term space microgravity on transcriptome of hiPSC-CMs by comparing cultures under the ISS μG condition with the ISS 1G condition. The 3-week cultures on the ISS revealed interesting changes in their transcriptome but were not without limitations. First, space experiment conducted on the ISS poses a common challenge of limited samples retrieved for further analysis. Greater numbers of replicates and cells returned would be desirable for future study to better understand the functional implications of DEGs observed. Nevertheless, the role of outstanding DEGs identified in this study remain as interesting targets for future research. Second, cellular composition of our 3D cultures contained mostly cardiomyocytes[18] and the role of cardiac fibroblasts in this process is lesser known. One interesting question to address in a future study may be to better understand the interaction occurring between cardiac cells and non-cardiac cells in 3D hiPSC-CMs, and how the cell composition may change in extended culture in space. Third, more advanced cell culture module with improved imaging and other characterization tools would help retrieve more visual data during cell culture and between medium change. For such purpose, future improvements in flight hardware with automated and more advanced imaging systems and other characterization tools would be desirable.

In conclusion, our transcriptomics analyses provide insight into developmental process of cryopreserved 3D cardiac progenitors under extended exposure to space microgravity. Our findings suggest that the combination of microgravity and the 3D culture leads to increased

differentiation and proliferation of cardiac progenitors, which could lead to highly desired future applications of hiPSC-CMs in therapy. Furthermore, genes and pathways identified here may be targets for future research on Earth to mimic the effects of space microgravity.

Methods

Cell Culture and Cryopreservation of Cardiac Progenitor Spheres

For the spaceflight experiment, we prepared cryopreserved cardiac progenitor spheres from IMR90 hiPSC lines (WiCell Research Institute) that were cultured in a feeder-free condition on Matrigel-coated plates and daily fed with mTeSR™1 medium. For the induction of IMR90 cardiomyocyte differentiation, hiPSCs were treated with growth factors[51,52]. Briefly, when compact colonies reached >95% confluence, cells were treated with 100 ng/mL activin A from differentiation day 0 till day 1 and then 10 ng/mL bone morphogenic protein-4 (BMP4) from day 1 till day 4 in RPMI/B27 insulin-free medium (RPMI 1640 with 2% B27 minus insulin). Cells were maintained in standard RPMI/B27 medium (RPMI 1640 with 2% B27 supplement with insulin) until differentiation day 6, when cardiac progenitor spheres were generated using microscale tissue engineering using Aggrewell 400 plates (STEMCELL Technologies)[53]. Briefly cells were dissociated using 0.25% trypsin-EDTA (Thermo Fisher Scientific), resuspended for cell counting, then seeded into the Aggrewell 400 plates at a concentration of $1.8x10^6$ cells/well (1,500 cells/microwell). Cells were cultured in RPMI/B27 medium and 10 μM ROCK inhibitor Y-27632 to facilitate cell survival following dissociation[54]. After 24 h, cardiac spheres were cryopreserved.

For cryopreservation, cardiac progenitor spheres were resuspended in 0.5 mL cryopreservation medium (90% fetal bovine serum and 10% dimethyl sulfoxide with 10 μM ROCK inhibitor) and transferred into cryosyringes at 0.5 mL/cryosyringe. Cardiac spheres were initially precooled at 4°C for up to 25 min to maximize cryopreservation efficiency, and then stored at -80˚C in a cooling box.

ISS Cell Culture Operation

For thawing cells on the ISS, cryosyringes containing cardiac progenitor spheres were placed in a thermoblock at 37°C for 5 min. The cells were then injected into cell culture chambers of the MVP modules containing a CO_2-independent medium with 10 μM ROCK inhibitor[55]. The MVP modules were re-installed into the MVP facility, which started with a medium flush cycle to replace the medium with new culture medium (20 mL per chamber; ~2x chamber volume), to flush out the DMSO in the cryopreserved cell solution (0.5 mL/cryosyringe). The cells were cultured at 37°C in the MVP system for 22 days on the ISS with medium exchange every other day.

At the end of the mission, live cultures were returned to ground via warm storage after having been cultured aboard the ISS. Upon arrival at Emory University, cardiac spheres were transferred immediately into an incubator and let recover overnight. The following day cardiac spheres were transferred from the collection bags into low adhesion dishes. Medium was changed from CO_2-independent medium to standard cardiomyocyte culture medium (RPMI/B27 medium), and cardiac spheres were then maintained overnight, followed by RNA isolation.

RNA-seq Analysis

RNA was isolated using RNeasy Mini Kit (Qiagen) as per manufacturer's instructions. RNA concentration was measured using NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific). RNA sequencing, quality control, and transcriptome mapping were done by Yerkes National Primate Research Center of Emory University. Total RNA quality was tested using an Agilent 4200 TapeStation and RNA 6000 Nano and Pico Chip (Agilent Technologies). RNA samples of triplicate ISS μG and ISS 1G cultures collected after the long-term exposure to space microgravity were subjected for library preparation and sequencing.

Two nanograms of total RNA were used as input for cDNA synthesis, using the Clontech SMART-Seq v4 Ultra Low Input RNA kit (Takara Bio) according to the manufacturer's instructions. Amplified cDNA was fragmented and appended with dual-indexed bar codes using

the NexteraXT DNA Library Preparation kit (Illumina). Libraries were validated by capillary electrophoresis on an Agilent 4200 TapeStation, pooled at equimolar concentrations, and sequenced on an Illumina NovaSeq 6000 at 100SR, yielding an average of 30 million reads per sample. Alignment was performed using STAR version 2.7.3a and transcripts were annotated using GRCh38. Transcript abundance estimates were calculated internal to the STAR aligner using the algorithm of htseq-count[56].

All downstream analyses were performed in R 4.1.2. Read count normalization and differential expression analyses were performed using DESeq2 R package 1.34.0[57]. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses of differentially expressed genes were done with a default p-value cutoff of 0.05 using clusterProfiler R package 4.2.2 and Chord diagrams were generated using GOplot 1.0.2[58,59].

Data Availability Statement

RNA-seq data reported in this paper is available at GEO: GEO accession number GSE228063.

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Competing Interests

J.F. and G.B. were employees of Techshot Inc. All other authors declare no competing interests.

Supplementary Information

Supplemental information includes Supplementary Tables 1-11 and Supplementary Figures S1-S2.

Author Contributions

A.R., K.M. and C.X. designed experiments. H.H., A.R., P.F. and D.L. performed experiments and analyzed data. J.F. and G.B. contributed to the MVP hardware design and testing. H.H. performed RNA-seq data analysis. H.H., A.R. and C.X. wrote the manuscript. All authors reviewed and approved the manuscript. H.H. and A.R. made equal contributions.

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Figure legends

Figure 1. Schematic of spaceflight operation.

Schematic of sample preparation, spaceflight operation, Multi-specimen Variable-gravity Platform (MVP) sample collection, and RNA extraction, sequencing, and gene expression analysis. Figures created with BioRender. MVP module image source: Techshot Inc, SpaceX-20 Dragon capsule and International Space Station image source: NicePNG (images adapted by the author).

Figure 2. Differentially expressed genes and gene ontology (GO) terms identified by RNA-sequencing analysis of hiPSC-CMs exposed to space microgravity.

(A) Volcano plot illustrating differentially expressed genes between ISS μG and ISS 1G samples (n=3 cultures) collected from IMR90 hiPSC differentiation cultures of 3 weeks on the ISS (long-term exposure to space microgravity). (B) Dot plot showing up- and downregulated GO terms of biological processes. GSEA, Gene Set Enrichment Analysis; ISS 1G, the 1G condition on the International Space Station; ISS μG, the microgravity condition on the International Space Station.

Figure 3. Chord diagrams showing the relationship between gene ontology (GO) terms and differentially expressed genes in hiPSC-CMs exposed to space microgravity.

(A) Chord diagram of selected upregulated GO terms and genes in ISS μG vs. ISS 1G of IMR90 hiPSC differentiation cultures of 3 weeks on the ISS. (B) Chord diagram of selected downregulated GO terms and genes. GO terms were presented on the right, genes on the left, colored squares on the left indicated $\log_2$(fold change) value from highest to lowest (n=3 cultures).

Figure 4. **Gene ontology (GO) terms identified by RNA-sequencing analysis of hiPSC-CMs exposed to space microgravity.**

(A) Dot plot showing upregulated and downregulated genes clustered by GO enrichment analysis of molecular function from ISS μG and ISS 1G samples (n=3 cultures) collected 3 weeks after thawing onboard ISS (long-term exposure to microgravity). (B) Dot plot showing up- and downregulated genes clustered by GO enrichment analysis of cellular components. GSEA, Gene Set Enrichment Analysis; ISS 1G, the 1G condition on the International Space Station; ISS μG, the microgravity condition on the International Space Station.

Figure 1

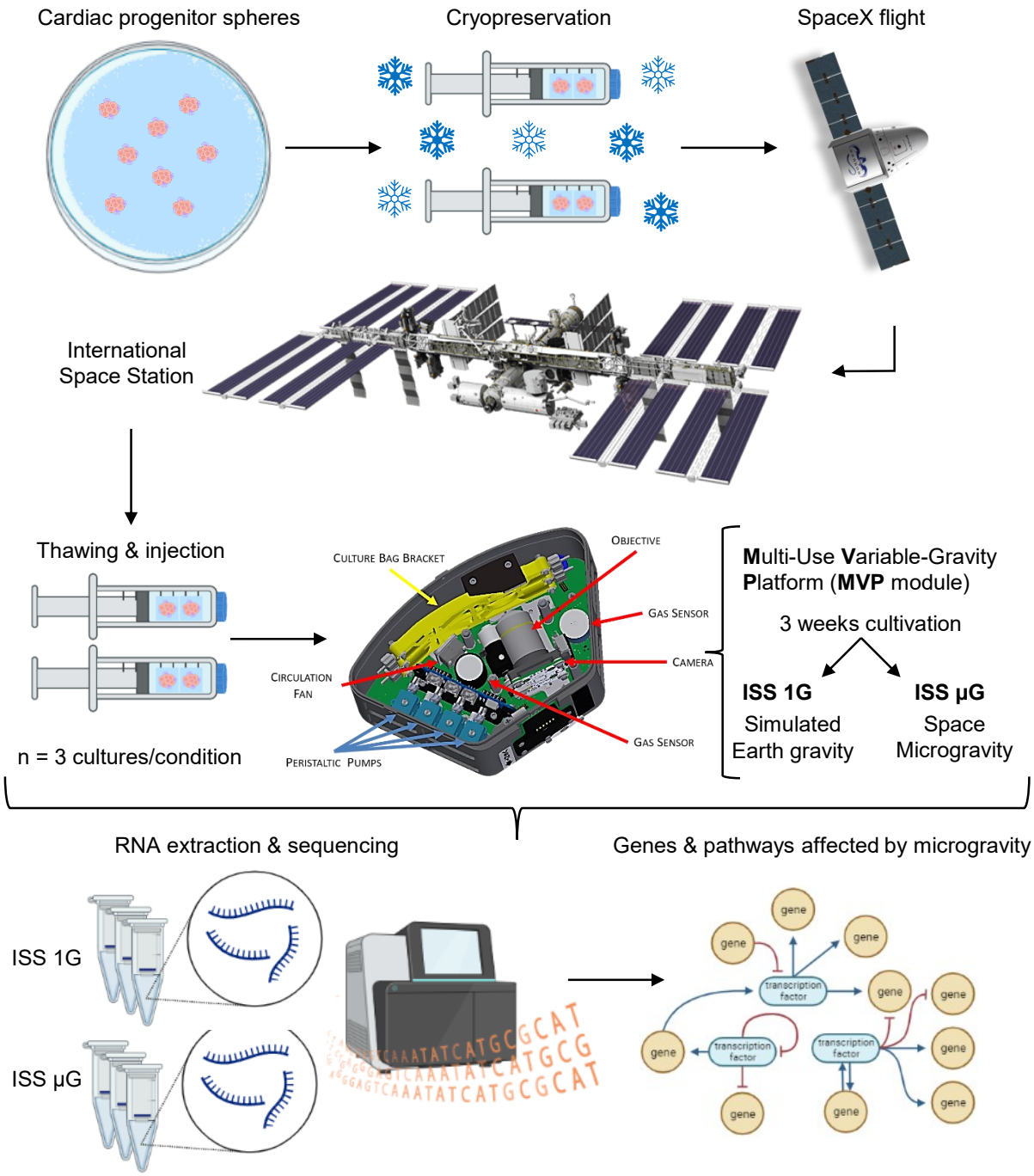

Cardiac progenitor spheres
Cryopreservation
SpaceX flight
International Space Station
Thawing & injection
n = 3 cultures/condition
Culture Bag Bracket
Objective
Gas Sensor
Camera
Circulation Fan
Peristaltic Pumps
Gas Sensor
Multi-Use Variable-Gravity Platform (MVP module)
3 weeks cultivation
ISS 1G
Simulated Earth gravity
ISS µG
Space Microgravity
RNA extraction & sequencing
Genes & pathways affected by microgravity
ISS 1G
ISS µG
gene
transcription factor

