COMBINATORIAL AND INDEPENDENT EFFECTS OF EXERCISE AND MYOSTATIN/ACTIVIN BLOCKING ON MUSCLE GENE EXPRESSION PROFILING

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ABSTRACT

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The administration of soluble ligand binding domain of type IIb activin receptor fused to the Fc domain (sActRIIB-Fc) has been recently shown to attenuate dystrophic pathology and to increase muscle mass, but also to supress aerobic metabolism. In contrast, aerobic exercise is known for promoting aerobic capacity. The aim of the present thesis was to investigate the effects of the combination of myostatin/activin blocking and aerobic exercise on muscle gene expression profile of a in Duchenne Muscular Dystrophy (DMD) model, the mdx mouse. Microarray analysis was conducted from the gastrocnemius muscle and Gene Set Enrichement Analysis (GSEA) was performed to examine the effects of the treatments and muscle dystrophy on gene sets and pathways. The level of significance was set at False Discovery Rate (FDR) < 0.05.

The results indicate that the beneficial effects of exercise in dystrophic muscle induce transcription responses towards the gene expression profile of the healthy muscle. Furthermore, the combination of exercise with myostatin/activin inhibition combines the benefits of each intervention alone, without any obvious side effect. The most profound changes were observed in aerobic metabolism pathways, where the shift in gene expression is presented in many different pathways. Moreover, the elevated expressions in glutathione and drug metabolism by cytochrome P450 pathways suggest a possible role for oxidative damage in DMD pathology and increases in BCAAs degradation and lipid metabolism indicate a possible connection between these two processes, but further research is needed to explore the role of oxidative damage in DMD pathology and their possible connections.

Key Words: exercise, DMD, myostatin/activin blocking, sActRIIB-Fc, gene expression profiling, microarray

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LIST OF ABBREVIATIONS

ActRIIB	Activin receptor type IIB
ALK	Activin-like kinase
AON	Antisense oligonucleotides
AMP	Adenosine monophosphate
AMPK	AMP-activated protein kinase
BCAAs	Branched-chain amino acids
BMP-1/TLD	Bone morphogenetic protein-1/Tolloid
CaMK	Calcium-calmodulin kinase
Cdk2	Cyclin-dependent kinase
DMD	Duchenne muscular dystrophy
DMEM	Dulbecco's modified Eagle media
ECM	Extracellular matrix
EDL	Extensor digitorum longus muscle
ERK ½	Extracellular kinase ½
ERRγ	Estrogen-related receptor γ
ES	Enrichment score
FCS	Fetal calf serum
FDR	False discovery rate
FoxO	Forkhead box O (a family of proteins)
GASP-1	Growth and differentiation factor-associated serum protein 1
GDF-8	Growth differentiation factor 8 (myostatin)
GPX	Glutathione peroxidase
GRMD	Golden retriever muscylar dystrophy dog
GSEA	Gene set enrichment analysis
GSH	Glutathione
IgG	Immunoglobulin G
IGF-1	Insulin-like Growth Factor-1
KEGG	Kyoto encyclopedia of genes and genomes
LTBP3	Latent TGF-β binding protein
Mstn	Myostatin
TGF-β	Tumour growth factor-β

RSSR	The sequence Arg-Ser-Arg-Arg
LTBP3	Latent TGF-β binding protein
MAPK	Mitogen-activated protein kinase
MEK	Mitogen-activated kinase kinase (also known as MAP2K)
MHC	Myosin heavy chain
MQF	Musculus quadriceps femoris muscle
MRF4	Muscle regulatory factor 4
mTOR	Mammalian target of rapamycin
NAC	N-acetylcysteine
NAPQI	N-acetyl-p-benzoquinone imine
NES	Normalized enrichment score
PBS	Phosphate buffered saline
PDK4	Pyruvate dehydrogenase kinase 4
PGC1-α	Peroxisome proliferator-activated receptor γ coactivator 1-α
PI3K	Phosphatidylinositol 3-kinase
PPAR	Peroxisome proliferator activating receptor
ROS	Reactive oxygen species
sActRIIB-Fc	soluble ligand binding domain of type IIb activin receptor (ActRIIB) fused to the Fc domain of IgG
SDH	Succinate dehydrogenase
SOD	Superoxide dismutase
TCA cycle	Tricarboxylic acid cycle (also known as Kreb's cycle and citric acid cycle)

CONTENTS

1 INTRODUCTION

The study and treatment of myopathies is an issue of interest for medical and biological sciences. Scientific research has brought to light different substances and mechanisms that are related to these disorders, providing scientists new ideas and manners in the struggle to cure the patients. The present thesis is about the effects of the combination of exercise and treatment on the muscle phenotype in the Duchenne muscular dystrophy background.

Duchenne muscular dystrophy (DMD) is a severe degenerative disorder of skeletal muscles caused by a mutation in the dystrophin gene. It affects almost exclusively boys, due to the location of the dystrophin gene in the X chromosome. A widely used genetic and biochemical mammalian model for DMD is the mdx mouse (Vainzof et al. 2008).
Myostatin is a protein which inhibits muscle growth by mechanisms that are not clear yet. Blocking of myostatin has been associated with increased protein synthesis and muscle mass in both normal and dystrophic subjects, but also with a decrease in oxidative and aerobic capability of the muscle (Welle et al. 2009a, Sartori et al. 2009, Rahimov et al. 2011, Matsakas et al. 2012, Hulmi et al. 2013a). The injection of a soluble ligand binding domain of type IIb activin receptor (ActRIIB) fused to the Fc domain of IgG (sActRIIB-Fc) is a method that effectively blocks myostatin and is currently in human clinical tests (Attie et al. 2012).

Exercise has beneficial effects on skeletal muscle size and function, as well as on the overall health. Recent studies show that exercise has positive effects on muscle, even when combined with myostatin blocking (LeBrasseur et al. 2009) or myostatin deletion (Savage and McPherron 2010, Matsakas et al. 2010). To date, we are not aware of any study examining the effects of exercise, combined with myostatin and activin blocking, on animal models of DMD or in DMD patients. In addition, gene expression profiling and pathway analysis gives us the opportunity to follow responses of thousands of genes to different treatments (Subramanian et al. 2005, Kivelä et al. 2010). Therefore, the aim of this thesis is to provide a description of the combination of exercise and myostatin blocking on the muscle gene expression profile of dystrophic (mdx) mice.

2 MYOSTATIN

In 1997, McPherron et al. described for the first time a new member of the tumour growth factor β (TGF-β) superfamily, the growth and differentiation factor-8. Since then, growth and differentiation factor-8 has become an issue of interest for many scientists around the world under the name myostatin (Mstn), which suggests its role primarily on the muscle tissue. Myostatin (fugure 1) and some related topics are described below.

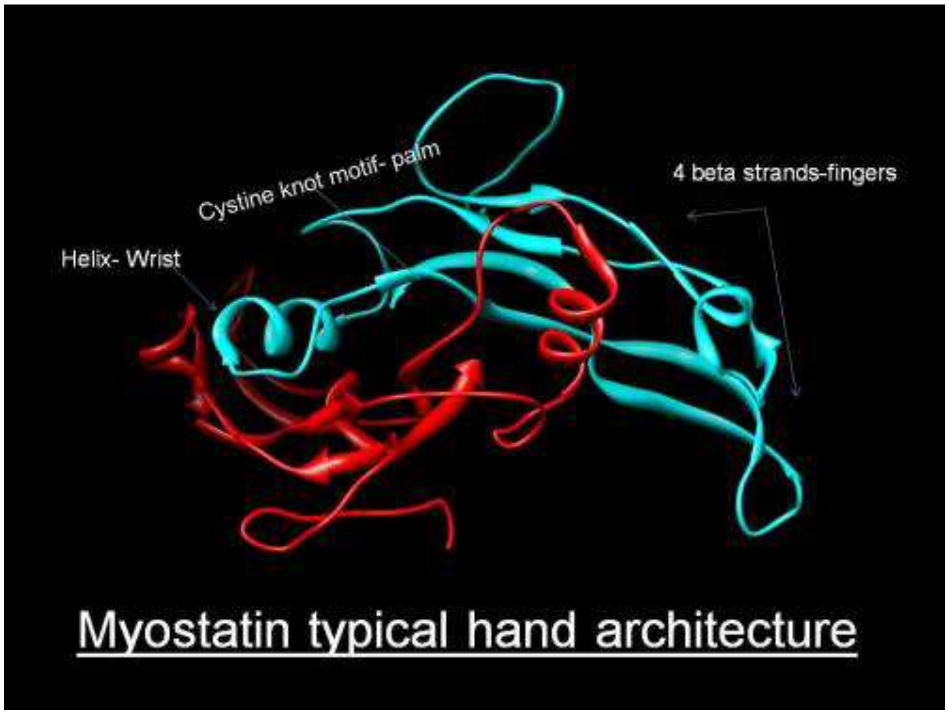

FIGURE 1. Myostatin dimer shown. Monomer A in cyan and monomer B in red. Notice how the molecule looks like hands folded within each other. There are 4 beta stranded fingers, a Cystine knot motif - palm and a helix wrist (Shikhel 2010).