Figure 2

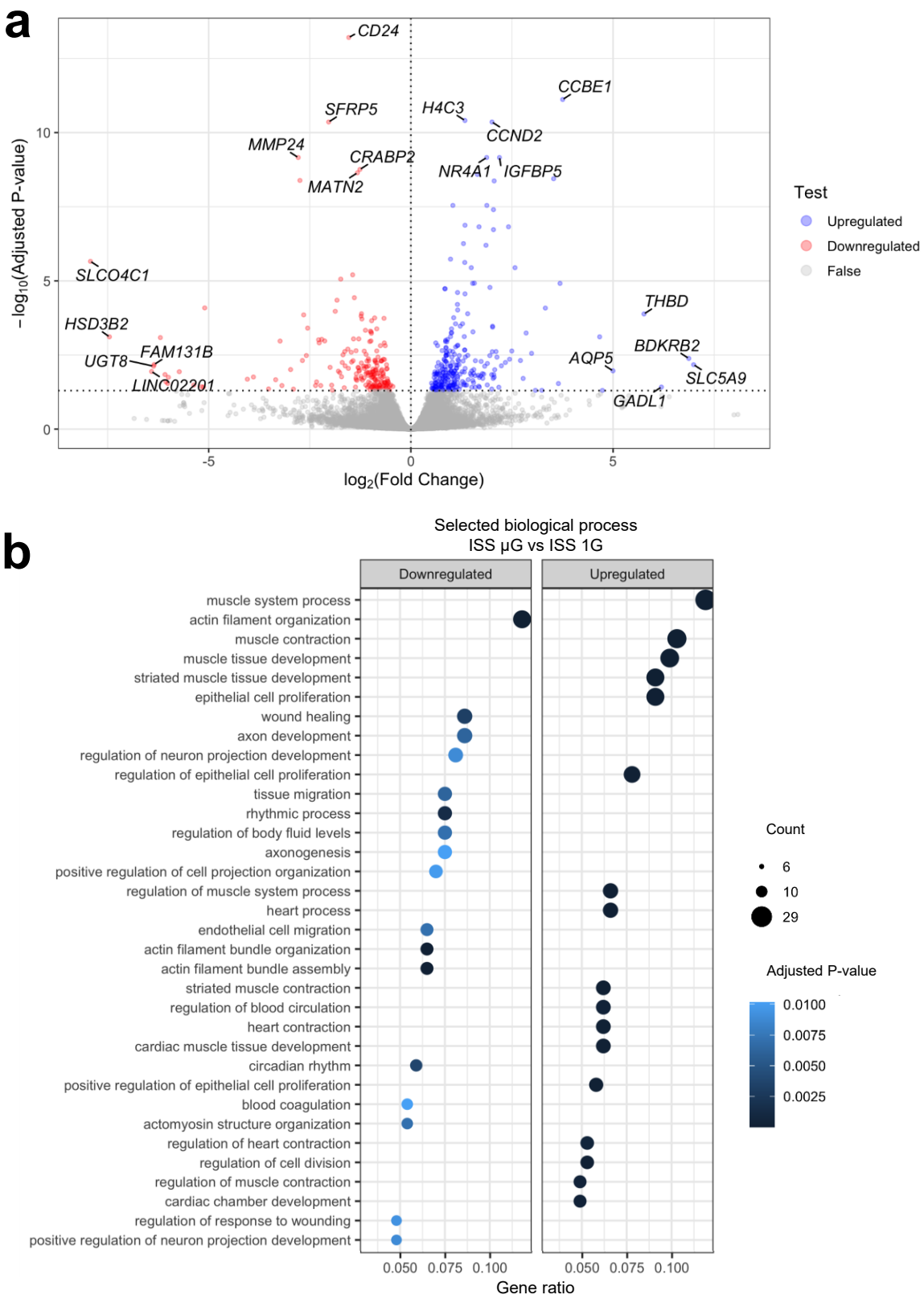
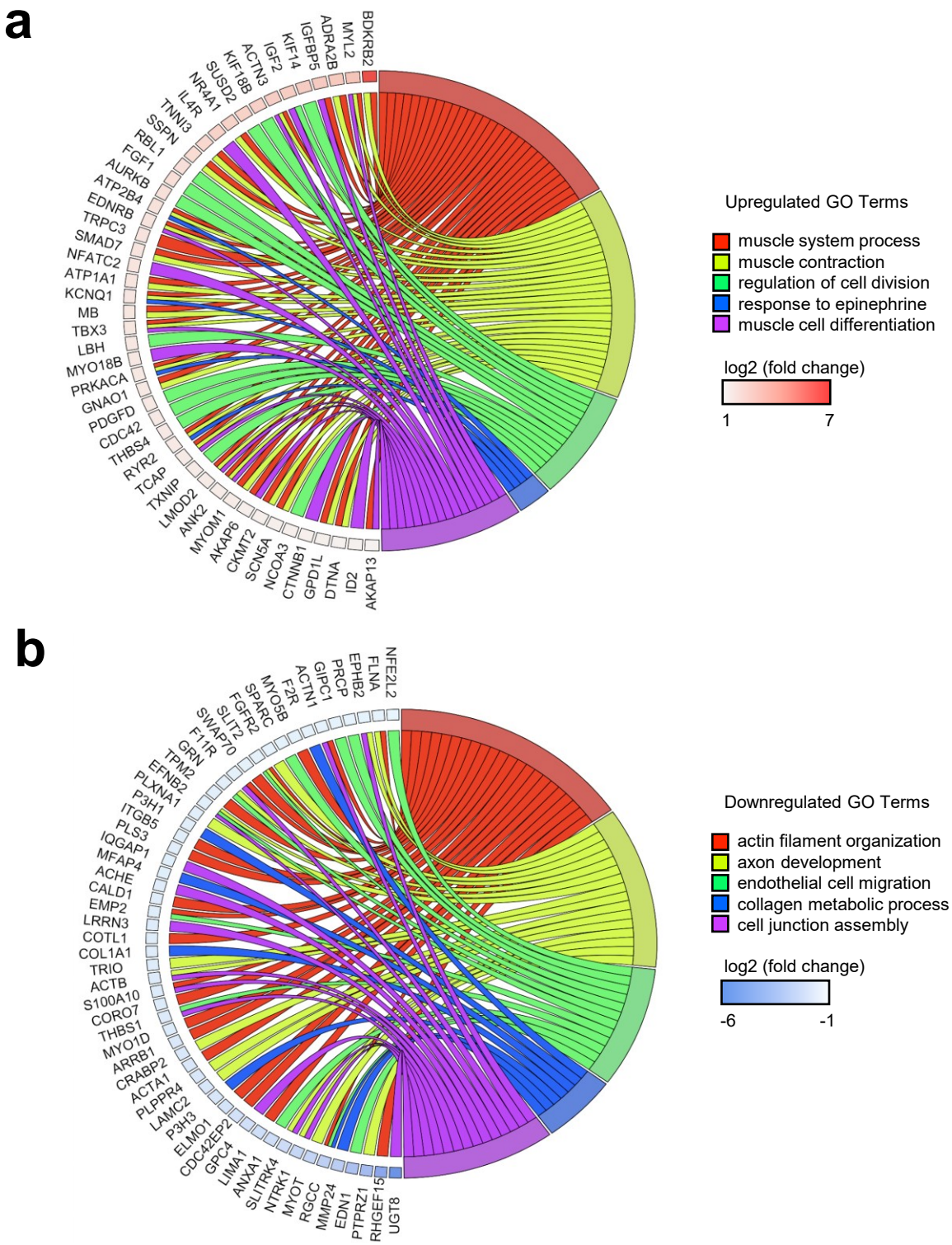

a
CD24
CCBE1
SFRP5
H4C3
CCND2
MMP24
CRABP2
NR4A1
IGFBP5
MATN2
SLCO4C1
THBD
HSD3B2
BDKRB2
FAM131B
UGT8
AQP5
SLC5A9
LINC02201
GADL1
−log10(Adjusted P-value)
log2(Fold Change)
Test
Upregulated
Downregulated
False
b
Selected biological process
ISS µG vs ISS 1G
Downregulated
Upregulated
muscle system process
actin filament organization
muscle contraction
muscle tissue development
striated muscle tissue development
epithelial cell proliferation
wound healing
axon development
regulation of neuron projection development
regulation of epithelial cell proliferation
tissue migration
rhythmic process
regulation of body fluid levels
axonogenesis
positive regulation of cell projection organization
regulation of muscle system process
heart process
endothelial cell migration
actin filament bundle organization
actin filament bundle assembly
striated muscle contraction
regulation of blood circulation
heart contraction
cardiac muscle tissue development
circadian rhythm
positive regulation of epithelial cell proliferation
blood coagulation
actomyosin structure organization
regulation of heart contraction
regulation of cell division
regulation of muscle contraction
cardiac chamber development
regulation of response to wounding
positive regulation of neuron projection development
0.050 0.075 0.100
Gene ratio
Count
6
10
29
Adjusted P-value
0.0100
0.0075
0.0050
0.0025

Figure 3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BDKRB2
MYL2
ADRA2B
IGFBP5
KIF14
IGF2
ACTN3
KIF18B
SUSD2
NR4A1
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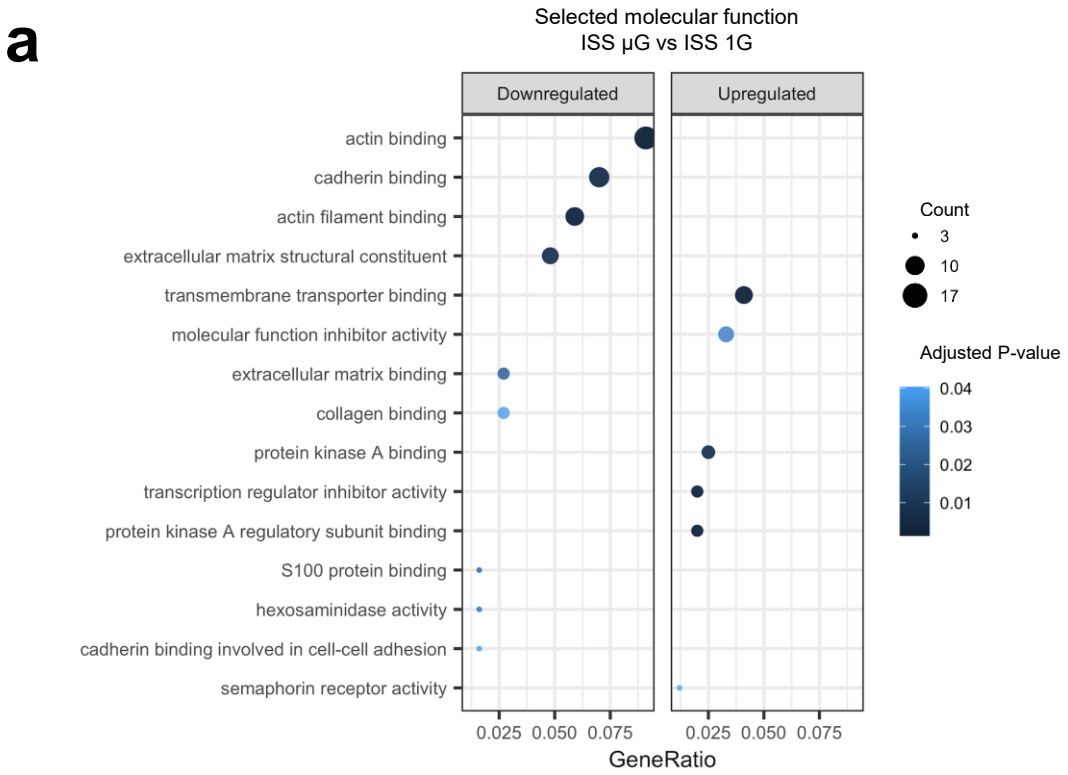
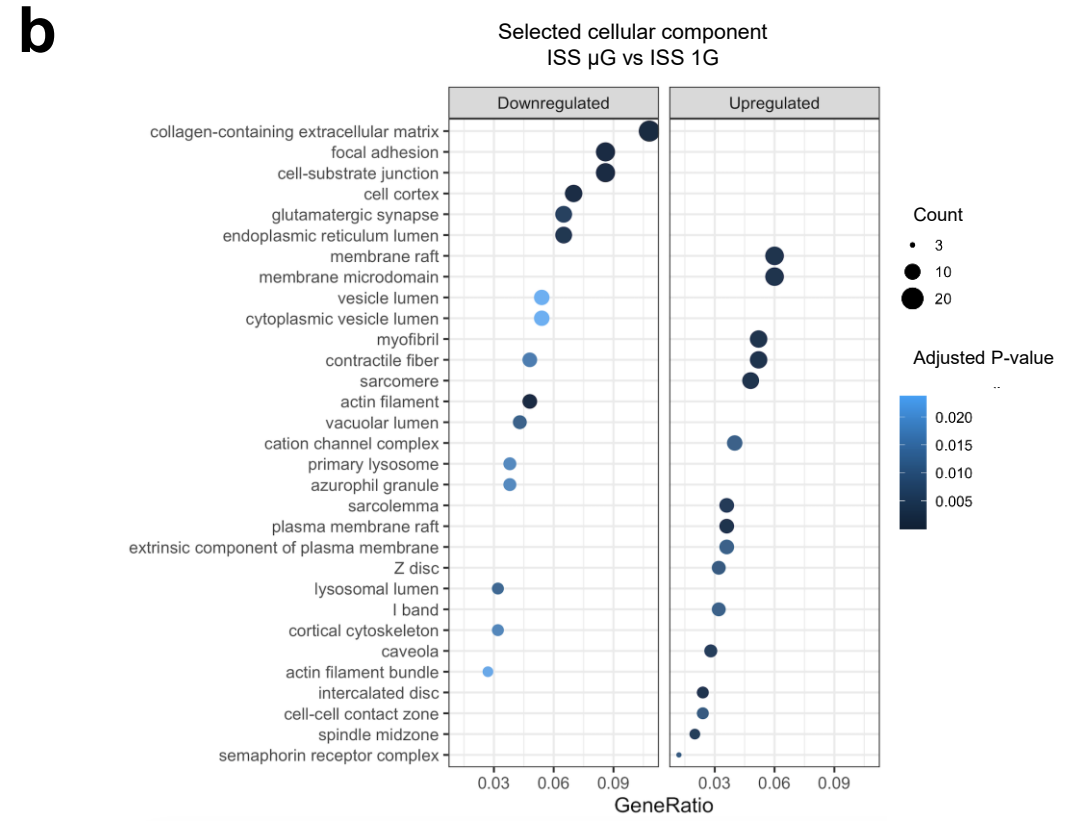
TNNI3
SSPN
RBL1
FGF1
AURKB
ATP2B4
EDNRB
TRPC3
SMAD7
NFATC2
ATP1A1
KCNQ1
MB
TBX3
LBH
MYO18B
PRKACA
GNAO1
PDGFD
CDC42
THBS4
RYR2
TCAP
TXNIP
LMOD2
ANK2
MYOM1
AKAP6
CKMT2
SCN5A
NCOA3
CTNNB1
GPD1L
DTNA
ID2
AKAP13
Upregulated GO Terms
muscle system process
muscle contraction
regulation of cell division
response to epinephrine
muscle cell differentiation
log2 (fold change)
1
7
b
NFE2L2
FLNA
EPHB2
PRCP
GIPC1
ACTN1
F2R
MYO5B
SPARC
FGFR2
SLIT2
SWAP70
F11R
GRN
TPM2
EFNB2
PLXNA1
P3H1
ITGB5
PLS3
IQGAP1
MFAP4
ACHE
CALD1
EMP2
LRRN3
COTL1
COL1A1
TRIO
ACTB
S100A10
CORO7
THBS1
MYO1D
ARRB1
CRABP2
ACTA1
PLPPR4
LAMC2
P3H3
ELMO1
CDC42EP2
GPC4
LIMA1
ANXA1
SLITRK4
NTRK1
MYOT
RGCC
MMP24
EDN1
PTPRZ1
RHGEF15
UGT8
Downregulated GO Terms
actin filament organization
axon development
endothelial cell migration
collagen metabolic process
cell junction assembly
log2 (fold change)
-6
-1