2.1 Basics of myostatin

Myostatin, or Growth and Differentiation Factor-8 (GDF-8), is a protein with a molecular weight of 25 kDa and is a member of the growth and differentiation factor

subgroup of the TGF-β superfamily. TGF-β includes a number of secreted factors that regulate cell growth and development through signal transduction (Elkina et al. 2011; Liu et al. 2012; Elliott et al. 2012). As a member of the TGF-β family, myostatin possesses several characteristics of the family, but it cannot be classified into any major TGF-β subfamily, such as inhibins, TGF-β and bone morphogenic proteins (Matsakas et al. 2005). Common features between myostatin and TGF-β superfamily members include: a precursor form that is proteolytically processed, a hydrophobic core of amino acids near the N-terminus that functions as a secretion signal, a conserved proteolytic processing signal of RSRR in the C-terminal half of the protein and nine cysteine residues in the C-terminal region which form disulfide bridges in a “cysteine knot” form (Sharma et al. 1999; Thomas et al. 2000, Huang et al. 2011).

Myostatin is expressed primarily in skeletal muscle, but lower levels could be detected in other tissues of mammals and other species (Huang et al. 2011; Liu et al. 2012). It is expressed by different cells in skeletal muscle like: satellite cells, fast muscle fibers and fibroblasts (McCroskery et al. 2003, Li et al. 2008, Chemello et al. 2011). It is highly expressed in embryonic and fetal stages of life and less in adult muscle tissue, but it can affect adult tissue as well (Matsakas et al. 2005). Myostatin role and regulation is discussed in following sections (2.3 and 3).

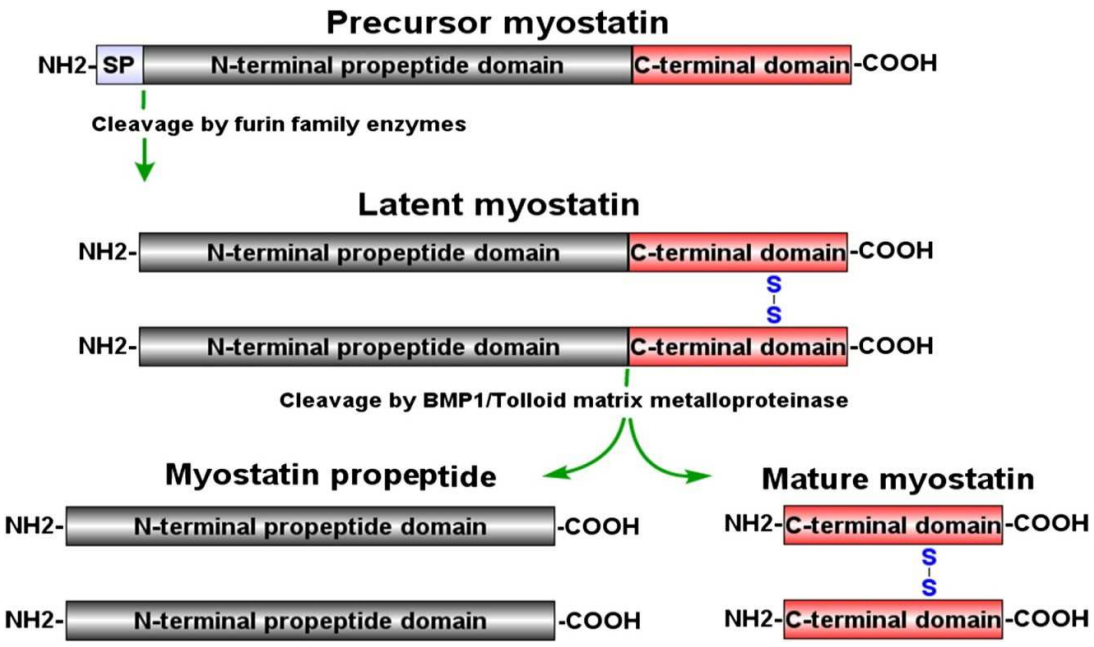

FIGURE 2. Proteolytic processing of myostatin protein (Huang et al 2011).

Active myostatin derives from a 375 amino acid precursor protein, after two proteolytic cleavages (figure 2). The first cleavage in extracellular matrix, by furin family enzymes, produces two polypeptide products, the NH2-terminal myostatin propeptide and the 110-amino-acid-long COOH-terminal mature myostatin. The two products remain noncovalently associated and along with other proteins form a latent complex, which is the circulating form of myostatin. The second cleavage, by bone morphogenetic protein-1/tolloid (BMP-1/TLD), occurs at the RSRR (Arg-Ser-Arg-Arg) site and is necessary to release the mature myostatin and activate it. Both the unprocessed and the mature active myostatin form disulfide-linked dimers (Hill et al. 2002; Gonzalez-Cadavid and Shalender 2004; Hennebry et al. 2009; Elkina et al. 2011; Liu et al. 2012).

2.2 Myostatin signalling

Most TGF-β family members signal through heteromeric complexes of type I and II serine/threonine kinase receptors, the activin receptors, which activate gene regulatory proteins called Smads. The Smad-mediated pathway is considered as the canonical signaling pathway for myostatin, but there are also Non-Smad pathways (figure 3) participate in myostatin signal transduction (Wagner 2005; Elkina et al. 2011; Huang et al. 2011). Inhibitors of myostatin include: the propeptide, growth and differentiation factor-associated serum protein 1 (GASP-1), latent TGF-β binding protein (LTBP3) and follistatin (Elkina 2011). Other regulators involved with myostatin signalling and action are described in the next section (2.3).

The canonical signal pathway mechanism of myostatin is believed to be similar to TGF-β signaling pathway. In that pathway, the signal transduction begins with TGF-β binding to the type II serine/threonine kinase receptor, which in turn associates with the type I serine/threonine kinase receptor and form a heterotetrameric receptor complex, which leads to the activation of the dormant type I receptor kinase. Then, the activated dormant type I receptor kinase phosphorylates receptor-regulated Smads (R-Smads), which form a complex with Smad 4 and enter the nucleus to regulate transcription (Wagner 2005; Alberts et al. 2008; Elkina et al. 2011; Huang et al. 2011).