Figure 4


a
Selected molecular function
ISS µG vs ISS 1G
Downregulated
Upregulated
actin binding
cadherin binding
actin filament binding
extracellular matrix structural constituent
transmembrane transporter binding
molecular function inhibitor activity
extracellular matrix binding
collagen binding
protein kinase A binding
transcription regulator inhibitor activity
protein kinase A regulatory subunit binding
S100 protein binding
hexosaminidase activity
cadherin binding involved in cell-cell adhesion
semaphorin receptor activity
0.025 0.050 0.075
0.025 0.050 0.075
GeneRatio
Count
3
10
17
Adjusted P-value
0.04
0.03
0.02
0.01


b
Selected cellular component
ISS µG vs ISS 1G
Downregulated
Upregulated
collagen-containing extracellular matrix
focal adhesion
cell-substrate junction
cell cortex
glutamatergic synapse
endoplasmic reticulum lumen
membrane raft
membrane microdomain
vesicle lumen
cytoplasmic vesicle lumen
myofibril
contractile fiber
sarcomere
actin filament
vacuolar lumen
cation channel complex
primary lysosome
azurophil granule
sarcolemma
plasma membrane raft
extrinsic component of plasma membrane
Z disc
lysosomal lumen
I band
cortical cytoskeleton
caveola
actin filament bundle
intercalated disc
cell-cell contact zone
spindle midzone
semaphorin receptor complex
0.03 0.06 0.09
0.03 0.06 0.09
GeneRatio
Count
3
10
20
Adjusted P-value
0.020
0.015
0.010
0.005

Supplementary Information

Space microgravity increases expression of genes associated with proliferation and differentiation in human cardiac spheres

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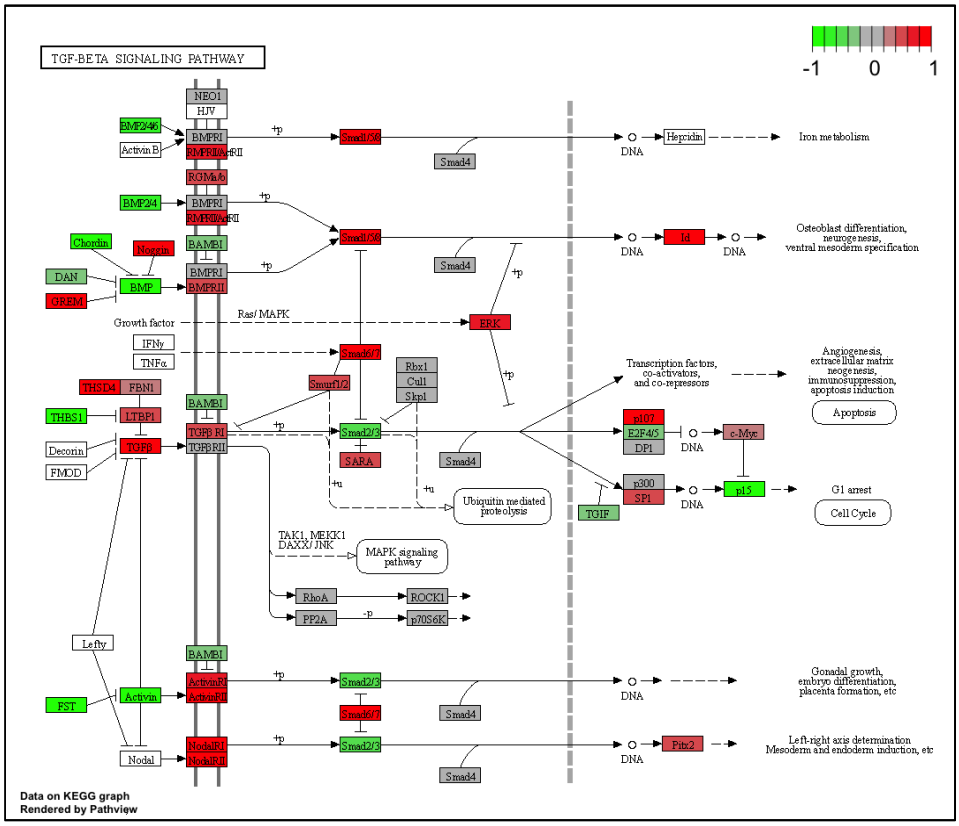
A



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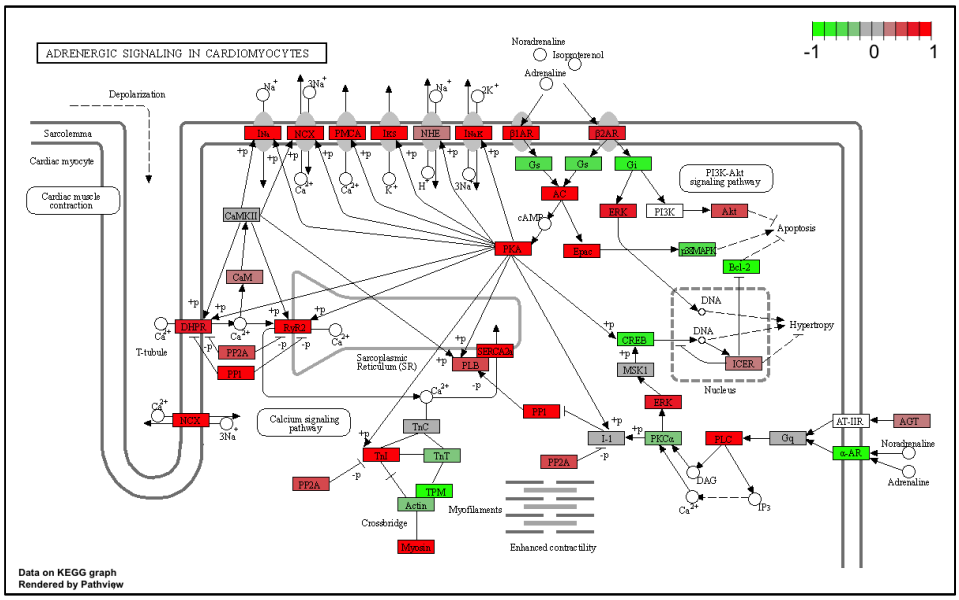
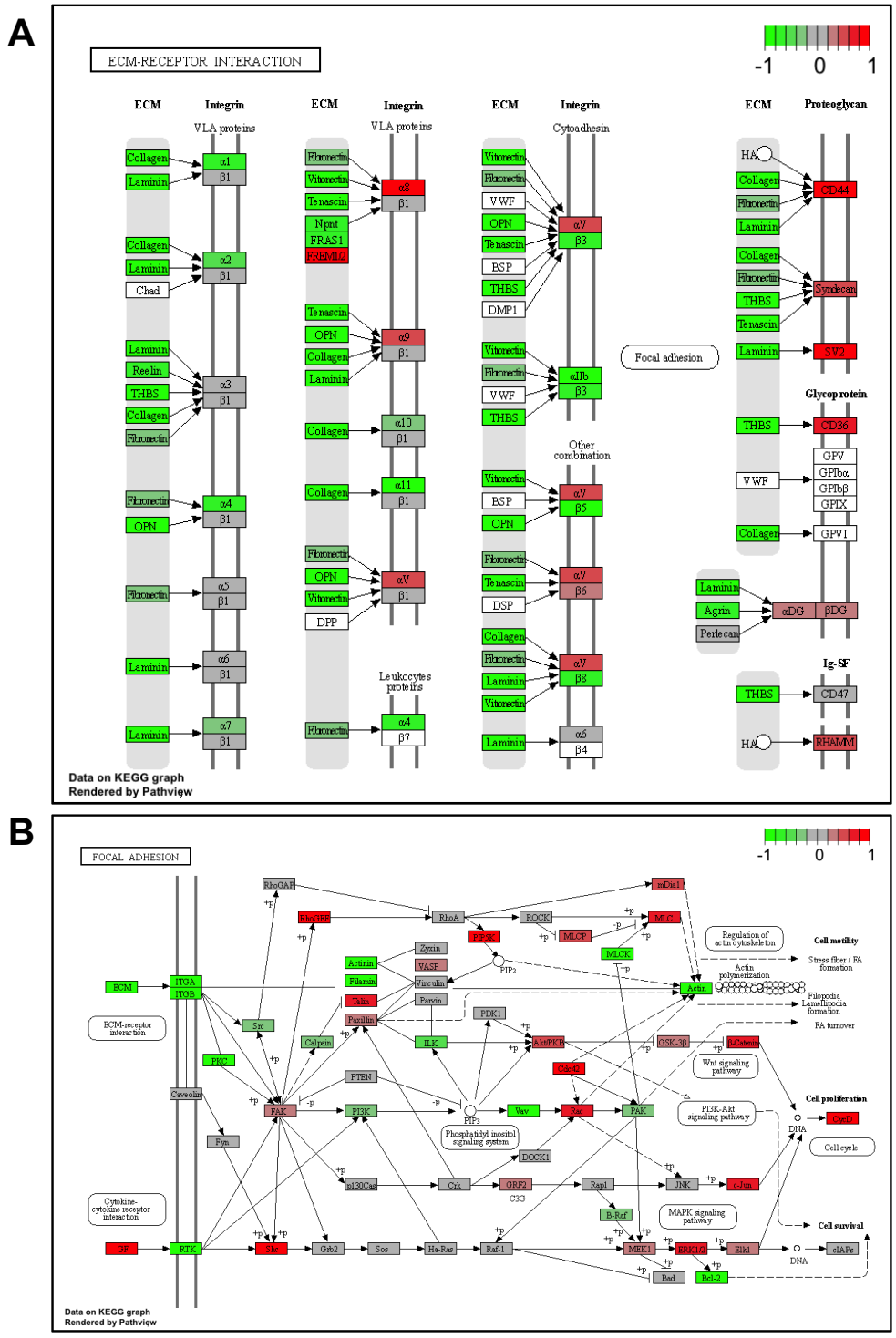


Supplementary Figure 1. KEGG mapping of TGF-beta signaling and adrenergic signaling pathways. Pathway maps were derived from KEGG analysis using our RNA-seq data set. Each gene is color-coded by the KEGG analysis, indicating the level of expression upregulated or downregulated by space microgravity. (A) TGF-beta signaling pathway in IMR90 cardiac spheres after 3 weeks in the ISS μG condition compared with the ISS 1G condition. (B) Adrenergic signaling pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes. ISS 1G, the 1G condition on the International Space Station; ISS μG, the microgravity condition on the International Space Station.



Supplementary Figure 2. KEGG mapping of ECM-receptor interaction and focal adhesion pathways. Pathway maps were derived from KEGG analysis using our RNA-seq data set. Each gene is color-coded by the KEGG analysis, indicating the level of expression upregulated or downregulated by space microgravity. (A) ECM-receptor interaction pathway in IMR90 cardiac spheres after 3 weeks in the ISS μG condition compared with the ISS 1G condition. (B) Focal adhesion pathway. KEGG, Kyoto Encyclopedia of Genes and Genomes. ISS 1G, the 1G condition on the International Space Station; ISS μG, the microgravity condition on the International Space Station.

Supplementary Table 1. 271 upregulated differentially expressed genes (DEGs) in the ISS μG condition compared with the ISS 1G condition.

Gene	Description	log2(Fold change)	adjusted p-value
SLC5A9	solute carrier family 5 member 9 [Source:HGNC Symbol;Acc:HGNC:22146]	6.99	6.70E-03
BDKRB2	bradykinin receptor B2 [Source:HGNC Symbol;Acc:HGNC:1030]	6.87	4.13E-03
GADL1	glutamate decarboxylase like 1 [Source:HGNC Symbol;Acc:HGNC:27949]	6.20	3.81E-02
THBD	thrombomodulin [Source:HGNC Symbol;Acc:HGNC:11784]	5.76	1.31E-04
AQP5	aquaporin 5 [Source:HGNC Symbol;Acc:HGNC:638]	5.00	1.08E-02
UPK1A	uroplakin 1A [Source:HGNC Symbol;Acc:HGNC:12577]	4.74	4.93E-02
COL11A1	collagen type XI alpha 1 chain [Source:HGNC Symbol;Acc:HGNC:2186]	4.67	7.71E-04
CCBE1	collagen and calcium binding EGF domains 1 [Source:HGNC Symbol;Acc:HGNC:29426]	3.75	7.67E-12
SOCS3	suppressor of cytokine signaling 3 [Source:HGNC Symbol;Acc:HGNC:19391]	3.69	1.22E-05
SNCB	synuclein beta [Source:HGNC Symbol;Acc:HGNC:11140]	3.65	2.90E-02
CTNNA2	catenin alpha 2 [Source:HGNC Symbol;Acc:HGNC:2510]	3.53	3.61E-09
ATOH8	atonal bHLH transcription factor 8 [Source:HGNC Symbol;Acc:HGNC:24126]	3.33	8.27E-05
SLC16A3	solute carrier family 16 member 3 [Source:HGNC Symbol;Acc:HGNC:10924]	3.24	4.91E-02
TRPA1	transient receptor potential cation channel subfamily A member 1 [Source:HGNC Symbol;Acc:HGNC:497]	3.08	4.91E-02
SPAG4	sperm associated antigen 4 [Source:HGNC Symbol;Acc:HGNC:11214]	2.98	2.78E-03
PNOC	prepronociceptin [Source:HGNC Symbol;Acc:HGNC:9163]	2.85	5.74E-04
BRIP1	BRCA1 interacting protein C-terminal helicase 1 [Source:HGNC Symbol;Acc:HGNC:20473]	2.78	1.95E-02
SEC14L6	SEC14 like lipid binding 6 [Source:HGNC Symbol;Acc:HGNC:40047]	2.71	4.85E-03
SHROOM4	shroom family member 4 [Source:HGNC Symbol;Acc:HGNC:29215]	2.57	3.60E-06
TAFA2	TAFA chemokine like family member 2 [Source:HGNC Symbol;Acc:HGNC:21589]	2.50	2.32E-03
MYL2	myosin light chain 2 [Source:HGNC Symbol;Acc:HGNC:7583]	2.44	4.39E-03
SLC26A9	solute carrier family 26 member 9 [Source:HGNC Symbol;Acc:HGNC:14469]	2.41	1.50E-07
EGR4	early growth response 4 [Source:HGNC Symbol;Acc:HGNC:3241]	2.34	5.66E-03
ADRA2B	adrenoceptor alpha 2B [Source:HGNC Symbol;Acc:HGNC:282]	2.24	7.31E-03
IGFBP5	insulin like growth factor binding protein 5 [Source:HGNC Symbol;Acc:HGNC:5474]	2.19	6.94E-10
RIN3	Ras and Rab interactor 3 [Source:HGNC Symbol;Acc:HGNC:18751]	2.17	3.87E-02
MT3	metallothionein 3 [Source:HGNC Symbol;Acc:HGNC:7408]	2.13	3.73E-03
KIF14	kinesin family member 14 [Source:HGNC Symbol;Acc:HGNC:19181]	2.11	2.42E-03
IGF2	insulin like growth factor 2 [Source:HGNC Symbol;Acc:HGNC:5466]	2.07	9.89E-03
ANKRD63	ankyrin repeat domain 63 [Source:HGNC Symbol;Acc:HGNC:40027]	2.06	1.77E-02
ADPRHL1	ADP-ribosylhydrolase like 1 [Source:HGNC Symbol;Acc:HGNC:21303]	2.06	4.28E-09
TMEM200B	transmembrane protein 200B [Source:HGNC Symbol;Acc:HGNC:33785]	2.05	1.44E-02
ACTN3	actinin alpha 3 (gene/pseudogene) [Source:HGNC Symbol;Acc:HGNC:165]	2.04	1.87E-07