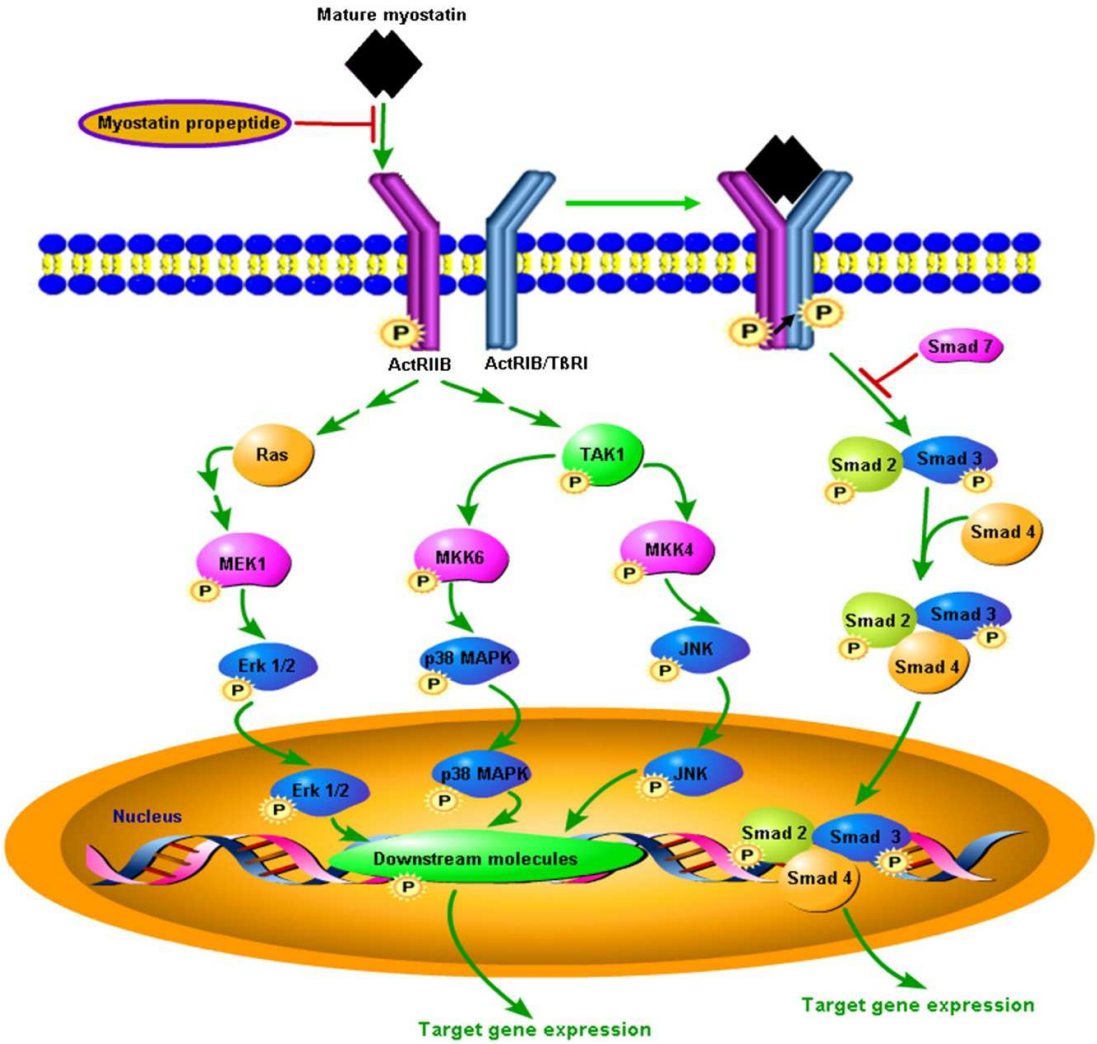

FIGURE 3. The myostatin signalling pathways (Huang et al. 2011).

For myostatin signaling pathway, activin receptor (ActR) IIB seems to be the type II receptor, while different type I receptors have been reported, including: the ActRIB, TβRI, activin-like kinase (ALK) receptor 4 and ALK5. Furthermore, the R-Smads involved seem to be Smad2 and Smad3, while Smad7 and maybe Smad6 seem to function as inhibitory Smads (I-Smads) and provide a negative feedback for the myostatin signal pathway (Wagner 2005; Huang et al. 2011; Kemaladewi et al. 2012). One of the known downstream targets of Smad signaling is MyoD, a transcriptional factor which is involved in muscle growth and repair. Myostatin downregulates MyoD and inhibits Pax3 expression, a possible upstream target of MyoD. Other Smad targets signaling include myf5 and myogenin (Hennebry et al. 2009; Elkina et al. 2011; Huang et al. 2011).

The non-Smad signaling pathways for myostatin involve mitogen—activated protein kinases (MAPK) signaling pathways and more particular p38 MAPK and extracellular kinase 1/2 (ERK1/2). Myostatin activates p38 via TGF-β kinase 1 (TAK-1)-MAPK kinase (MAPKK or MKK) 6 cascade. The activation of ERK1/2, in C2C12 cells, involves the participation of Ras and another MAPK kinase, the MEK-1 (Elkina et al. 2011; Huang et al. 2011). On the other hand, Hulmi et al. (2013a) report that blocking of myostatin with sActRIIB-Fc inhibits MAPK signaling but does not affect total and phosphorylated Smad2.

2.3 Myostatin effects on muscle phenotype and mRNA profiling

A vast number of papers have already established myostatin as a negative regulator of skeletal muscle development and growth. Most of these studies showed spectacular increases in muscle mass for animals lacking myostatin (myostatin null), due to both hyperplasia and hypertrophy. (McPherron et al. 1997b; Gonzalez-Cadavid and Shalender 2004; Mosher et al. 2007; Elkina et al. 2011; Huang et al. 2011). It was suggested that myostatin may regulate fiber-type composition by causing a switch in myogenic fiber type and especially from slow to fast type, but fast-twitch fiber protein gene expression does not seem to increase (Steelman et al. 2006; Hennebry et al. 2009; Rahimov 2011). In addition, myostatin appears to play an important role in metabolism and has been associated with improved glucose metabolism (Matsakas and Diel 2005; Chen et al. 2010; Huang et al. 2011; Elliott et al. 2012; Ploquin et al. 2012).

In the developmental stage of muscle, myostatin seems to regulate skeletal muscle development through inhibition of myoblast proliferation and differentiation and the maintenance of satellite cells in a quiescent state. It also upregulates the levels of cyclin-dependent kinase (Cdk) inhibitor p21 protein and decreases the levels and activity of Cdk2 protein suggesting that myostatin inhibits the G_1 to S phase transition and maintains the satellite cells in a quiescent state. Myoblast proliferation inhibition by myostatin is associated with upregulation of Cdk inhibitor p21, the downregulation of Cdk2, the decrease in Cdk2 activity and the decrease in phosphorylation of retinoblastoma (Rb). Furthermore, myostatin appears to inhibit myoblast differentiation by downregulating the activity of creatine kinase and the expression of differentiation

genes: MyoD, myogenin and Myf5 (Hennebry et al. 2009; Elkina et al. 2011; Huang et al. 2011). In addition, blocking of myostatin may decrease capillary density, at least during rapid muscle growth (Matsakas et al. 2012).

Unlike in developmental stage, in adult muscle, the role of proliferating cells and their differentiation by blocking myostatin or activins is questionable. Instead, blocking myostatin seems to affect the adult muscle mainly by enhancing muscle protein synthesis (McPherron et al. 1997; Welle et al. 2009a; Hulmi et al. 2012; Lee et al. 2012, Wang and McPherron 2012).

Insulin-like growth factor 1 (IGF-1) regulates myostatin signalling in normal conditions, by a mechanism which includes the phosphatidylinositol 3-kinase (PI3K)/Akt pathway, but it seems inhibited when myostatin is overexpressed (Chelh et al. 2009; Elkina et al. 2011). Akt is a protein associated with hypertrophic response to insulin and IGF-1 and is the crossing point between the IGF-1/myostatin pathways. Myostatin seems to be a negative regulator of the Akt/mammalian target of rapamycin (mTOR) signaling pathway and decrease protein synthesis (Chelh et al. 2009; Elkina et al. 2011; Huang et al. 2011), while it activates Akt/Forkhead box O (Akt/FoxO) pathway and thus upregulates proteasome ubiquitin ligases MuRF-1 and atrogin-1, which are involved in protein degradation (Elkina et al. 2011).

MicroRNAs are a type of non-coding RNAs which regulate gene expression by blocking translation of selective mRNAs. Myostatin has been associated with different microRNAs, such as: miR-208 a, MiR-208 b, miR-27 a, miR-27 b, miR-1, miR-133 a, miR-133 b and miR-206 (Huang 2011), while the ingestion of essential amino acids (EAAs) (Drummond et al. 2009) and diet with n-3 polyunsatured fatty acids (Davidson et al. 2009) have been reported to modulate microRNAs and the expression of their target genes.

Microarray approach targeting genes affected by the presence or absence of myostatin is limited in literature. Findings show a large number of gene expression differences between the presence and absence of myostatin. The downregulation of Wnt signaling through β-catenin and the upregulation of Wnt/calcium pathway with loss of myostatin,

the repression of satellite cell proliferation by myostatin inhibition of Wnt signaling, the promotion of glycolysis and glucose uptake by myostatin, the decrease of glycogen content in C2C12 myotubes by myostatin, the suppression of mitochondrial and oxidative phosphorylation genes and the lack of increases in fast-twitch fiber protein expression in the deficiency of myostatin have been reported (Steelman et al. 2006; Chelh et al. 2009; Chen et al. 2010; Wicik et al. 2011; Rahimov et al. 2011).