SORL1	sortilin related receptor 1 [Source:HGNC Symbol;Acc:HGNC:11185]	2.04	3.97E-08
KCNQ3	potassium voltage-gated channel subfamily Q member 3 [Source:HGNC Symbol;Acc:HGNC:6297]	2.03	1.95E-02
LOXL3	lysyl oxidase like 3 [Source:HGNC Symbol;Acc:HGNC:13869]	2.02	3.64E-04
MIR210HG	MIR210 host gene [Source:HGNC Symbol;Acc:HGNC:39524]	2.02	3.38E-04
LINC00880	long intergenic non-protein coding RNA 880 [Source:HGNC Symbol;Acc:HGNC:27948]	2.01	2.45E-03
CCND2	cyclin D2 [Source:HGNC Symbol;Acc:HGNC:1583]	2.01	4.41E-11
ADAMTS17	ADAM metallopeptidase with thrombospondin type 1 motif 17 [Source:HGNC Symbol;Acc:HGNC:17109]	2.00	4.44E-02
LDHAP4	lactate dehydrogenase A pseudogene 4 [Source:HGNC Symbol;Acc:HGNC:6539]	2.00	1.46E-02
EGLN3	egl-9 family hypoxia inducible factor 3 [Source:HGNC Symbol;Acc:HGNC:14661]	1.95	1.66E-05
AC107068.2	novel transcript, antisense to CORIN and NFXL1	1.95	3.31E-03
ID1	inhibitor of DNA binding 1, HLH protein [Source:HGNC Symbol;Acc:HGNC:5360]	1.89	6.10E-03
KIF18B	kinesin family member 18B [Source:HGNC Symbol;Acc:HGNC:27102]	1.89	3.23E-02
SUSD2	sushi domain containing 2 [Source:HGNC Symbol;Acc:HGNC:30667]	1.88	2.86E-08
NR4A1	nuclear receptor subfamily 4 group A member 1 [Source:HGNC Symbol;Acc:HGNC:7980]	1.87	6.94E-10
IL4R	interleukin 4 receptor [Source:HGNC Symbol;Acc:HGNC:6015]	1.86	6.31E-07
KCNN1	potassium calcium-activated channel subfamily N member 1 [Source:HGNC Symbol;Acc:HGNC:6290]	1.82	7.53E-03
FOXM1	forkhead box M1 [Source:HGNC Symbol;Acc:HGNC:3818]	1.80	1.39E-02
NEK2	NIMA related kinase 2 [Source:HGNC Symbol;Acc:HGNC:7745]	1.78	1.93E-02
CDCA2	cell division cycle associated 2 [Source:HGNC Symbol;Acc:HGNC:14623]	1.77	1.34E-02
LINC01470	long intergenic non-protein coding RNA 1470 [Source:HGNC Symbol;Acc:HGNC:51105]	1.74	5.21E-03
AC015522.1	novel transcript	1.72	1.61E-02
VWC2	von Willebrand factor C domain containing 2 [Source:HGNC Symbol;Acc:HGNC:30200]	1.70	1.81E-02
PDCD6IPP2	PDCD6IP pseudogene 2 [Source:HGNC Symbol;Acc:HGNC:49873]	1.69	3.85E-02
ID3	inhibitor of DNA binding 3, HLH protein [Source:HGNC Symbol;Acc:HGNC:5362]	1.68	1.50E-07
OPCML	opioid binding protein/cell adhesion molecule like [Source:HGNC Symbol;Acc:HGNC:8143]	1.68	3.54E-02
AC021678.2	novel transcript	1.67	1.53E-02
TNNI3	troponin I3, cardiac type [Source:HGNC Symbol;Acc:HGNC:11947]	1.64	2.58E-09
GCNT1	glucosaminyl (N-acetyl) transferase 1 [Source:HGNC Symbol;Acc:HGNC:4203]	1.59	4.93E-03
FAIM2	Fas apoptotic inhibitory molecule 2 [Source:HGNC Symbol;Acc:HGNC:17067]	1.59	3.89E-02
GREM2	gremlin 2, DAN family BMP antagonist [Source:HGNC Symbol;Acc:HGNC:17655]	1.59	1.57E-02
PRUNE2	prune homolog 2 with BCH domain [Source:HGNC Symbol;Acc:HGNC:25209]	1.57	1.20E-05
ARHGAP45	Rho GTPase activating protein 45 [Source:HGNC Symbol;Acc:HGNC:17102]	1.56	9.78E-03
CDH13	cadherin 13 [Source:HGNC Symbol;Acc:HGNC:1753]	1.55	1.32E-02

ERCC6L	ERCC excision repair 6 like, spindle assembly checkpoint helicase [Source:HGNC Symbol;Acc:HGNC:20794]	1.55	2.69E-02
STARD13	StAR related lipid transfer domain containing 13 [Source:HGNC Symbol;Acc:HGNC:19164]	1.53	5.24E-04
APOLD1	apolipoprotein L domain containing 1 [Source:HGNC Symbol;Acc:HGNC:25268]	1.53	2.29E-02
ARHGEF37	Rho guanine nucleotide exchange factor 37 [Source:HGNC Symbol;Acc:HGNC:34430]	1.53	1.22E-05
MAB21L3	mab-21 like 3 [Source:HGNC Symbol;Acc:HGNC:26787]	1.51	3.87E-02
KIFC1	kinesin family member C1 [Source:HGNC Symbol;Acc:HGNC:6389]	1.50	1.09E-02
LMNB1	lamin B1 [Source:HGNC Symbol;Acc:HGNC:6637]	1.49	1.55E-02
HHATL	hedgehog acyltransferase like [Source:HGNC Symbol;Acc:HGNC:13242]	1.49	3.60E-06
SSPN	sarcospan [Source:HGNC Symbol;Acc:HGNC:11322]	1.47	1.33E-02
NFIB	nuclear factor I B [Source:HGNC Symbol;Acc:HGNC:7785]	1.42	2.14E-02
TOP2A	DNA topoisomerase II alpha [Source:HGNC Symbol;Acc:HGNC:11989]	1.41	3.24E-03
C2orf88	chromosome 2 open reading frame 88 [Source:HGNC Symbol;Acc:HGNC:28191]	1.40	1.28E-04
KLHL29	kelch like family member 29 [Source:HGNC Symbol;Acc:HGNC:29404]	1.38	3.27E-02
ASB11	ankyrin repeat and SOCS box containing 11 [Source:HGNC Symbol;Acc:HGNC:17186]	1.35	3.70E-03
BCL11A	BAF chromatin remodeling complex subunit BCL11A [Source:HGNC Symbol;Acc:HGNC:13221]	1.34	3.27E-02
ARTN	artemin [Source:HGNC Symbol;Acc:HGNC:727]	1.34	8.95E-03
NMRK2	nicotinamide riboside kinase 2 [Source:HGNC Symbol;Acc:HGNC:17871]	1.34	1.34E-07
H1-0	H1.0 linker histone [Source:HGNC Symbol;Acc:HGNC:4714]	1.34	2.75E-05
H4C3	H4 clustered histone 3 [Source:HGNC Symbol;Acc:HGNC:4787]	1.34	3.92E-11
TRH	thyrotropin releasing hormone [Source:HGNC Symbol;Acc:HGNC:12298]	1.33	2.14E-03
PLEKHA4	pleckstrin homology domain containing A4 [Source:HGNC Symbol;Acc:HGNC:14339]	1.33	2.40E-06
NLGN1	neuroligin 1 [Source:HGNC Symbol;Acc:HGNC:14291]	1.33	2.14E-03
LRRC14B	leucine rich repeat containing 14B [Source:HGNC Symbol;Acc:HGNC:37268]	1.32	7.57E-04
RBL1	RB transcriptional corepressor like 1 [Source:HGNC Symbol;Acc:HGNC:9893]	1.32	1.19E-02
CCDC151	coiled-coil domain containing 151 [Source:HGNC Symbol;Acc:HGNC:28303]	1.32	2.76E-02
SCD5	stearoyl-CoA desaturase 5 [Source:HGNC Symbol;Acc:HGNC:21088]	1.31	3.15E-02
FGF1	fibroblast growth factor 1 [Source:HGNC Symbol;Acc:HGNC:3665]	1.30	5.54E-07
IER2	immediate early response 2 [Source:HGNC Symbol;Acc:HGNC:28871]	1.29	5.05E-03
SMCO1	single-pass membrane protein with coiled-coil domains 1 [Source:HGNC Symbol;Acc:HGNC:27407]	1.28	2.79E-04
AURKB	aurora kinase B [Source:HGNC Symbol;Acc:HGNC:11390]	1.28	2.47E-02
OLFML2A	olfactomedin like 2A [Source:HGNC Symbol;Acc:HGNC:27270]	1.27	1.15E-02
FGF16	fibroblast growth factor 16 [Source:HGNC Symbol;Acc:HGNC:3672]	1.23	3.50E-02
RGS9	regulator of G protein signaling 9 [Source:HGNC Symbol;Acc:HGNC:10004]	1.22	3.36E-04
PRRG3	proline rich and Gla domain 3 [Source:HGNC Symbol;Acc:HGNC:30798]	1.21	1.41E-02
ATP2B4	ATPase plasma membrane Ca2+ transporting 4 [Source:HGNC Symbol;Acc:HGNC:817]	1.20	3.31E-03

CCNB2	cyclin B2 [Source:HGNC Symbol;Acc:HGNC:1580]	1.19	4.58E-02
ZFP36	ZFP36 ring finger protein [Source:HGNC Symbol;Acc:HGNC:12862]	1.19	6.71E-03
PGK1	phosphoglycerate kinase 1 [Source:HGNC Symbol;Acc:HGNC:8896]	1.18	4.93E-03
TSPAN9	tetraspanin 9 [Source:HGNC Symbol;Acc:HGNC:21640]	1.16	4.55E-03
THSD4	thrombospondin type 1 domain containing 4 [Source:HGNC Symbol;Acc:HGNC:25835]	1.16	1.48E-03
SMAD6	SMAD family member 6 [Source:HGNC Symbol;Acc:HGNC:6772]	1.16	9.62E-05
LRRC52-AS1	LRRC52 antisense RNA 1 [Source:HGNC Symbol;Acc:HGNC:54044]	1.16	1.69E-02
ADAMTSL1	ADAMTS like 1 [Source:HGNC Symbol;Acc:HGNC:14632]	1.15	1.96E-02
A2M	alpha-2-macroglobulin [Source:HGNC Symbol;Acc:HGNC:7]	1.15	2.47E-05
PPP1R3A	protein phosphatase 1 regulatory subunit 3A [Source:HGNC Symbol;Acc:HGNC:9291]	1.15	6.23E-05
SOX11	SRY-box transcription factor 11 [Source:HGNC Symbol;Acc:HGNC:11191]	1.15	2.72E-02
PPFIA4	PTPRF interacting protein alpha 4 [Source:HGNC Symbol;Acc:HGNC:9248]	1.15	1.53E-03
EDNRB	endothelin receptor type B [Source:HGNC Symbol;Acc:HGNC:3180]	1.15	6.90E-03
VSNL1	visinin like 1 [Source:HGNC Symbol;Acc:HGNC:12722]	1.14	3.87E-02
B3GNT9	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 9 [Source:HGNC Symbol;Acc:HGNC:28714]	1.13	8.80E-03
TRPC3	transient receptor potential cation channel subfamily C member 3 [Source:HGNC Symbol;Acc:HGNC:12335]	1.12	8.80E-03
P4HA1	prolyl 4-hydroxylase subunit alpha 1 [Source:HGNC Symbol;Acc:HGNC:8546]	1.12	1.31E-02
FXYD6	FXYD domain containing ion transport regulator 6 [Source:HGNC Symbol;Acc:HGNC:4030]	1.11	6.79E-03
CENPF	centromere protein F [Source:HGNC Symbol;Acc:HGNC:1857]	1.11	3.81E-02
SDK2	sidekick cell adhesion molecule 2 [Source:HGNC Symbol;Acc:HGNC:19308]	1.11	1.69E-03
SMAD7	SMAD family member 7 [Source:HGNC Symbol;Acc:HGNC:6773]	1.10	2.81E-04
TSPAN15	tetraspanin 15 [Source:HGNC Symbol;Acc:HGNC:23298]	1.09	4.72E-02
HS3ST3B1	heparan sulfate-glucosamine 3-sulfotransferase 3B1 [Source:HGNC Symbol;Acc:HGNC:5198]	1.09	2.31E-02
BIRC5	baculoviral IAP repeat containing 5 [Source:HGNC Symbol;Acc:HGNC:593]	1.09	3.13E-02
WDHD1	WD repeat and HMG-box DNA binding protein 1 [Source:HGNC Symbol;Acc:HGNC:23170]	1.08	1.11E-02
NFATC2	nuclear factor of activated T cells 2 [Source:HGNC Symbol;Acc:HGNC:7776]	1.08	2.67E-02
PDK1	pyruvate dehydrogenase kinase 1 [Source:HGNC Symbol;Acc:HGNC:8809]	1.08	1.08E-02
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1 [Source:HGNC Symbol;Acc:HGNC:799]	1.08	3.00E-03
NUSAP1	nucleolar and spindle associated protein 1 [Source:HGNC Symbol;Acc:HGNC:18538]	1.08	1.47E-02
EPN3	epsin 3 [Source:HGNC Symbol;Acc:HGNC:18235]	1.06	2.04E-02
PRICKLE2	prickle planar cell polarity protein 2 [Source:HGNC Symbol;Acc:HGNC:20340]	1.06	1.75E-04
PHF19	PHD finger protein 19 [Source:HGNC Symbol;Acc:HGNC:24566]	1.06	2.90E-02
INTS2	integrator complex subunit 2 [Source:HGNC Symbol;Acc:HGNC:29241]	1.05	3.21E-02
C14orf132	chromosome 14 open reading frame 132 [Source:HGNC Symbol;Acc:HGNC:20346]	1.05	1.33E-02

ST8SIA2	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 2 [Source:HGNC Symbol;Acc:HGNC:10870]	1.05	4.69E-02
FUT11	fucosyltransferase 11 [Source:HGNC Symbol;Acc:HGNC:19233]	1.04	3.36E-02
ACVR2B	activin A receptor type 2B [Source:HGNC Symbol;Acc:HGNC:174]	1.04	3.28E-02
PLXNA4	plexin A4 [Source:HGNC Symbol;Acc:HGNC:9102]	1.04	1.29E-02
CKM	creatine kinase, M-type [Source:HGNC Symbol;Acc:HGNC:1994]	1.04	2.86E-08
FAM162A	family with sequence similarity 162 member A [Source:HGNC Symbol;Acc:HGNC:17865]	1.04	2.42E-03
KCNQ1	potassium voltage-gated channel subfamily Q member 1 [Source:HGNC Symbol;Acc:HGNC:6294]	1.04	1.67E-02
MB	myoglobin [Source:HGNC Symbol;Acc:HGNC:6915]	1.03	1.55E-02
TBX3	T-box transcription factor 3 [Source:HGNC Symbol;Acc:HGNC:11602]	1.02	5.48E-03
PLCB2	phospholipase C beta 2 [Source:HGNC Symbol;Acc:HGNC:9055]	1.02	4.06E-02
PHACTR1	phosphatase and actin regulator 1 [Source:HGNC Symbol;Acc:HGNC:20990]	1.01	3.73E-03
NUCKS1	nuclear casein kinase and cyclin dependent kinase substrate 1 [Source:HGNC Symbol;Acc:HGNC:29923]	1.01	5.58E-04
STING1	stimulator of interferon response cGAMP interactor 1 [Source:HGNC Symbol;Acc:HGNC:27962]	1.01	4.14E-04
TENM3	teneurin transmembrane protein 3 [Source:HGNC Symbol;Acc:HGNC:29944]	1.00	3.38E-02
DOCK11	dedicator of cytokinesis 11 [Source:HGNC Symbol;Acc:HGNC:23483]	1.00	2.65E-02
CD44	CD44 molecule (Indian blood group) [Source:HGNC Symbol;Acc:HGNC:1681]	1.00	1.05E-02
TIPARP	TCDD inducible poly(ADP-ribose) polymerase [Source:HGNC Symbol;Acc:HGNC:23696]	1.00	5.73E-04
WDR54	WD repeat domain 54 [Source:HGNC Symbol;Acc:HGNC:25770]	0.99	1.14E-02
PLXNA2	plexin A2 [Source:HGNC Symbol;Acc:HGNC:9100]	0.99	7.53E-03
DUSP5	dual specificity phosphatase 5 [Source:HGNC Symbol;Acc:HGNC:3071]	0.99	1.09E-02
ADAMTS9	ADAM metallopeptidase with thrombospondin type 1 motif 9 [Source:HGNC Symbol;Acc:HGNC:13202]	0.98	7.36E-03
LRP5	LDL receptor related protein 5 [Source:HGNC Symbol;Acc:HGNC:6697]	0.98	7.57E-04
CCND1	cyclin D1 [Source:HGNC Symbol;Acc:HGNC:1582]	0.98	1.85E-06
NCALD	neurocalcin delta [Source:HGNC Symbol;Acc:HGNC:7655]	0.97	3.38E-02
LBH	LBH regulator of WNT signaling pathway [Source:HGNC Symbol;Acc:HGNC:29532]	0.97	7.71E-03
MYO18B	myosin XVIIIB [Source:HGNC Symbol;Acc:HGNC:18150]	0.96	2.31E-03
VPS13A	vacuolar protein sorting 13 homolog A [Source:HGNC Symbol;Acc:HGNC:1908]	0.96	1.26E-02
PRKACA	protein kinase cAMP-activated catalytic subunit alpha [Source:HGNC Symbol;Acc:HGNC:9380]	0.95	4.73E-02
GNAO1	G protein subunit alpha o1 [Source:HGNC Symbol;Acc:HGNC:4389]	0.95	4.62E-02
GEM	GTP binding protein overexpressed in skeletal muscle [Source:HGNC Symbol;Acc:HGNC:4234]	0.94	3.41E-02
HNRNPA0	heterogeneous nuclear ribonucleoprotein A0 [Source:HGNC Symbol;Acc:HGNC:5030]	0.94	4.13E-02
JPT2	Jupiter microtubule associated homolog 2 [Source:HGNC Symbol;Acc:HGNC:14137]	0.94	4.18E-02
RRM2	ribonucleotide reductase regulatory subunit M2 [Source:HGNC Symbol;Acc:HGNC:10452]	0.94	3.70E-02

IRF6	interferon regulatory factor 6 [Source:HGNC Symbol;Acc:HGNC:6121]	0.93	4.68E-02
PEX26	peroxisomal biogenesis factor 26 [Source:HGNC Symbol;Acc:HGNC:22965]	0.93	1.72E-02
SYNDIG1	synapse differentiation inducing 1 [Source:HGNC Symbol;Acc:HGNC:15885]	0.93	2.19E-02
PDGFD	platelet derived growth factor D [Source:HGNC Symbol;Acc:HGNC:30620]	0.93	1.28E-02
ETV3	ETS variant transcription factor 3 [Source:HGNC Symbol;Acc:HGNC:3492]	0.92	2.95E-02
TAB3	TGF-beta activated kinase 1 (MAP3K7) binding protein 3 [Source:HGNC Symbol;Acc:HGNC:30681]	0.92	4.16E-02
SRSF6	serine and arginine rich splicing factor 6 [Source:HGNC Symbol;Acc:HGNC:10788]	0.92	3.06E-03
FKBP5	FKBP prolyl isomerase 5 [Source:HGNC Symbol;Acc:HGNC:3721]	0.91	1.49E-02
CDC42	cell division cycle 42 [Source:HGNC Symbol;Acc:HGNC:1736]	0.90	9.44E-04
CBX7	chromobox 7 [Source:HGNC Symbol;Acc:HGNC:1557]	0.90	2.01E-02
THBS4	thrombospondin 4 [Source:HGNC Symbol;Acc:HGNC:11788]	0.90	5.96E-03
KIF1C	kinesin family member 1C [Source:HGNC Symbol;Acc:HGNC:6317]	0.89	1.13E-03
FAM219A	family with sequence similarity 219 member A [Source:HGNC Symbol;Acc:HGNC:19920]	0.89	3.28E-02
RYR2	ryanodine receptor 2 [Source:HGNC Symbol;Acc:HGNC:10484]	0.88	3.29E-03
MSX2	msh homeobox 2 [Source:HGNC Symbol;Acc:HGNC:7392]	0.88	2.46E-03
TFRC	transferrin receptor [Source:HGNC Symbol;Acc:HGNC:11763]	0.88	2.65E-03
SC5D	sterol-C5-desaturase [Source:HGNC Symbol;Acc:HGNC:10547]	0.88	3.22E-03
DMXL2	Dmx like 2 [Source:HGNC Symbol;Acc:HGNC:2938]	0.87	1.79E-03
ZNF385B	zinc finger protein 385B [Source:HGNC Symbol;Acc:HGNC:26332]	0.87	5.17E-03
CREB3L2	cAMP responsive element binding protein 3 like 2 [Source:HGNC Symbol;Acc:HGNC:23720]	0.87	9.86E-03
USP9X	ubiquitin specific peptidase 9 X-linked [Source:HGNC Symbol;Acc:HGNC:12632]	0.86	1.79E-03
SSH2	slingshot protein phosphatase 2 [Source:HGNC Symbol;Acc:HGNC:30580]	0.86	1.25E-02
YEATS2	YEATS domain containing 2 [Source:HGNC Symbol;Acc:HGNC:25489]	0.86	3.29E-03
ABCA2	ATP binding cassette subfamily A member 2 [Source:HGNC Symbol;Acc:HGNC:32]	0.86	5.17E-03
CCDC141	coiled-coil domain containing 141 [Source:HGNC Symbol;Acc:HGNC:26821]	0.86	1.01E-03
TCAP	titin-cap [Source:HGNC Symbol;Acc:HGNC:11610]	0.85	1.11E-03
TXNIP	thioredoxin interacting protein [Source:HGNC Symbol;Acc:HGNC:16952]	0.85	1.55E-02
PLCXD3	phosphatidylinositol specific phospholipase C X domain containing 3 [Source:HGNC Symbol;Acc:HGNC:31822]	0.85	1.94E-02
KDM3A	lysine demethylase 3A [Source:HGNC Symbol;Acc:HGNC:20815]	0.85	7.95E-03
BANCR	BRAF-activated non-protein coding RNA [Source:HGNC Symbol;Acc:HGNC:43877]	0.85	1.85E-05
TMEM65	transmembrane protein 65 [Source:HGNC Symbol;Acc:HGNC:25203]	0.84	3.86E-02
LCLAT1	lysocardiolipin acyltransferase 1 [Source:HGNC Symbol;Acc:HGNC:26756]	0.84	2.37E-02
NEFL	neurofilament light [Source:HGNC Symbol;Acc:HGNC:7739]	0.84	1.69E-02
MCC	MCC regulator of WNT signaling pathway [Source:HGNC Symbol;Acc:HGNC:6935]	0.84	1.85E-05
ADD2	adducin 2 [Source:HGNC Symbol;Acc:HGNC:244]	0.83	2.36E-02

LINC00881	long intergenic non-protein coding RNA 881 [Source:HGNC Symbol;Acc:HGNC:48567]	0.83	9.41E-03
CPNE5	copine 5 [Source:HGNC Symbol;Acc:HGNC:2318]	0.83	4.74E-03
LMOD2	leiomodin 2 [Source:HGNC Symbol;Acc:HGNC:6648]	0.82	4.36E-03
CBX6	chromobox 6 [Source:HGNC Symbol;Acc:HGNC:1556]	0.81	2.63E-02
GNG7	G protein subunit gamma 7 [Source:HGNC Symbol;Acc:HGNC:4410]	0.81	1.71E-02
FAM122B	family with sequence similarity 122B [Source:HGNC Symbol;Acc:HGNC:30490]	0.80	1.23E-02
CACNB1	calcium voltage-gated channel auxiliary subunit beta 1 [Source:HGNC Symbol;Acc:HGNC:1401]	0.79	1.28E-02
ELOVL6	ELOVL fatty acid elongase 6 [Source:HGNC Symbol;Acc:HGNC:15829]	0.79	4.79E-02
ANK2	ankyrin 2 [Source:HGNC Symbol;Acc:HGNC:493]	0.79	4.68E-02
KDM2B	lysine demethylase 2B [Source:HGNC Symbol;Acc:HGNC:13610]	0.78	1.84E-02
DCAF17	DDB1 and CUL4 associated factor 17 [Source:HGNC Symbol;Acc:HGNC:25784]	0.78	4.62E-02
PKM	pyruvate kinase M1/2 [Source:HGNC Symbol;Acc:HGNC:9021]	0.78	9.55E-03
SLC35F1	solute carrier family 35 member F1 [Source:HGNC Symbol;Acc:HGNC:21483]	0.78	3.23E-02
LRRC20	leucine rich repeat containing 20 [Source:HGNC Symbol;Acc:HGNC:23421]	0.78	1.55E-02
PFKL	phosphofructokinase, liver type [Source:HGNC Symbol;Acc:HGNC:8876]	0.77	3.87E-02
TMTC3	transmembrane O-mannosyltransferase targeting cadherins 3 [Source:HGNC Symbol;Acc:HGNC:26899]	0.77	2.25E-03
DGAT2	diacylglycerol O-acyltransferase 2 [Source:HGNC Symbol;Acc:HGNC:16940]	0.77	2.69E-02
MYOM1	myomesin 1 [Source:HGNC Symbol;Acc:HGNC:7613]	0.76	3.27E-02
HEATR1	HEAT repeat containing 1 [Source:HGNC Symbol;Acc:HGNC:25517]	0.76	2.49E-02
DEPP1	DEPP1 autophagy regulator [Source:HGNC Symbol;Acc:HGNC:23355]	0.76	7.53E-03
PDE4DIP	phosphodiesterase 4D interacting protein [Source:HGNC Symbol;Acc:HGNC:15580]	0.76	6.71E-03
ZKSCAN2	zinc finger with KRAB and SCAN domains 2 [Source:HGNC Symbol;Acc:HGNC:25677]	0.74	3.15E-02
C4orf3	chromosome 4 open reading frame 3 [Source:HGNC Symbol;Acc:HGNC:19225]	0.74	3.81E-02
MIDEAS	mitotic deacetylase associated SANT domain protein [Source:HGNC Symbol;Acc:HGNC:19853]	0.74	3.42E-02
MLH3	mutL homolog 3 [Source:HGNC Symbol;Acc:HGNC:7128]	0.73	4.05E-02
C20orf194	chromosome 20 open reading frame 194 [Source:HGNC Symbol;Acc:HGNC:17721]	0.73	1.95E-02
IGF2R	insulin like growth factor 2 receptor [Source:HGNC Symbol;Acc:HGNC:5467]	0.72	1.08E-02
MAPK8IP3	mitogen-activated protein kinase 8 interacting protein 3 [Source:HGNC Symbol;Acc:HGNC:6884]	0.72	2.00E-02
SMCHD1	structural maintenance of chromosomes flexible hinge domain containing 1 [Source:HGNC Symbol;Acc:HGNC:29090]	0.72	2.86E-02
NFYB	nuclear transcription factor Y subunit beta [Source:HGNC Symbol;Acc:HGNC:7805]	0.71	4.58E-02
PRKAA2	protein kinase AMP-activated catalytic subunit alpha 2 [Source:HGNC Symbol;Acc:HGNC:9377]	0.70	1.33E-02
EXOC6B	exocyst complex component 6B [Source:HGNC Symbol;Acc:HGNC:17085]	0.70	3.21E-02
AKAP6	A-kinase anchoring protein 6 [Source:HGNC Symbol;Acc:HGNC:376]	0.69	4.63E-02