Myostatin null mice or mice treated with an inhibitor of myostatin (ActRIIB-Fc) show reduced expression levels of transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α), Nos1, Ddah1, Zmynd17 genes and upregulations of the α1-syntrophin, Ctnna3, Cdh4, Igf2 and Igfbp5 genes (Chelh et al. 2009; Rahimov et al. 2011). In addition, differential expression was detected in mRNAs encoding PIK3R3, PIK3CG, PLCG1, GSK-3β, indicating activation of PI3K/Akt pathway (Chelh et al. 2009). Myostatin also alters glucose metabolism genes (Glu1, Glut 4, HK2 and IL-6) expression through AMP-activated protein kinase (AMPK) signal pathway (Chen et al. 2010). Furthermore, downregulation of key muscle genes (Mef2, Hgf, Ilb1, Itgb1, Edn1, Ppargc) was detected with the supplementation of myostatin in C2C12 myoblasts during cell culture (Wicik et al. 2011). The list of differentiated genes is even larger, but a full description of the genes affected by myostatin is beyond the limits of the present thesis.

3 MYOSTATIN BLOCKING

There are various methods and strategies for the inhibition of myostatin action or expression targeting different stages of myostatin signalling pathways (figure 4). These approaches include: myostatin antibodies, overexpression of the propeptide or follistatin, expression of a dominant negative myostatin or negative ActRIIB, antisense oligonucleotides (AON) and sActRIIB-Fc. A number of pharmaceutical compounds have been developed to serve these strategies resulting in blocking myostatin or ActRIIB or other TGF-β ligands (figure 5), myostatin exon skipping, myostatin mRNA interference/decay and defective synthesis of ActRIIB or Smad2/3 (Amthor 2012). This section describes the effects of the soluble ligand binding domain of type IIb activin receptor (ActRIIB) fused to the Fc domain of IgG (sActRIIB-Fc) strategy, which was used in our study.

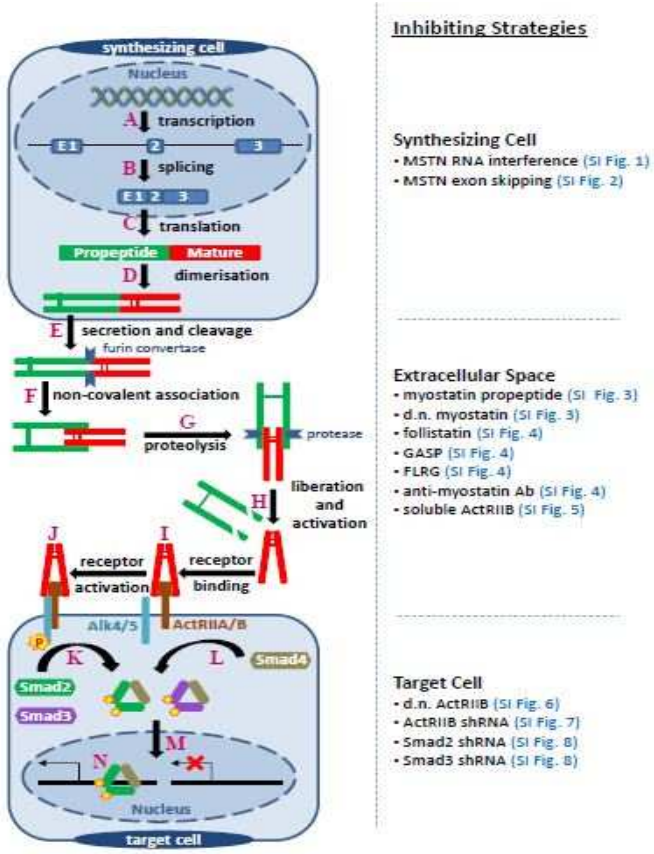

FIGURE 4. Summary of myostatin signaling (Amthor and Hoogars 2012).

The effects of sActRIIB-Fc on myostatin were first described in 2005 by Lee at al. The administration of sActRIIB-Fc increases muscle mass, body weight, total protein and DNA contents (Lee et al. 2005), improves hypoxia-induced muscle dysfunction (Pistilli et al. 2010) and normalize (LPS or activin A overexpression induced) lung pathology in a mouse model (Apostolou et al. 2012). The increases in muscle mass by sActRIIB-Fc occur also in myostatin null mice suggesting that there is at least one other ligand, except myostatin, which functions to suppress muscle development and antagonizing sActRIIB-Fc. Growth Differentiation Factor 11 (GDF11) or Bone Morphogenic Protein 11 (BMP11) has already been identified as one ligand which also binds with ActRIIB, but its role in muscle growth remains unclear (Lee et al. 2005; Pistilli et al. 2011; Rahimov et al. 2011). More likely candidates are activins and especially activin (Act) A, the overexpression of which was reported to induce atrophy. Activins seem to regulate differentiation of muscle cells and and have a common receptor and a common inhibitor with myostatin, ActRIIB and follistatin respectively (Souza et al. 2008, Gilson et al. 2009, Lee et al. 2010). sActRIIB-Fc effectively blocks ActRIIB ligands, such as myostatin, GDF 11 and activins A, B and AB (Souza et al. 2008, Sako et al. 2010).

Blockade of activin receptor type IIB has been shown to attenuate dystrophic pathology of a mouse model for Duchenne muscular dystrophy, the mdx mouse in some (Morine et al. 2010; Pistilli et al. 2011), but not all studies (Hoogaars et al. 2012). The treatment with sActRIIB-Fc in mdx mouse leads to increased skeletal muscle mass, body weight, and myofiber size and stimulates muscle growth. It also seems to increase absolute, but not specific (force per muscle size or body mass) muscle force production, although an adaptation period seems to be needed for the muscle to adjust to its new size (Morine et al. 2010; Pistilli et al. 2011; Hoogaars et al. 2012; Hulmi et al. 2012). The mdx mouse model was used in the present thesis and is described in section 4, along with Duchenne muscular dystrophy.

Although there are studies describing gene expression profiles in presence or absence of myostatin (Steelman et al. 2006; Chelh et al. 2009; Welle et al. 2009; Wicik et al. 2011), there is only one study which describes gene expression profiles of skeletal muscles treated with sActRIIB-Fc for two weeks (Rahimov et al. 2011). In the study of Rahimov et al. (2011), the reported overall similarity between the gene expression in chronic

ActRIIB-Fc treated and myostatin null mice (66%) indicates that the biological effect of ActRIIB-Fc on muscle occurs via inhibition of a common TGF-β pathway, shared by myostatin and other ActRIIB-Fc binding ligands. In addition, two weeks of ActRIIB-Fc treatment seems to reduce the expression of genes encoding a number of isoforms of slow type muscle fiber proteins, but not the genes encoding fast-twitch fiber gene expression. Furthermore, ActRIIB-Fc treatment seems to suppress mitochondrial and oxidative phosphorylation genes, the Nos1 gene, the expression levels of the estrogen-related receptor α (Errα) gene, the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) gene, the Ddah1 gene and the Zmynd17 gene. In contrast, it seems to upregulate Ctnna3, Cdh4, Igf2 and Igfbp5 genes. Finally, the pathways that seemed to be the most affected by ActRIIB-Fc treatment are: oxidative phosphorylation, mitochondrial function and ubiquitin-proteasome proteolytic pathway (Rahimov et al. 2011). The present thesis describes for the first time the gene expression profiles of mdx mouse skeletal muscles treated with sActRIIB-Fc in combination with exercise.

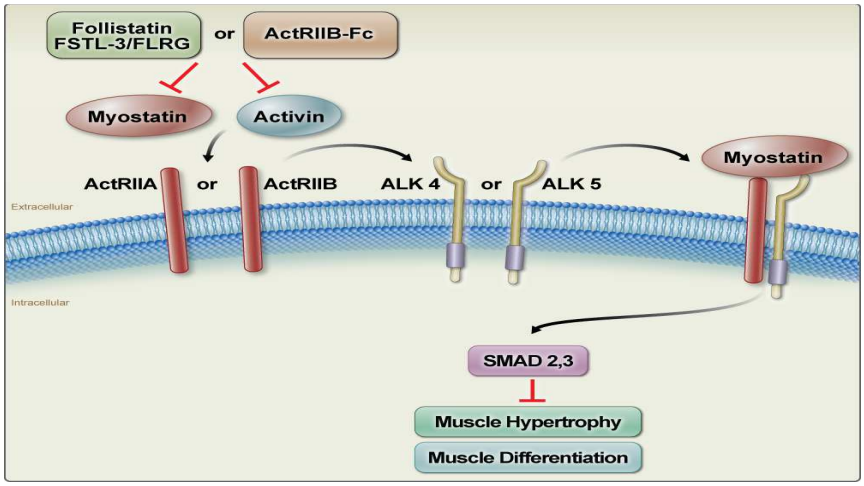

FIGURE 5. Inhibition of myostatin and activin signalling by the soluble activin type IIB receptor (ActRIIB). Myostatin and activin signal to target cells by binding initially to the two activin type II receptors, ActRIIA and/or ActRIIB (also called Acvr2 and Acvr2b, respectively) and then to the type I receptors, ALK4 and/or ALK5. The activated type I receptors phosphorylate the intracellular mediators of signalling, Smad2 and/or Smad3. Signaling through this pathway results in the inhibition of muscle differentiation and growth. The activities of myostatin and activin are regulated normally by a number of different extracellular binding proteins, such as follistatin and FSTL-3. The soluble form of ActRIIB (ActRIIB/Fc) can act as a ligand trap by binding MSTN and activin and preventing the ligands from binding to their true receptors (Lee and Glass 2011).

4 DUCHENNE MUSCULAR DYSTROPHY

Duchenne muscular dystrophy (DMD) is a fatal, genetic disease characterised by progressive muscle wasting and weakness (Chakkalakal et al. 2005; Markert et al. 2011). It is linked to the X-chromosome (Xp21) and affects 1 in every 3,600 to 6,000 newborn boys, but clinical symptoms are not evident until the 3-5 years of age. The continuous and progressive muscle wasting usually leaves DMD patients in a wheelchair bound at the age of 11 or 12, before they succumb to the disease, usually by the second or third decade of life (Chakkalakal et al. 2005; Pistilli et al. 2011; Beytía et al. 2012).

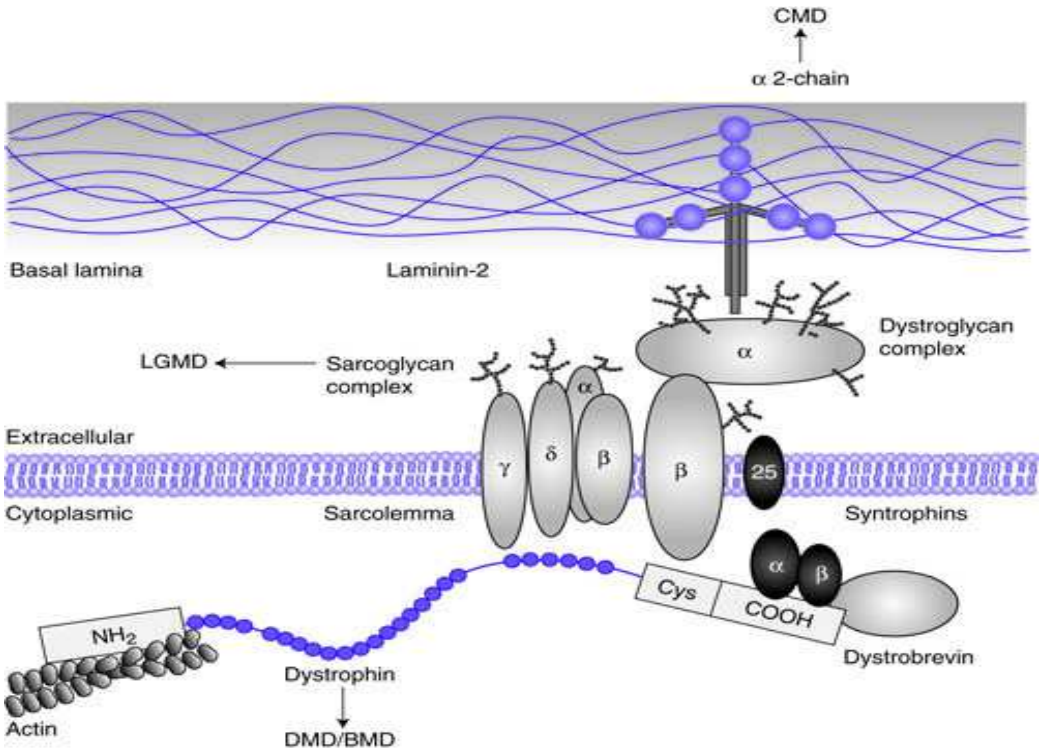

FIGURE 6. Diagram of muscle cell membrane (sarcolemma), dystrophin, and the dystrophin-associated protein complex. Dystrophin is located inside the cell and binds actin at its N-terminus and the syntrophins, sarcoglycans, and dystrobrevin at the C-terminus. Mutations in dystrophin are responsible for Duchenne muscular dystrophy/ Becker muscular dystrophy (DMD/BMD). Mutations in the various sarcoglycans give rise to limb-girdle muscular dystrophy (LGMD), and laminin mutations result in congenital muscular dystrophy (CMD). The image was derived from Bönnemann et al. (1996) and the text derived from Korf (2006).

The cause of DMD is a mutation of the dystrophin gene, at locus Xp21, which encodes dystrophin (Chakkalakal et al. 2005; Turk et al. 2005a). Dystrophin is a subsarcolemmal protein functioning within the dystrophin-associated glycoprotein complex (DGC), which connects the intracellular cytoskeleton to the extracellular matrix and confers the transmission of force, across the muscle fiber (figure 6). The absence of functional dystrophin in muscle fibers of DMD patients, results in membrane instability and sarcolemmal ruptures and eventually in progressive damage of muscle (Turk et al. 2005a; Pistilli et al. 2011). The main stages of the null-mutation of the dystrophin gene is shown in figure 7, but a full description of DMD is beyond the limits of the present thesis.

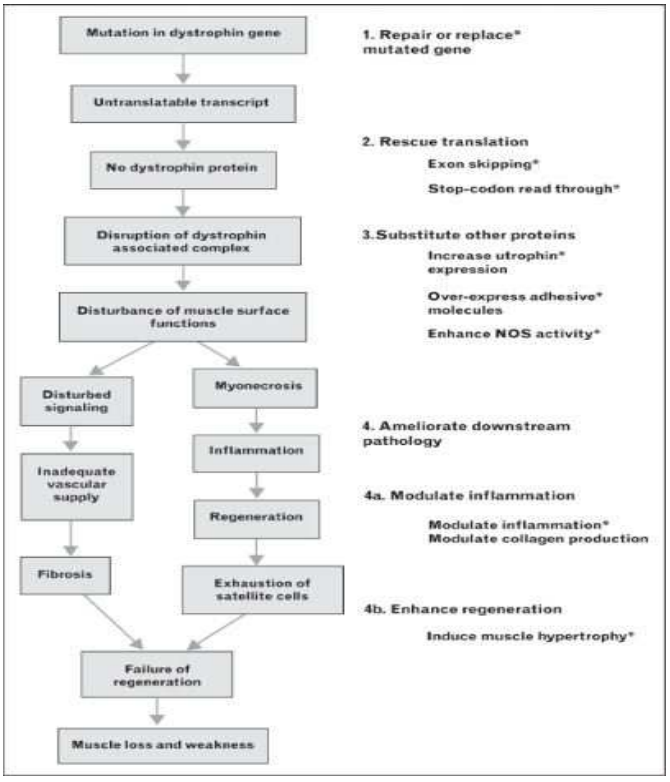

FIGURE 7. The main stages in the pathological cascade arising from a null-mutation of the dystrophin gene, to the left, together with the potential therapeutic actions to combat each of these stages, to the right (Partridge T.A. 2011). NOS= Nitric oxide synthase *Those strategies that have advanced to the point where human trials are current or imminent.

There are different animal models in which dystrophin gene mutations lead to muscular dystrophy and therefore used in study of DMD. The mammalian models include several canine X-linked muscular dystrophy (CXMD) models, mouse and cat models. The most close to DMD, at multiple levels, is the golden retriever muscular dystrophy (GRMD)

dog. GRMD dog along with the mdx mouse are the most notable mammalian models for DMD (Collins and Morgan 2003; Vainzof et al. 2008; Kornegay et al. 2012a). The present thesis used the mdx mouse model.