PHLDA1	pleckstrin homology like domain family A member 1 [Source:HGNC Symbol;Acc:HGNC:8933]	0.69	4.15E-02
PRSS35	serine protease 35 [Source:HGNC Symbol;Acc:HGNC:21387]	0.68	5.69E-03
CKMT2	creatine kinase, mitochondrial 2 [Source:HGNC Symbol;Acc:HGNC:1996]	0.67	1.47E-02
INSR	insulin receptor [Source:HGNC Symbol;Acc:HGNC:6091]	0.66	1.15E-02
SCN5A	sodium voltage-gated channel alpha subunit 5 [Source:HGNC Symbol;Acc:HGNC:10593]	0.66	1.53E-03
NRP1	neuropilin 1 [Source:HGNC Symbol;Acc:HGNC:8004]	0.65	1.39E-02
NCOA3	nuclear receptor coactivator 3 [Source:HGNC Symbol;Acc:HGNC:7670]	0.65	2.54E-02
SLC20A2	solute carrier family 20 member 2 [Source:HGNC Symbol;Acc:HGNC:10947]	0.65	7.79E-03
PIFO	primary cilia formation [Source:HGNC Symbol;Acc:HGNC:27009]	0.64	1.33E-02
CTNNB1	catenin beta 1 [Source:HGNC Symbol;Acc:HGNC:2514]	0.64	4.68E-02
ANP32A	acidic nuclear phosphoprotein 32 family member A [Source:HGNC Symbol;Acc:HGNC:13233]	0.64	3.81E-02
GPD1L	glycerol-3-phosphate dehydrogenase 1 like [Source:HGNC Symbol;Acc:HGNC:28956]	0.62	3.62E-02
PDCD11	programmed cell death 11 [Source:HGNC Symbol;Acc:HGNC:13408]	0.62	2.76E-02
MCL1	MCL1 apoptosis regulator, BCL2 family member [Source:HGNC Symbol;Acc:HGNC:6943]	0.62	1.57E-02
LZTS3	leucine zipper tumor suppressor family member 3 [Source:HGNC Symbol;Acc:HGNC:30139]	0.62	3.85E-02
GLUL	glutamate-ammonia ligase [Source:HGNC Symbol;Acc:HGNC:4341]	0.62	1.23E-03
MFGE8	milk fat globule-EGF factor 8 protein [Source:HGNC Symbol;Acc:HGNC:7036]	0.62	2.76E-02
DOCK7	dedicator of cytokinesis 7 [Source:HGNC Symbol;Acc:HGNC:19190]	0.61	2.21E-02
SMARCC1	SWI/SNF related, matrix associated, actin dependent regulator of chromatin subfamily c member 1 [Source:HGNC Symbol;Acc:HGNC:11104]	0.61	1.33E-02
PWWP3B	PWWP domain containing 3B [Source:HGNC Symbol;Acc:HGNC:26583]	0.61	4.80E-02
DARS1	aspartyl-tRNA synthetase 1 [Source:HGNC Symbol;Acc:HGNC:2678]	0.61	3.62E-02
AC245297.1	phosphodiesterase 4D interacting protein (myomegalin) (PDE4DIP) pseudogene	0.60	2.63E-02
PARP4	poly(ADP-ribose) polymerase family member 4 [Source:HGNC Symbol;Acc:HGNC:271]	0.60	3.65E-02
TTC28	tetratricopeptide repeat domain 28 [Source:HGNC Symbol;Acc:HGNC:29179]	0.60	3.41E-02
ACADSB	acyl-CoA dehydrogenase short/branched chain [Source:HGNC Symbol;Acc:HGNC:91]	0.59	1.16E-02
MASP1	mannan binding lectin serine peptidase 1 [Source:HGNC Symbol;Acc:HGNC:6901]	0.58	4.44E-02
SET	SET nuclear proto-oncogene [Source:HGNC Symbol;Acc:HGNC:10760]	0.58	4.47E-02
DTNA	dystrobrevin alpha [Source:HGNC Symbol;Acc:HGNC:3057]	0.58	1.39E-02
ID2	inhibitor of DNA binding 2 [Source:HGNC Symbol;Acc:HGNC:5361]	0.58	1.81E-02
CBLB	Cbl proto-oncogene B [Source:HGNC Symbol;Acc:HGNC:1542]	0.56	4.26E-02
AKAP13	A-kinase anchoring protein 13 [Source:HGNC Symbol;Acc:HGNC:371]	0.55	4.48E-02
XPR1	xenotropic and polytropic retrovirus receptor 1 [Source:HGNC Symbol;Acc:HGNC:12827]	0.54	3.29E-02
GPR162	G protein-coupled receptor 162 [Source:HGNC Symbol;Acc:HGNC:16693]	0.53	4.18E-02
H3-3B	H3.3 histone B [Source:HGNC Symbol;Acc:HGNC:4765]	0.52	4.17E-02

NDRG2	NDRG family member 2 [Source:HGNC Symbol;Acc:HGNC:14460]	0.51	2.81E-02

Supplementary Table 2. 199 downregulated DEGs in the ISS μG condition compared with the ISS 1G condition.

Gene	Description	log_2(Fold change)	adjusted p-value
SLCO4C1	solute carrier organic anion transporter family member 4C1 [Source:HGNC Symbol;Acc:HGNC:23612]	-7.93	2.2E-06
HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 2 [Source:HGNC Symbol;Acc:HGNC:5218]	-7.46	7.7E-04
LINC02201	long intergenic non-protein coding RNA 2201 [Source:HGNC Symbol;Acc:HGNC:53067]	-6.42	1.1E-02
UGT8	UDP glycosyltransferase 8 [Source:HGNC Symbol;Acc:HGNC:12555]	-6.36	7.5E-03
FAM131B	family with sequence similarity 131 member B [Source:HGNC Symbol;Acc:HGNC:22202]	-6.35	6.6E-03
DNAH2	dynein axonemal heavy chain 2 [Source:HGNC Symbol;Acc:HGNC:2948]	-6.20	8.2E-04
MUC4	mucin 4, cell surface associated [Source:HGNC Symbol;Acc:HGNC:7514]	-6.08	1.4E-02
PCSK9	proprotein convertase subtilisin/kexin type 9 [Source:HGNC Symbol;Acc:HGNC:20001]	-6.06	2.5E-02
ITIH5	inter-alpha-trypsin inhibitor heavy chain 5 [Source:HGNC Symbol;Acc:HGNC:21449]	-6.01	2.9E-02
VIPR1	vasoactive intestinal peptide receptor 1 [Source:HGNC Symbol;Acc:HGNC:12694]	-6.00	1.8E-02
COL4A4	collagen type IV alpha 4 chain [Source:HGNC Symbol;Acc:HGNC:2206]	-5.73	1.2E-02
SATB2	SATB homeobox 2 [Source:HGNC Symbol;Acc:HGNC:21637]	-5.37	3.2E-02
DNAH12	dynein axonemal heavy chain 12 [Source:HGNC Symbol;Acc:HGNC:2943]	-5.18	3.7E-02
ARHGEF15	Rho guanine nucleotide exchange factor 15 [Source:HGNC Symbol;Acc:HGNC:15590]	-5.13	3.8E-02
GABRP	gamma-aminobutyric acid type A receptor subunit pi [Source:HGNC Symbol;Acc:HGNC:4089]	-5.10	8.2E-05
C5orf46	chromosome 5 open reading frame 46 [Source:HGNC Symbol;Acc:HGNC:33768]	-4.05	2.1E-02
PTPRZ1	protein tyrosine phosphatase receptor type Z1 [Source:HGNC Symbol;Acc:HGNC:9685]	-3.90	1.7E-02
EDN1	endothelin 1 [Source:HGNC Symbol;Acc:HGNC:3176]	-3.52	4.4E-02
GRAMD1C	GRAM domain containing 1C [Source:HGNC Symbol;Acc:HGNC:25252]	-3.34	2.2E-02
TRPC4	transient receptor potential cation channel subfamily C member 4 [Source:HGNC Symbol;Acc:HGNC:12336]	-3.24	1.1E-03
Z97832.2	novel transcript, antisense to SCUBE3	-3.19	3.4E-02
CDHR1	cadherin related family member 1 [Source:HGNC Symbol;Acc:HGNC:14550]	-2.98	9.8E-03
SLC38A5	solute carrier family 38 member 5 [Source:HGNC Symbol;Acc:HGNC:18070]	-2.90	4.5E-02
MMP24	matrix metallopeptidase 24 [Source:HGNC Symbol;Acc:HGNC:7172]	-2.79	6.9E-10
C1R	complement C1r [Source:HGNC Symbol;Acc:HGNC:1246]	-2.74	4.1E-09
RGCC	regulator of cell cycle [Source:HGNC Symbol;Acc:HGNC:20369]	-2.68	4.8E-03
ITGA11	integrin subunit alpha 11 [Source:HGNC Symbol;Acc:HGNC:6136]	-2.65	1.4E-04
MFSD2A	major facilitator superfamily domain containing 2A [Source:HGNC Symbol;Acc:HGNC:25897]	-2.59	3.3E-03

NECAB2	N-terminal EF-hand calcium binding protein 2 [Source:HGNC Symbol;Acc:HGNC:23746]	-2.55	3.9E-04
MYOT	myotilin [Source:HGNC Symbol;Acc:HGNC:12399]	-2.36	3.4E-02
PROCR	protein C receptor [Source:HGNC Symbol;Acc:HGNC:9452]	-2.33	2.6E-02
COL26A1	collagen type XXVI alpha 1 chain [Source:HGNC Symbol;Acc:HGNC:18038]	-2.28	9.7E-04
NTRK1	neurotrophic receptor tyrosine kinase 1 [Source:HGNC Symbol;Acc:HGNC:8031]	-2.27	1.2E-03
HPX	hemopexin [Source:HGNC Symbol;Acc:HGNC:5171]	-2.23	3.4E-02
PHF24	PHD finger protein 24 [Source:HGNC Symbol;Acc:HGNC:29180]	-2.21	9.4E-04
AC018647.1	novel transcript	-2.13	1.1E-03
SFRP5	secreted frizzled related protein 5 [Source:HGNC Symbol;Acc:HGNC:10779]	-2.03	4.4E-11
ENC1	ectodermal-neural cortex 1 [Source:HGNC Symbol;Acc:HGNC:3345]	-2.00	8.3E-03
AC023481.1	novel transcript	-1.96	3.3E-02
SLITRK4	SLIT and NTRK like family member 4 [Source:HGNC Symbol;Acc:HGNC:23502]	-1.94	4.8E-02
NIPAL4	NIPA like domain containing 4 [Source:HGNC Symbol;Acc:HGNC:28018]	-1.87	1.1E-04
ANXA1	annexin A1 [Source:HGNC Symbol;Acc:HGNC:533]	-1.83	4.5E-05
LIMA1	LIM domain and actin binding 1 [Source:HGNC Symbol;Acc:HGNC:24636]	-1.79	1.1E-02
HPSE2	heparanase 2 (inactive) [Source:HGNC Symbol;Acc:HGNC:18374]	-1.76	1.2E-02
GPC4	glypican 4 [Source:HGNC Symbol;Acc:HGNC:4452]	-1.74	8.7E-06
INHBA	inhibin subunit beta A [Source:HGNC Symbol;Acc:HGNC:6066]	-1.71	1.7E-02
ERICH5	glutamate rich 5 [Source:HGNC Symbol;Acc:HGNC:26823]	-1.70	5.7E-03
SLC35F2	solute carrier family 35 member F2 [Source:HGNC Symbol;Acc:HGNC:23615]	-1.68	3.1E-03
AC109583.1	Probable threonine protease PRSS50 [Source:UniProtKB/Swiss-Prot;Acc:Q9UI38]	-1.65	4.0E-02
SNCAIP	synuclein alpha interacting protein [Source:HGNC Symbol;Acc:HGNC:11139]	-1.64	2.4E-03
CDC42EP2	CDC42 effector protein 2 [Source:HGNC Symbol;Acc:HGNC:16263]	-1.63	7.3E-03
RASGRP1	RAS guanyl releasing protein 1 [Source:HGNC Symbol;Acc:HGNC:9878]	-1.56	2.0E-03
MYRF	myelin regulatory factor [Source:HGNC Symbol;Acc:HGNC:1181]	-1.55	8.9E-03
CD24	CD24 molecule [Source:HGNC Symbol;Acc:HGNC:1645]	-1.54	6.2E-14
GPR176	G protein-coupled receptor 176 [Source:HGNC Symbol;Acc:HGNC:32370]	-1.48	2.5E-03
ELMO1	engulfment and cell motility 1 [Source:HGNC Symbol;Acc:HGNC:16286]	-1.47	5.5E-03
CTSV	cathepsin V [Source:HGNC Symbol;Acc:HGNC:2538]	-1.44	6.3E-06
TCIRG1	T cell immune regulator 1, ATPase H+ transporting V0 subunit a3 [Source:HGNC Symbol;Acc:HGNC:11647]	-1.43	4.9E-03
LDB2	LIM domain binding 2 [Source:HGNC Symbol;Acc:HGNC:6533]	-1.42	9.1E-04
TAGLN	transgelin [Source:HGNC Symbol;Acc:HGNC:11553]	-1.40	3.7E-05
EFHD1	EF-hand domain family member D1 [Source:HGNC Symbol;Acc:HGNC:29556]	-1.37	3.8E-02
PPM1H	protein phosphatase, Mg2+/Mn2+ dependent 1H [Source:HGNC Symbol;Acc:HGNC:18583]	-1.36	1.9E-02
ACAT2	acetyl-CoA acetyltransferase 2 [Source:HGNC Symbol;Acc:HGNC:94]	-1.36	2.3E-03

P3H3	prolyl 3-hydroxylase 3 [Source:HGNC Symbol;Acc:HGNC:19318]	-1.36	2.1E-02
NDNF	neuron derived neurotrophic factor [Source:HGNC Symbol;Acc:HGNC:26256]	-1.35	1.4E-02
LAMC2	laminin subunit gamma 2 [Source:HGNC Symbol;Acc:HGNC:6493]	-1.33	2.3E-02
MATN2	matrilin 2 [Source:HGNC Symbol;Acc:HGNC:6908]	-1.32	2.3E-09
PLPPR4	phospholipid phosphatase related 4 [Source:HGNC Symbol;Acc:HGNC:23496]	-1.31	8.3E-03
HCN3	hyperpolarization activated cyclic nucleotide gated potassium channel 3 [Source:HGNC Symbol;Acc:HGNC:19183]	-1.29	8.6E-03
ACTA1	actin alpha 1, skeletal muscle [Source:HGNC Symbol;Acc:HGNC:129]	-1.29	9.1E-04
RELB	RELB proto-oncogene, NF-kB subunit [Source:HGNC Symbol;Acc:HGNC:9956]	-1.28	1.1E-02
KMT2D	lysine methyltransferase 2D [Source:HGNC Symbol;Acc:HGNC:7133]	-1.27	1.0E-02
CRABP2	cellular retinoic acid binding protein 2 [Source:HGNC Symbol;Acc:HGNC:2339]	-1.26	1.8E-09
DDIT3	DNA damage inducible transcript 3 [Source:HGNC Symbol;Acc:HGNC:2726]	-1.23	1.7E-02
MT-ND6	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 6 [Source:HGNC Symbol;Acc:HGNC:7462]	-1.23	1.5E-04
ARRB1	arrestin beta 1 [Source:HGNC Symbol;Acc:HGNC:711]	-1.23	1.9E-02
RNF19B	ring finger protein 19B [Source:HGNC Symbol;Acc:HGNC:26886]	-1.23	5.9E-03
RAI14	retinoic acid induced 14 [Source:HGNC Symbol;Acc:HGNC:14873]	-1.22	1.3E-04
TKT	transketolase [Source:HGNC Symbol;Acc:HGNC:11834]	-1.21	1.9E-04
EHD2	EH domain containing 2 [Source:HGNC Symbol;Acc:HGNC:3243]	-1.20	2.2E-02
SEC31B	SEC31 homolog B, COPII coat complex component [Source:HGNC Symbol;Acc:HGNC:23197]	-1.17	7.5E-03
ASXL3	ASXL transcriptional regulator 3 [Source:HGNC Symbol;Acc:HGNC:29357]	-1.16	1.9E-02
GNA11	G protein subunit alpha 11 [Source:HGNC Symbol;Acc:HGNC:4379]	-1.16	8.9E-03
MYO1D	myosin ID [Source:HGNC Symbol;Acc:HGNC:7598]	-1.15	2.3E-03
MGST1	microsomal glutathione S-transferase 1 [Source:HGNC Symbol;Acc:HGNC:7061]	-1.12	4.6E-04
THBS1	thrombospondin 1 [Source:HGNC Symbol;Acc:HGNC:11785]	-1.12	7.7E-03
BRD1	bromodomain containing 1 [Source:HGNC Symbol;Acc:HGNC:1102]	-1.12	3.3E-02
CMTM5	CKLF like MARVEL transmembrane domain containing 5 [Source:HGNC Symbol;Acc:HGNC:19176]	-1.11	1.3E-02
TP53I3	tumor protein p53 inducible protein 3 [Source:HGNC Symbol;Acc:HGNC:19373]	-1.11	3.0E-03
CORO7	coronin 7 [Source:HGNC Symbol;Acc:HGNC:26161]	-1.10	1.2E-02
S100A10	S100 calcium binding protein A10 [Source:HGNC Symbol;Acc:HGNC:10487]	-1.09	7.9E-03
ACTB	actin beta [Source:HGNC Symbol;Acc:HGNC:132]	-1.09	5.0E-03
H2BC4	H2B clustered histone 4 [Source:HGNC Symbol;Acc:HGNC:4757]	-1.07	3.8E-02
IDI1	isopentenyl-diphosphate delta isomerase 1 [Source:HGNC Symbol;Acc:HGNC:5387]	-1.06	1.3E-02
VAMP1	vesicle associated membrane protein 1 [Source:HGNC Symbol;Acc:HGNC:12642]	-1.06	1.5E-02
CAPN5	calpain 5 [Source:HGNC Symbol;Acc:HGNC:1482]	-1.05	4.2E-03