The mdx mouse is the most widely used animal model for DMD. It is a good genetic and biochemical model for DMD and presents a total deficiency of dystrophin protein in the muscle (Bulfield et al. 1984; Vainzof et al. 2008; Kornegay et al. 2012a). In contrast, the mdx mouse is not a very useful DMD model for clinical evaluation in therapeutic trials, due to its very mild phenotype (Collins and Morgan 2003; Vainzof et al. 2008; Kornegay et al. 2012a). On the other hand, the mdx model presents variability in the severity of muscle damage, which seems to be affected by factors such as: age, sex, exercise and the muscle itself (Collins and Morgan 2003; Smythe and White 2012; Kornegay et al. 2012b). The pathology of the mdx mouse is characterised by histologically well-defined stages similar to the human pathology (Turk et al. 2005a). Muscle pathology in mdx mouse is most pronounced between 2 and 8 weeks of age and varies between different muscles (Collins and Morgan 2003; Kornegay et al. 2012b). The effects of exercise on mdx muscle are discussed in section 5.3.

There is no current cure for DMD and the treatment is generally aimed to control the onset of symptoms and improve the quality of life, but there are limitations due to adverse effects associated with each drug (Angelini C. 2007; Pistilli et al. 2011; Beytía et al. 2012). A number of studies describe benefits in muscle by myostatin and ActRIIB blocking treatment (see section 3). Treatment with an ActRIIB blockade in the mdx mouse increased skeletal muscle mass and force production of extensor digitorum longus (EDL), while it reduced serum creatine kinase (Morine et al. 2010). Several potential drugs are currently being investigated including the myostatin inhibitor ActRIIB-Fc, which is used in our study. First clinical trial in humans showed that treatment with sActRIIB-Fc increases muscle mass without significant adverse events (Attie et al. 2013). In addition, a number of studies investigated the effects of exercise in the mdx mouse indicating several benefits from a training program (described in section 5.3). The present thesis describes the combination of myostatin blocking with ActRIIB-Fc combined with endurance training, as voluntary wheel running, in the gene expression profile of the mdx mouse muscle.

5 EFFECTS OF ENDURANCE EXERCISE ON MUSCLE

Endurance exercise is characterised by prolonged continuous or intermittent periods of contractile activity against low resistance and it can also be found in literature by the term “aerobic exercise” due to the predominant mean of energy production (Mougios 2006). The most important peripheral effects of endurance exercise in skeletal muscle include the transformation of myofibers and increases in mitochondrial and capillary densities (Yan 2009).

Qualitative and quantitative changes in gene expression, due to responses to endurance exercise, result in reversible fiber type transitions towards more oxidative metabolism in a spectrum of pure and hybrid type fibers between type I and type IIB fibers (Baar K. 2006). Furthermore, endurance exercise results in a general shift from the fast to slow myosin heavy chain (MHC) isoform expression (Short et al. 2005) and increases of skeletal muscle content of mitochondria and GLUT4 (Ojuka 2004; Holloszy 2008; Higashida et al. 2011).

Physical activity and aerobic exercise have positive effects on diseases such as diabetes and muscular dystrophies, which both are characterized by a reduction in oxidative gene expression (Timmons et al. 2005). Furthermore, a number of genes have been associated with endurance athletic performance and muscle performance (Lippi et al. 2010). This section describes the effects of endurance exercise on muscle (voluntary wheel running), focusing on: RNA profile, presence or absence of myostatin, the Duchenne muscular dystrophy and the mdx mouse.

5.1 Effects on muscle and RNA profile

The signaling transcription pathways of skeletal muscle adaptation to endurance exercise are not fully elucidated yet (Pietrangelo et al. 2012). However, several studies support that endurance exercise enhances the activity and expression of molecules such as: calcium-calmodulin kinase (CaMK), peroxisome-proliferator activating receptor (PPAR) γ coactivator 1 α and β (PGC-1 α and β), hexokinase, uncoupling protein 3,

adenosine monophosphate-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor delta (PPARδ) and directly alter metabolic outcomes in mice affecting adiposity, glucose tolerance, oxidative capacity and resistance to fatigue (Booth et al. 2002; Ojuka 2004; Nader 2006; Röckl et al. 2007; Bernardo et al. 2009; Yan 2009; Lira et al. 2010; Safdar et al. 2011; Ying et al. 2011). Furthermore, endurance exercise seems to improve antioxidant activity to balance reactive oxygen species (ROS), by increasing total superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities, a response that depends on exercise intensity (Powers et al. 1999; Call et al. 2008). The differences between molecular pathways activated by resistance and endurance exercise are summarized and shown in figure 8.

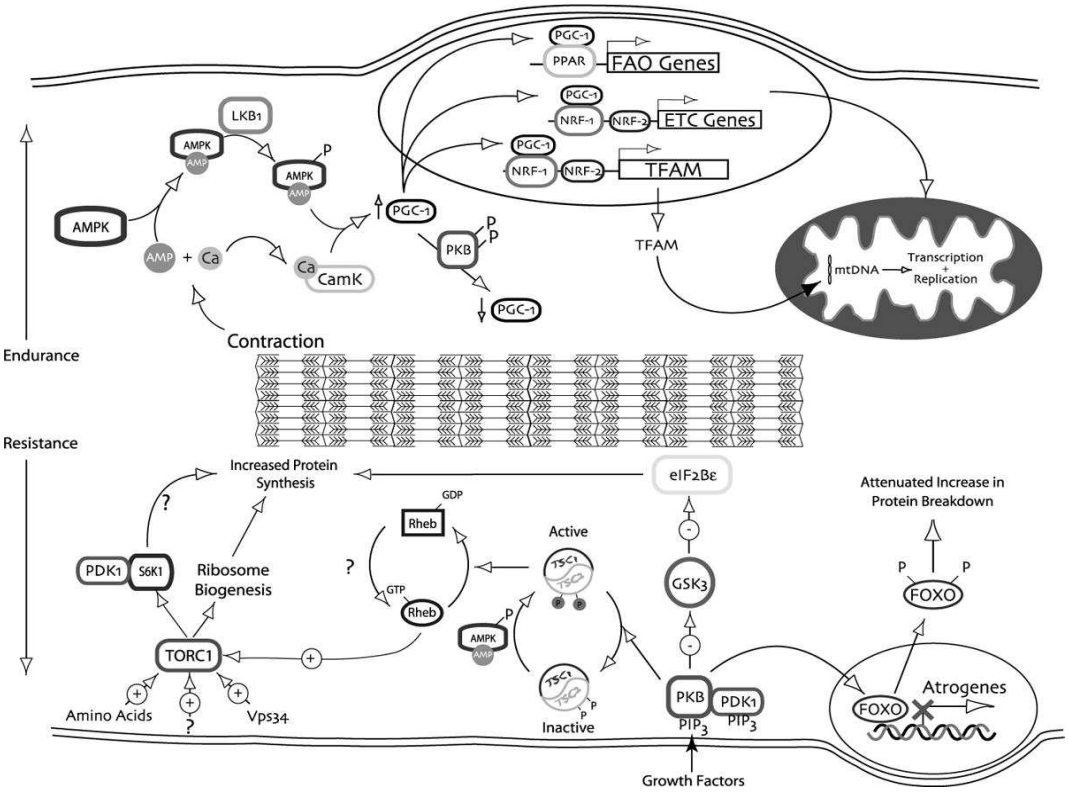

FIGURE 8. Representation of the molecular pathways activated by (top) endurance and (bottom) resistance exercise: Adenosine monophosphate (AMP), AMP-activated protein kinase (AMPK), Calcium (Ca), calcium-calmodulin kinase (CamK), eukaryotic initiation factor 2B (eIF2B), forkhead transcription factor (FOXO); glycogen synthase kinase (GSK3), serine-threonine kinase 11 (LKB1), mammalian target of rapamycin complex 1 (TORC1), nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2), peroxisome proliferator activating receptor (PPAR), PPARγ coactivator (PGC-1), 3-phosphoinositide-dependent protein kinase (PDPK1), protein kinase B/akt (PKB), ras-homolog protein enriched in brain (Rheb), 70KDa ribosomal S6 protein kinase (p70S6K). The tuberosclerosis complex includes hamartin (TSC1) and tuberin (TSC2). The interaction of the two types of exercise is denoted by the ability of PKB to decrease PGC-1α in the top half of the figure and the ability of AMPK to activate TSC2 in the bottom half of the figure (Baar 2006).

Inside the cell, endurance exercise results in progressive increases of the AMP:ATP ratio and intracellular free calcium, which in turn result in increases of AMPK activity and in levels of both PGC-1α mRNA and protein. The latter co-regulates the expression of respiratory genes, mitochondrial transcription factor A, GLUT 4, the fatty acid-oxidation enzymes (Baar 2006; Little et al. 2010). Increases in muscle protein synthesis, fractional rate of protein synthesis (FSR) and phosphorylation of mTOR after intensive endurance exercise (65-77% of maximal oxygen uptake) have been reported. The effects of intensive endurance exercise on 70KDa ribosomal S6 protein kinase (p70S6K) are not clear yet and may depend on the intensity and mode of the exercise and training state (Booth et al. 2002; Coffey et al. 2006, Harber et al. 2009; Mascher et al. 2011). On the other hand, the effects of endurance exercise on eukaryotic elongation factor-2 (eEF2) phosphorylation seem to depend on the type of the muscle fiber and the nutritional status (Rose et al. 2009; Van Proeyen et al. 2011). In addition, feeding seems to be important on muscle regulatory factor 4 (MRF4) mRNA expression after intensive endurance exercise (Harber et al. 2010).

The transcriptome responses to endurance exercise seem to be influenced by the training state (Schmutz et al. 2006), but other exercise factors, such as the intensity of the exercise and the nutrition following the exercise may play a role (Booth et al. 2002; Van Proeyen et al. 2011; Mascher et al. 2011; Matsakas et al. 2012). The effects of endurance exercise on human skeletal muscle gene expression profile have first been described in wide level using microarray techniques in 2005 (Timmons et al. 2005; Mahoney et al. 2005) and important DNA microarray results are shown in figure 9 (Mahoney and Tarnopolsky 2005). In the study of Timmons et al. (2005), about 500 genes were modulated after aerobic training. Furthermore, in the same study, the manner of regulation of approximately 100 genes responsive to endurance exercise was similar to Duchenne muscular dystrophy (DMD) when compared to healthy muscle and a large number of extracellular matrix (ECM) genes were upregulated by endurance exercise training (Timmons et al. 2005). Mahoney et al. (2005) describe mRNA expression during recovery from endurance exercise identifying genes involved in: metabolism, mitochondrial biogenesis, oxidant stress management and signalling, cell stress and damage, cell growth and death, electrolyte handling and proteolysis. The administration of protein after endurance exercise affects the transcriptome involved in

skeletal muscle development, energy metabolism, slow–myofibril remodelling, immunity and defence, while it affects genes of muscle contraction, extracellular matrix-signalling and structure and nucleoside, nucleotide and nucleic acid metabolism, developmental processes, glycolysis and lipid and fatty acid metabolism (Rowlands et al. 2011). Transcriptome analysis of the skeletal muscle in older subjects (70 to 73 years old) showed 74 significantly regulated genes in response to endurance training (Pietrangelo et al. 2012). The present thesis studies the effects of endurance training alone and in combination with myostatin blocking in mdx mice.

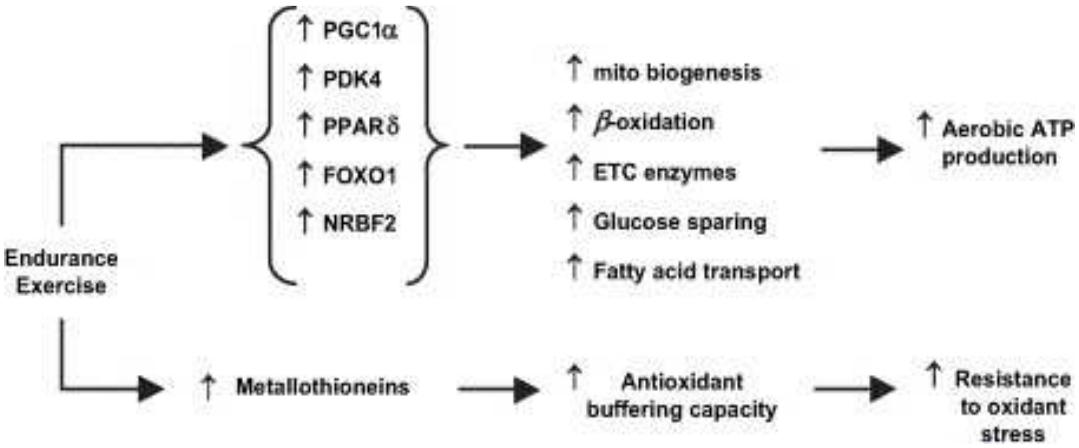

FIGURE 9. Schematic representation of important DNA microarray results after endurance exercise study. Endurance exercise rapidly activated PGC1α, PDK4, PPARδ, FOXO1, and NRBF2, which seem to be important for exercise-mediated mitochondrial expansion and altered fuel selection, which enhances aerobic ATP-generating capacity in skeletal muscle. Endurance exercise also rapidly activated the metallothionein gene family, which seems to have a role in oxidant management and may confer protection from exercise-induced oxidant stress (Mahoney and Tarnopolsky 2005).

5.2 Effects associated with myostatin

The effects of myostatin on muscle phenotype and mRNA profiling are described in section 2.3. Myostatin null mice are, in theory, better suited for anaerobic type of exercise, but they are able to adapt to aerobic training by increasing oxidative metabolism. Untrained $Mstn^{-/-}$ mice reported to show lower work output (38%) compared with wild-type ($Mstn^{+/+}$), but this difference seems to be eliminated after endurance exercise training (Savage and McPherron 2010; Matsakas et al. 2010).

Furthermore, myostatin null mice have a greater percentage of fast/glycolytic fibers, as well as greater muscle mass and fiber cross-sectional area than $Mstn^{+}/^{+}$ mice and show reduced expression of cytochrome c oxidase, mitochondrial content and citrate synthase activity, altered respiration of intermyofibrillar (IMF) mitochondria and mitochondrial metabolism (Savage and McPherron 2010; Ploquin et al. 2012). In addition, myostatin null mice showed a similar response to endurance exercise training (voluntary wheel running training) with wild-type mice in IIb-to-IIa shift in myosin heavy chain (MHC) expression (Matsakas et al. 2010).

Several studies have demonstrated a decrease in myostatin gene expression by chronic endurance training (Matsakas and Diel 2005; Kopple et al. 2006; Hittel et al. 2010; Allen et al. 2011) or even a single bout of endurance exercise (Harber et al. 2009; Allen et al. 2011). Moderate endurance training for 9 weeks in hemodialysis patients and in overweight sedentary subjects showed a decrease of 50% in myostatin mRNA and protein levels (Kopple et al. 2006; Hittel et al. 2010), but not in healthy and physically active people (Schiffer et al. 2011). Short-term endurance exercise training has been reported to prevent the increase of muscle mass in mice treated with a myostatin inhibitor and to decrease muscle mass, without causing muscle damage in both $Mstn^{-}/^{-}$ and $Mstn^{+}/^{+}$ mice (LeBrasseur et al. 2009; Savage and McPherron 2010). Similarly to endurance training with myostatin deficiency, increased frequency and duration of endurance training, but not very high-increased intensity, can also decrease adaptations to resistance training (Wilson et al. 2012).

Endurance exercise training normalizes key phenotypic alterations of myostatin deficient skeletal muscle and it has been reported that it increases the nuclear/cytoplasmic ratio (which correlated with tetanic and specific force) and oxidative metabolic phenotype and capillary density, decreases muscle mass and improves the force generation (Matsakas et al. 2012). In addition, wheel running and swim training seems to induce the autophagic marker Bnip3 and estrogen-related receptor γ (ERRγ) and to promote SDH activity, mitochondrial enlargement, angiogenesis and slow MHC expression in $Mstn^{-}/^{-}$ mice (Matsakas et al. 2012).

To the author's knowledge there is no study describing the gene expression profile of the combination of exercise and blocking of myostatin activity, with either genetic deletion or postnatal inhibition. Another novelty of this thesis is that describes this gene expression profile in the mdx mouse.

5.3 Effects on Duchenne muscular dystrophy muscle and mdx mouse

The effects of endurance exercise on Duchenne muscular dystrophy muscle have been minimally explored, resulting in a limited number of studies. Most of these studies were conducted in the last 5 years. The available technology and the knowledge provided by recent studies allow us to study the effects of endurance exercise in combination with myostatin blocking on the genetic expression profile of mdx mouse muscle for the first time.

In the mdx mouse, long-term voluntary exercise has been reported to stabilize the pathology of skeletal muscle (Landisch et al. 2008), to increase the force-generating ability of the diaphragm and leave the contractile properties of the soleus muscle unaffected (Dupont-Versteegden et al. 1994; Hayes and Williams 1996). Furthermore, voluntary endurance exercise increases resistance to fatigue (Hayes et al. 1993; Hayes and Williams 1996), the proportion of type I fibers in soleus muscle, type IIa fibers in EDL muscle, while not altering the percentage of area of type I and type IIa fibers on the soleus mdx muscle (Hayes and Williams 1996). The administration of green tea extract in combination with endurance exercise enhances endurance capacity in mdx mice (Call et al. 2008).