TRIO	trio Rho guanine nucleotide exchange factor [Source:HGNC Symbol;Acc:HGNC:12303]	-1.05	7.3E-03
COL1A1	collagen type I alpha 1 chain [Source:HGNC Symbol;Acc:HGNC:2197]	-1.05	2.6E-02
COTL1	coactosin like F-actin binding protein 1 [Source:HGNC Symbol;Acc:HGNC:18304]	-1.04	4.9E-03
TMEM97	transmembrane protein 97 [Source:HGNC Symbol;Acc:HGNC:28106]	-1.03	3.4E-02
CLK1	CDC like kinase 1 [Source:HGNC Symbol;Acc:HGNC:2068]	-1.02	1.1E-03
ADA	adenosine deaminase [Source:HGNC Symbol;Acc:HGNC:186]	-1.02	1.2E-02
INHA	inhibin subunit alpha [Source:HGNC Symbol;Acc:HGNC:6065]	-1.01	4.7E-02
LRRN3	leucine rich repeat neuronal 3 [Source:HGNC Symbol;Acc:HGNC:17200]	-1.01	2.8E-02
NAGLU	N-acetyl-alpha-glucosaminidase [Source:HGNC Symbol;Acc:HGNC:7632]	-1.00	5.0E-04
TCN2	transcobalamin 2 [Source:HGNC Symbol;Acc:HGNC:11653]	-0.99	1.5E-02
EHD3	EH domain containing 3 [Source:HGNC Symbol;Acc:HGNC:3244]	-0.99	1.1E-03
TXNL4B	thioredoxin like 4B [Source:HGNC Symbol;Acc:HGNC:26041]	-0.98	3.9E-02
MSMO1	methylsterol monooxygenase 1 [Source:HGNC Symbol;Acc:HGNC:10545]	-0.98	1.3E-02
CLK4	CDC like kinase 4 [Source:HGNC Symbol;Acc:HGNC:13659]	-0.98	5.2E-04
PRSS23	serine protease 23 [Source:HGNC Symbol;Acc:HGNC:14370]	-0.98	5.9E-04
PAOX	polyamine oxidase [Source:HGNC Symbol;Acc:HGNC:20837]	-0.98	1.8E-02
EMP2	epithelial membrane protein 2 [Source:HGNC Symbol;Acc:HGNC:3334]	-0.97	2.2E-04
CEP164	centrosomal protein 164 [Source:HGNC Symbol;Acc:HGNC:29182]	-0.96	3.8E-02
ZNF467	zinc finger protein 467 [Source:HGNC Symbol;Acc:HGNC:23154]	-0.95	1.4E-02
CALD1	caldesmon 1 [Source:HGNC Symbol;Acc:HGNC:1441]	-0.95	3.6E-02
ACHE	acetylcholinesterase (Cartwright blood group) [Source:HGNC Symbol;Acc:HGNC:108]	-0.95	1.7E-02
LIMK2	LIM domain kinase 2 [Source:HGNC Symbol;Acc:HGNC:6614]	-0.94	1.2E-02
FAM189A2	family with sequence similarity 189 member A2 [Source:HGNC Symbol;Acc:HGNC:24820]	-0.94	9.4E-03
USP7	ubiquitin specific peptidase 7 [Source:HGNC Symbol;Acc:HGNC:12630]	-0.94	1.1E-02
GBP2	guanylate binding protein 2 [Source:HGNC Symbol;Acc:HGNC:4183]	-0.93	1.7E-02
MFAP4	microfibril associated protein 4 [Source:HGNC Symbol;Acc:HGNC:7035]	-0.93	3.3E-02
IQGAP1	IQ motif containing GTPase activating protein 1 [Source:HGNC Symbol;Acc:HGNC:6110]	-0.92	3.7E-02
FAM89A	family with sequence similarity 89 member A [Source:HGNC Symbol;Acc:HGNC:25057]	-0.92	1.4E-02
NAGPA	N-acetylglucosamine-1-phosphodiester alpha-N-acetylglucosaminidase [Source:HGNC Symbol;Acc:HGNC:17378]	-0.90	2.1E-02
RHPN1	rhophilin Rho GTPase binding protein 1 [Source:HGNC Symbol;Acc:HGNC:19973]	-0.90	1.5E-02
PLS3	plastin 3 [Source:HGNC Symbol;Acc:HGNC:9091]	-0.90	1.8E-04
SGK1	serum/glucocorticoid regulated kinase 1 [Source:HGNC Symbol;Acc:HGNC:10810]	-0.90	4.4E-02
GLRX	glutaredoxin [Source:HGNC Symbol;Acc:HGNC:4330]	-0.90	3.0E-02
GSDME	gasdermin E [Source:HGNC Symbol;Acc:HGNC:2810]	-0.89	4.7E-03
SQLE	squalene epoxidase [Source:HGNC Symbol;Acc:HGNC:11279]	-0.89	1.0E-02

ITGB5	integrin subunit beta 5 [Source:HGNC Symbol;Acc:HGNC:6160]	-0.89	3.6E-02
P3H1	prolyl 3-hydroxylase 1 [Source:HGNC Symbol;Acc:HGNC:19316]	-0.89	2.0E-02
DAB2	DAB adaptor protein 2 [Source:HGNC Symbol;Acc:HGNC:2662]	-0.88	3.4E-02
ASS1	argininosuccinate synthase 1 [Source:HGNC Symbol;Acc:HGNC:758]	-0.88	5.5E-03
CSRP2	cysteine and glycine rich protein 2 [Source:HGNC Symbol;Acc:HGNC:2470]	-0.88	4.9E-03
PLXNA1	plexin A1 [Source:HGNC Symbol;Acc:HGNC:9099]	-0.88	4.7E-02
ARSA	arylsulfatase A [Source:HGNC Symbol;Acc:HGNC:713]	-0.88	1.0E-02
FHL1	four and a half LIM domains 1 [Source:HGNC Symbol;Acc:HGNC:3702]	-0.87	2.3E-02
HSD17B14	hydroxysteroid 17-beta dehydrogenase 14 [Source:HGNC Symbol;Acc:HGNC:23238]	-0.86	4.2E-02
MAFG	MAF bZIP transcription factor G [Source:HGNC Symbol;Acc:HGNC:6781]	-0.86	9.9E-03
EFNB2	ephrin B2 [Source:HGNC Symbol;Acc:HGNC:3227]	-0.84	2.3E-03
TPM2	tropomyosin 2 [Source:HGNC Symbol;Acc:HGNC:12011]	-0.84	4.1E-04
VAT1L	vesicle amine transport 1 like [Source:HGNC Symbol;Acc:HGNC:29315]	-0.84	5.8E-03
GRN	granulin precursor [Source:HGNC Symbol;Acc:HGNC:4601]	-0.84	7.3E-03
C3orf70	chromosome 3 open reading frame 70 [Source:HGNC Symbol;Acc:HGNC:33731]	-0.83	3.7E-02
FBXL6	F-box and leucine rich repeat protein 6 [Source:HGNC Symbol;Acc:HGNC:13603]	-0.83	2.1E-02
PLAAT3	phospholipase A and acyltransferase 3 [Source:HGNC Symbol;Acc:HGNC:17825]	-0.83	1.4E-02
GUCY1A1	guanylate cyclase 1 soluble subunit alpha 1 [Source:HGNC Symbol;Acc:HGNC:4685]	-0.83	2.5E-02
TENT5A	terminal nucleotidyltransferase 5A [Source:HGNC Symbol;Acc:HGNC:18345]	-0.81	3.7E-03
P2RY14	purinergic receptor P2Y14 [Source:HGNC Symbol;Acc:HGNC:16442]	-0.80	1.3E-02
HTRA1	HtrA serine peptidase 1 [Source:HGNC Symbol;Acc:HGNC:9476]	-0.79	3.8E-02
PAXIP1-AS1	PAXIP1 antisense RNA 1 (head to head) [Source:HGNC Symbol;Acc:HGNC:27328]	-0.79	3.4E-02
LGR4	leucine rich repeat containing G protein-coupled receptor 4 [Source:HGNC Symbol;Acc:HGNC:13299]	-0.78	4.2E-02
SRPX	sushi repeat containing protein X-linked [Source:HGNC Symbol;Acc:HGNC:11309]	-0.78	3.3E-02
MVD	mevalonate diphosphate decarboxylase [Source:HGNC Symbol;Acc:HGNC:7529]	-0.77	3.3E-02
PUM3	pumilio RNA binding family member 3 [Source:HGNC Symbol;Acc:HGNC:29676]	-0.76	4.1E-02
TENM4	teneurin transmembrane protein 4 [Source:HGNC Symbol;Acc:HGNC:29945]	-0.76	1.7E-02
ULK3	unc-51 like kinase 3 [Source:HGNC Symbol;Acc:HGNC:19703]	-0.75	1.5E-03
MOV10	Mov10 RISC complex RNA helicase [Source:HGNC Symbol;Acc:HGNC:7200]	-0.74	4.9E-03
F11R	F11 receptor [Source:HGNC Symbol;Acc:HGNC:14685]	-0.73	5.1E-03
RSRP1	arginine and serine rich protein 1 [Source:HGNC Symbol;Acc:HGNC:25234]	-0.73	1.1E-02
CXXC5	CXXC finger protein 5 [Source:HGNC Symbol;Acc:HGNC:26943]	-0.73	2.4E-02
LRRC8D	leucine rich repeat containing 8 VRAC subunit D [Source:HGNC Symbol;Acc:HGNC:16992]	-0.72	2.7E-02

CERCAM	cerebral endothelial cell adhesion molecule [Source:HGNC Symbol;Acc:HGNC:23723]	-0.72	4.9E-03
SUMF1	sulfatase modifying factor 1 [Source:HGNC Symbol;Acc:HGNC:20376]	-0.72	9.8E-03
MVK	mevalonate kinase [Source:HGNC Symbol;Acc:HGNC:7530]	-0.72	1.8E-02
SWAP70	switching B cell complex subunit SWAP70 [Source:HGNC Symbol;Acc:HGNC:17070]	-0.71	3.8E-02
SLIT2	slit guidance ligand 2 [Source:HGNC Symbol;Acc:HGNC:11086]	-0.69	1.4E-02
S100A11	S100 calcium binding protein A11 [Source:HGNC Symbol;Acc:HGNC:10488]	-0.68	1.3E-02
FGFR2	fibroblast growth factor receptor 2 [Source:HGNC Symbol;Acc:HGNC:3689]	-0.68	4.4E-03
SPARC	secreted protein acidic and cysteine rich [Source:HGNC Symbol;Acc:HGNC:11219]	-0.68	1.7E-02
MYO5B	myosin VB [Source:HGNC Symbol;Acc:HGNC:7603]	-0.67	3.7E-02
HEXA	hexosaminidase subunit alpha [Source:HGNC Symbol;Acc:HGNC:4878]	-0.67	3.4E-02
PRDX1	peroxiredoxin 1 [Source:HGNC Symbol;Acc:HGNC:9352]	-0.67	1.8E-04
F2R	coagulation factor II thrombin receptor [Source:HGNC Symbol;Acc:HGNC:3537]	-0.66	2.6E-02
ACTN1	actinin alpha 1 [Source:HGNC Symbol;Acc:HGNC:163]	-0.66	1.2E-03
GIPC1	GIPC PDZ domain containing family member 1 [Source:HGNC Symbol;Acc:HGNC:1226]	-0.65	4.5E-03
MACROH2A1	macroH2A.1 histone [Source:HGNC Symbol;Acc:HGNC:4740]	-0.64	2.6E-02
KIF13A	kinesin family member 13A [Source:HGNC Symbol;Acc:HGNC:14566]	-0.64	2.8E-02
TP53I11	tumor protein p53 inducible protein 11 [Source:HGNC Symbol;Acc:HGNC:16842]	-0.63	1.5E-03
PRCP	prolylcarboxypeptidase [Source:HGNC Symbol;Acc:HGNC:9344]	-0.62	3.6E-02
TULP3	TUB like protein 3 [Source:HGNC Symbol;Acc:HGNC:12425]	-0.62	2.8E-02
CNN1	calponin 1 [Source:HGNC Symbol;Acc:HGNC:2155]	-0.61	4.0E-02
CERS5	ceramide synthase 5 [Source:HGNC Symbol;Acc:HGNC:23749]	-0.60	9.4E-03
ANXA2	annexin A2 [Source:HGNC Symbol;Acc:HGNC:537]	-0.60	6.7E-03
SERPINI1	serpin family I member 1 [Source:HGNC Symbol;Acc:HGNC:8943]	-0.60	3.3E-02
ANO10	anoctamin 10 [Source:HGNC Symbol;Acc:HGNC:25519]	-0.59	2.8E-02
EPHB2	EPH receptor B2 [Source:HGNC Symbol;Acc:HGNC:3393]	-0.59	9.1E-03
FLNA	filamin A [Source:HGNC Symbol;Acc:HGNC:3754]	-0.58	2.0E-02
AKR1B1	aldo-keto reductase family 1 member B [Source:HGNC Symbol;Acc:HGNC:381]	-0.58	2.4E-02
ACTA2	actin alpha 2, smooth muscle [Source:HGNC Symbol;Acc:HGNC:130]	-0.56	3.8E-02
PRKRIP1	PRKR interacting protein 1 [Source:HGNC Symbol;Acc:HGNC:21894]	-0.56	2.5E-02
TRIM54	tripartite motif containing 54 [Source:HGNC Symbol;Acc:HGNC:16008]	-0.56	2.8E-02
NFE2L2	nuclear factor, erythroid 2 like 2 [Source:HGNC Symbol;Acc:HGNC:7782]	-0.53	4.9E-02
RCN1	reticulocalbin 1 [Source:HGNC Symbol;Acc:HGNC:9934]	-0.53	5.0E-02
DDX28	DEAD-box helicase 28 [Source:HGNC Symbol;Acc:HGNC:17330]	-0.52	3.5E-02
ABHD4	abhydrolase domain containing 4 [Source:HGNC Symbol;Acc:HGNC:20154]	-0.50	3.3E-02
TSC22D3	TSC22 domain family member 3 [Source:HGNC Symbol;Acc:HGNC:3051]	-0.45	3.6E-02

Supplementary Table 3. Select upregulated GO terms in the ISS μG condition compared with the ISS 1G condition.

Ontology	ID	Description	adjusted p-value	Count
BP	GO:0003012	muscle system process	4.91E-09	29
BP	GO:0006936	muscle contraction	7.77E-09	25
BP	GO:0060537	muscle tissue development	7.19E-07	24
BP	GO:0014706	striated muscle tissue development	5.92E-06	22
BP	GO:0006941	striated muscle contraction	9.27E-06	15
BP	GO:0006942	regulation of striated muscle contraction	2.55E-05	11
BP	GO:0050673	epithelial cell proliferation	3.41E-05	22
BP	GO:0003015	heart process	8.21E-05	16
BP	GO:0090257	regulation of muscle system process	8.21E-05	16
BP	GO:0048738	cardiac muscle tissue development	1.54E-04	15
BP	GO:0003229	ventricular cardiac muscle tissue development	1.54E-04	8
BP	GO:0050679	positive regulation of epithelial cell proliferation	1.54E-04	14
BP	GO:0051302	regulation of cell division	1.54E-04	13
BP	GO:0050678	regulation of epithelial cell proliferation	1.54E-04	19
BP	GO:0060047	heart contraction	1.54E-04	15
BP	GO:0003205	cardiac chamber development	2.90E-04	12
BP	GO:1903522	regulation of blood circulation	2.90E-04	15
BP	GO:0006937	regulation of muscle contraction	4.44E-04	12
BP	GO:0008016	regulation of heart contraction	5.91E-04	13
BP	GO:0003231	cardiac ventricle development	8.53E-04	10
MF	GO:0140416	transcription regulator inhibitor activity	2.94E-03	5
MF	GO:0044325	transmembrane transporter binding	2.94E-03	10
MF	GO:0034237	protein kinase A regulatory subunit binding	2.94E-03	5
MF	GO:0051018	protein kinase A binding	7.32E-03	6
MF	GO:0140678	molecular function inhibitor activity	3.26E-02	8
MF	GO:0017154	semaphorin receptor activity	4.03E-02	3
CC	GO:0030016	myofibril	1.76E-03	13
CC	GO:0043292	contractile fiber	1.76E-03	13
CC	GO:0044853	plasma membrane raft	1.76E-03	9
CC	GO:0030017	sarcomere	1.76E-03	12
CC	GO:0045121	membrane raft	1.86E-03	15
CC	GO:0098857	membrane microdomain	1.86E-03	15

Ontology	ID	Description	adjusted p-value	Count
CC	GO:0014704	intercalated disc	2.19E-03	6
CC	GO:0042383	sarcolemma	3.43E-03	9
CC	GO:0051233	spindle midzone	3.72E-03	5
CC	GO:0005901	caveola	3.79E-03	7
CC	GO:0030018	Z disc	9.17E-03	8
CC	GO:0002116	semaphorin receptor complex	9.17E-03	3
CC	GO:0044291	cell-cell contact zone	9.17E-03	6
CC	GO:0034703	cation channel complex	1.04E-02	10
CC	GO:0019897	extrinsic component of plasma membrane	1.04E-02	9
CC	GO:0031674	I band	1.05E-02	8

Supplementary Table 4. DEGs associated with regulation of cell division.

Gene	Description	log_2(fold change)	adjusted p-value
KIF14	kinesin family member 14	2.11	2.42E-03
IGF2	insulin like growth factor 2	2.07	9.89E-03
KIF18B	kinesin family member 18B	1.89	3.23E-02
SUSD2	sushi domain containing 2	1.88	2.86E-08
RBL1	RB transcriptional corepressor like 1	1.32	1.19E-02
FGF1	fibroblast growth factor 1	1.3	5.54E-07
AURKB	aurora kinase B	1.28	2.47E-02
LBH	LBH regulator of WNT signaling pathway	0.97	7.71E-03
PDGFD	platelet derived growth factor D	0.93	1.28E-02
CDC42	cell division cycle 42	0.9	9.44E-04
THBS4	thrombospondin 4	0.9	5.96E-03
TXNIP	thioredoxin interacting protein	0.85	1.55E-02
NCOA3	nuclear receptor coactivator 3	0.65	2.54E-02

Supplementary Table 5. DEGs associated with muscle cell differentiation.

Gene	Description	log_2(fold change)	adjusted p-value
MYL2	myosin light chain 2	2.44	4.39E-03
IGFBP5	insulin like growth factor binding protein 5	2.19	6.94E-10
IGF2	IL4R (interleukin 4 receptor	1.86	6.31E-07
EDNRB	endothelin receptor type B	1.15	6.90E-03
NFATC2	nuclear factor of activated T cells 2	1.08	2.67E-02
MYO18B	myosin XVIIIB	0.96	2.31E-03
LMOD2	leiomodin 2	0.82	4.36E-03
ANK2	ankyrin 2	0.79	4.68E-02
AKAP6	A-kinase anchoring protein 6	0.69	4.63E-02
CTNNB1	catenin beta 1	0.64	4.68E-02
ID2	inhibitor of DNA binding 2	0.58	1.81E-02
AKAP13	A-kinase anchoring protein 13	0.55	4.48E-02

Supplementary Table 6. Select downregulated GO terms in in the ISS μG condition compared with the ISS 1G condition.

Ontology	ID	Description	adjusted p-value	Count
BP	GO:0007015	actin filament organization	1.75E-06	22
BP	GO:0051017	actin filament bundle assembly	7.69E-05	12
BP	GO:0061572	actin filament bundle organization	7.69E-05	12
BP	GO:0048511	rhythmic process	1.36E-03	14
BP	GO:0042060	wound healing	3.17E-03	16
BP	GO:0050818	regulation of coagulation	3.43E-03	7
BP	GO:0007623	circadian rhythm	3.72E-03	11
BP	GO:0090130	tissue migration	6.22E-03	14
BP	GO:0061564	axon development	6.22E-03	16
BP	GO:0043542	endothelial cell migration	7.05E-03	12
BP	GO:0050878	regulation of body fluid levels	7.05E-03	14
BP	GO:0031032	actomyosin structure organization	7.05E-03	10
BP	GO:0010976	positive regulation of neuron projection development	8.87E-03	9
BP	GO:0010975	regulation of neuron projection development	8.87E-03	15
BP	GO:1903034	regulation of response to wounding	9.34E-03	9
BP	GO:0030193	regulation of blood coagulation	9.53E-03	6
BP	GO:0031346	positive regulation of cell projection organization	9.92E-03	13
BP	GO:1900046	regulation of hemostasis	1.00E-02	6
BP	GO:0007596	blood coagulation	1.02E-02	10
BP	GO:0007409	axonogenesis	1.02E-02	14
MF	GO:0003779	actin binding	1.34E-03	17
MF	GO:0051015	actin filament binding	3.38E-03	11
MF	GO:0045296	cadherin binding	5.84E-03	13
MF	GO:0005201	extracellular matrix structural constituent	8.06E-03	9
MF	GO:0050840	extracellular matrix binding	2.45E-02	5
MF	GO:0044548	S100 protein binding	2.73E-02	3
MF	GO:0015929	hexosaminidase activity	2.90E-02	3
MF	GO:0005518	collagen binding	3.96E-02	5
MF	GO:0098641	cadherin binding involved in cell-cell adhesion	3.96E-02	3
CC	GO:0062023	collagen-containing extracellular matrix	1.45E-06	20
CC	GO:0005884	actin filament	2.31E-04	9
CC	GO:0005925	focal adhesion	2.59E-04	16
CC	GO:0030055	cell-substrate junction	2.59E-04	16
CC	GO:0005938	cell cortex	5.33E-04	13
CC	GO:0005788	endoplasmic reticulum lumen	2.55E-03	12

Ontology	ID	Description	adjusted p-value	Count
CC	GO:0098978	glutamatergic synapse	4.54E-03	12
CC	GO:0005775	vacuolar lumen	1.06E-02	8
CC	GO:0043202	lysosomal lumen	1.17E-02	6
CC	GO:0043292	contractile fiber	1.55E-02	9
CC	GO:0030863	cortical cytoskeleton	1.70E-02	6
CC	GO:0005766	primary lysosome	1.76E-02	7
CC	GO:0042582	azurophil granule	1.76E-02	7
CC	GO:0032432	actin filament bundle	2.27E-02	5
CC	GO:0060205	cytoplasmic vesicle lumen	2.38E-02	10
CC	GO:0031983	vesicle lumen	2.38E-02	10

Supplementary Table 7. DEGs associated with cardiac contraction and conduction.

Gene	Description	log_2(fold change)	adjusted p-value
MYL2	myosin light chain 2	2.44	4.39E-03
TNNI3	troponin I3	1.64	2.58E-09
ATP2B4	ATPase plasma membrane Ca2+ transporting 4	1.2	2.52E-05
PRKACA	protein kinase cAMP-activated catalytic subunit a	0.95	4.73E-02
RYR2	ryanodine receptor 2	0.88	3.29E-03
TCAP	titin-cap	0.85	1.11E-03
ANK2	ankyrin 2	0.79	4.68E-02
SCN5A	sodium voltage-gated channel alpha subunit 5	0.66	1.53E-03

Supplementary Table 8. DEGs associated with regulation of ECMs.

Gene	Description	log_2(fold change)	adjusted p-value
ITIH5	inter-alpha-trypsin inhibitor heavy chain 5	-6.01	2.88E-02
COL4A4	collagen type IV alpha 4 chain	-5.73	1.16E-02
PTPRZ1	protein tyrosine phosphatase receptor type Z1	-3.9	1.74E-02
COL26A1	collagen type XXVI alpha 1 chain	-2.28	9.69E-04
HPX	hemopexin	-2.23	3.38E-02
ANXA1	annexin A1	-1.83	4.48E-05
GPC4	glypican 4	-1.74	8.72E-06
LAMC2	laminin subunit gamma 2	-1.33	2.26E-02
MATN2	matrilin 2	-1.32	2.28E-09
THBS1	thrombospondin 1	-1.12	7.71E-03
S100A10	S100 calcium binding protein A10	-1.09	7.92E-03
COL1A1	collagen type I alpha 1 chain	-1.05	2.57E-02
ACHE	acetylcholinesterase	-0.95	1.74E-02
MFAP4	microfibril associated protein 4	-0.93	3.32E-02
P3H1	prolyl 3-hydroxylase 1	-0.89	2.02E-02
HTRA1	HtrA serine peptidase 1	-0.79	3.81E-02
SRPX	sushi repeat containing protein X-linked	-0.78	3.29E-02
FGFR2	fibroblast growth factor receptor 2	-0.68	4.39E-03
SPARC	secreted protein acidic and cysteine rich	-0.68	1.67E-02
ANXA2	annexin A2	-0.6	6.74E-03

Supplementary Table 9. DEGs associated with focal adhesion.

Gene	Description	log_2(fold change)	adjusted p-value
ITGA11	integrin subunit alpha 11	-2.65	1.40E-04
PROCR	protein C receptor	-2.33	2.55E-02
ANXA1	annexin A1	-1.83	4.48E-05
LIMA1	LIM domain and actin binding 1	-1.79	1.11E-02
ACTB	actin beta	-1.09	5.05E-03
CAPN5	calpain 5	-1.05	4.23E-03
EHD3	EH domain containing 3	-0.99	1.06E-03
IQGAP1	IQ motif containing GTPase activating protein 1	-0.92	3.73E-02
ITGB5	integrin subunit beta 5	-0.89	3.62E-02
DAB2	DAB adaptor protein 2	-0.88	3.36E-02
CSRP2	cysteine and glycine rich protein 2	-0.88	4.93E-03
FHL1	four and a half LIM domains 1	-0.87	2.26E-02
EFNB2	ephrin B2	-0.84	2.31E-03
ACTN1	actinin alpha 1	-0.66	1.23E-03
CNN1	calponin 1	-0.61	4.01E-02

Supplementary Table 10. Common upregulated DEGs between short-term and long-term exposure to space microgravity.

Gene	Description	log_2(fold change)	adjusted p-value
ARTN	artemin [Source:HGNC Symbol;Acc:HGNC:727]	1.32	2.76E-02
PRSS35	serine protease 35 [Source:HGNC Symbol;Acc:HGNC:21387]	0.90	1.34E-03
ATP2B4	ATPase plasma membrane Ca2+ transporting 4 [Source:HGNC Symbol;Acc:HGNC:817]	0.89	1.60E-04
MYO18B	myosin XVIIIB [Source:HGNC Symbol;Acc:HGNC:18150]	0.82	2.59E-02
TFRC	transferrin receptor [Source:HGNC Symbol;Acc:HGNC:11763]	0.76	2.32E-03
CCND2	cyclin D2 [Source:HGNC Symbol;Acc:HGNC:1583]	0.67	2.34E-03
EXOC6B	exocyst complex component 6B [Source:HGNC Symbol;Acc:HGNC:17085]	0.61	1.77E-03
XPR1	xenotropic and polytropic retrovirus receptor 1 [Source:HGNC Symbol;Acc:HGNC:12827]	0.54	1.01E-02
FGFR2	fibroblast growth factor receptor 2 [Source:HGNC Symbol;Acc:HGNC:3689]	0.54	3.98E-02
CSRP2	cysteine and glycine rich protein 2 [Source:HGNC Symbol;Acc:HGNC:2470]	0.52	1.59E-02
MGST1	microsomal glutathione S-transferase 1 [Source:HGNC Symbol;Acc:HGNC:7061]	0.52	2.64E-02

Supplementary Table 11. Common downregulated DEGs between short-term and long-term exposure to space microgravity.

Gene	Description	log_2(fold change)	adjusted p-value
H2BC4	H2B clustered histone 4 [Source:HGNC Symbol;Acc:HGNC:4757]	-2.47	1.19E-02
EFEMP1	EGF containing fibulin extracellular matrix protein 1 [Source:HGNC Symbol;Acc:HGNC:3218]	-1.78	1.06E-02
MT-ND6	mitochondrially encoded NADH:ubiquinone oxidoreductase core subunit 6 [Source:HGNC Symbol;Acc:HGNC:7462]	-1.34	4.19E-02
DDIT3	DNA damage inducible transcript 3 [Source:HGNC Symbol;Acc:HGNC:2726]	-1.14	8.63E-05
ACTG1	actin gamma 1 [Source:HGNC Symbol;Acc:HGNC:144]	-0.92	1.49E-05
CDC42EP2	CDC42 effector protein 2 [Source:HGNC Symbol;Acc:HGNC:16263]	-0.84	1.28E-03
LIMA1	LIM domain and actin binding 1 [Source:HGNC Symbol;Acc:HGNC:24636]	-0.80	4.09E-02
EFNB2	ephrin B2 [Source:HGNC Symbol;Acc:HGNC:3227]	-0.73	4.23E-02
TKT	transketolase [Source:HGNC Symbol;Acc:HGNC:11834]	-0.47	3.80E-02