6 RESEARCH QUESTIONS

One important aspect of muscle and body metabolism is the oxidative metabolism. Treatment with sActRIIB-Fc has been reported to suppress the expression of oxidative metabolism-related genes, while endurance exercise promotes it (Timmons et al 2005; Matsakas et al. 2010; Rahimov et al. 2011; Hulmi et al. 2013b). The questions that arise are how sActRIIB-Fc treatment alone or in combination with exercise and how exercise alone or in combination with sActRIIB-Fc treatment affect the oxidative metabolism gene expression in muscle. Furthermore, broadening these questions to the whole transcriptional level, the question formed is: how are the above mentioned interventions affecting, alone or in combination, the global gene expression profile.

Elevated oxidative stress has been proposed, recently, as a mediator between dystrophin deficiency and pathology (Kaczor et al. 2007; Renjini et al 2012; Kim et al. 2013). Given the beneficial effects of the treatment with sActRIIB-Fc and the beneficial effects of endurance exercise in dystrophic muscle, the combination of sActRIIB-Fc treatment and exercise is hypothesized to be more beneficial for the dystrophic muscle, than each intervention alone.

The present thesis aims in identifying the pathways affected by the treatment with sActRIIB-Fc, exercise and the combination of them in dystrophic muscle and to compare those responses with the healthy muscle. The global gene expression profiling approach allows us to simultaneously examine the expression of thousands of genes responding to sActRIIB-Fc treatment, exercise and the combination of them. In addition, pathway analysis makes the identification of the role of the affected genes faster and provides reliable answers on the above mentioned questions.

Hypotheses

a. Exercise and sActRIIB-Fc have opposite effects on aerobic gene expression profiling in dystrophic skeletal muscle and exercise induces transcriptional responses towards the aerobic gene expression profile of the healthy muscle

(Timmons et al 2005; Matsakas et al. 2010; Rahimov et al. 2011; Hulmi et al. 2013b).

b. Treatment with sActRIIB-Fc affects differently on the global gene expression profile of the exercised in comparison with the sedentary mdx muscle (Timmons et al 2005; Matsakas et al. 2010; Rahimov et al. 2011; Hulmi et al. 2013b).

c. Exercise affects differently on the global gene expression profile of the muscle treated with sActRIIB-Fc in comparison with the untreated mdx muscle (Timmons et al 2005; Matsakas et al. 2010; Rahimov et al. 2011; Hulmi et al. 2013b).

7 METHODS

7.1 Animals

The mice used in the study were male, 6-7 weeks old, from a C57Bl/10SnJ background. All mice were from Jackson Laboratory (Bar Harbor, Maine, USA). They were housed in individual cages with standard conditions (temperature 22°C, light from 8:00 AM to 8:00 PM). The mice had free access to tap water and food pellets (R36, 4% fat, 55.7% carbohydrate, 18.5% protein, 3 kcal/g, Labfor, Stockholm Sweden).

7.2 Ethics statement

The treatment of the animals was in strict accordance with the European convention for the protection of vertebrate animals used for experimental and other scientific purposes. The protocol was approved by the national animal experiment board (permit number: ESLH-2009-08528/Ym-23). All efforts were made to minimize suffering.

7.3 Experimental design

The mdx mice were randomly divided into 4 groups (n=8 in each): PBS (P), PBS running (PR), sActRIIB-Fc (A) and sActRIIB-Fc running (AR). sActRIIB-Fc or PBS (5 mg/kg) was injected intraperitoneally once a week for 7 weeks. In addition, PBS was administered to wild mice (n=5), which was used as a healthy control group (Control). A schematic representation of the experimental design is shown in figure 10.

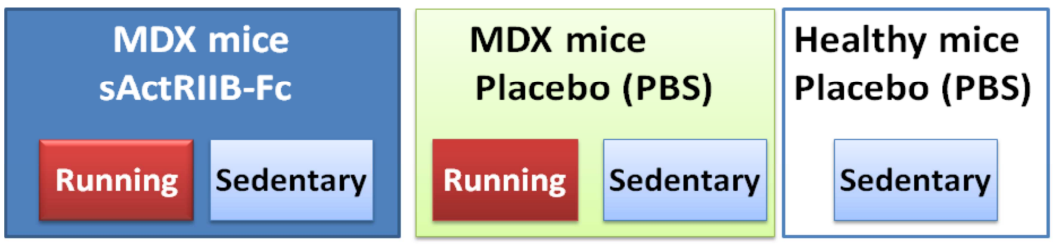

FIGURE 10. Schematic representation of the 5 groups: sActRIIB-Fc, sActRIIB-Fc running, PBS, PBS running and healthy control.

Voluntary wheel running was chosen as exercise modality, during the 7 weeks of the study. The running wheels were locked during the first injection day and the next day to prevent mice from exercising and to allow the treatment take effect. The mice did not have access to the running wheels during the last two days, so that the outcome effects would not reflect the acute effects of exercise.

During the experiment all conditions were standardized. The mice were sacrificed after the experiment by cervical dislocation and blood and tissue samples were collected.

7.4 sActRIIB-Fc production

The recombinant fusion protein was produced and purified *in house* at the University of Helsinki, as it is described in Hoogars et al. 2012 and Hulmi et al. 2013. It has been reported previously to normalize (LPS- or activin A overexpression-induced) lung pathology in a mouse model (5) and to increase muscle size in mdx mice (24). The ectodomain (ecd) of human sActRIIB was amplified via PCR with the following primers: 5'-GGACTAGTAACATGACGGCGCCCTGG-3' and 5'-CCAGATCTGCGGTGGGGGCTGTCGG-3' from a plasmid containingthe human ActRIIB sequence (in pCR-Blunt II-TOPO AM2 G17 ActRIIB, IMAGE clone no. 40005760; The IMAGE Consortium). A human IgG1 Fc domain with a COOH-terminal His6 tag was amplified by PCR (5'-GCAGATCTAATCGAAGGTCGTGGTGATCCCAAATCTTGTGAC-3' and 5'-TCCCTGTCTCCGGGTAAACACCATCACCATCACCATTGAGCGGCCGCTT-3') from the pIgPlus expression plasmid. The subcloning of these products was done into the pGEM-T easy (Promega) vectors, sequenced, and fused before cloning into the expression vector pEFIRES-p. For the final protein production, chinese hamster ovary (CHO) cells were transfected with the above-mentioned ActRIIBecd-FcHis6 expression vector via lipofection (Fugene 6; Roche) and selected with puromycin (Sigma-Aldrich, Lyon, France). During selection, cells were grown in Dulbecco's modified Eagle media (DMEM) supplemented with 2 mmol/l L-glutamine, 100 g/ml streptomycin, 100 IU/ml penicillin, and 10% fetal calf serum (FCS). For large-scale expression, cells were adapted to CD OptiCHO medium (Gibco) supplemented with 2 mmol/l L-glutamine

and grown in suspension in an orbital shaker. Cell culture supernatants were clarified by filtration through a 0.22-m membrane (Steritop; Millipore). Next, NaCl and imidazole were added, and the solution was pumped through a Ni2-loaded HiTrap Chelating column (GE Healthcare Life Sciences, Uppsala, Sweden) at 4°C. Protein was eluted by raising imidazole concentrations, dialyzed against PBS, and finally concentrated with Amicon Ultra concentrator (30 000 MWCO; Millipore). The purity of our sActRIIB-Fc preparation after IMAC purification was estimated to be 90% based on silver-stained SDS-PAGE.

7.5 Voluntary wheel running and feed intake

Voluntary wheel running may offer benefits in mdx mice (Call et al. 2008; Landisch et al. 2008), whereas forced exercise, such as running, may even contribute to the exacerbation of the dystrophy in dystrophic mice or humans (Fraysse et al. 2004; Grange and Call 2007). Therefore, the chosen exercise modality in this study is voluntary wheel running.

The mice were housed individually in cages where they had free access to custom-made running wheels (diameter 24 cm, width 8cm) 24 h/day. The sedentary animals were housed in similar cages without the running wheel. Total running distance was recorded daily, while body mass and feed consumption were measured weekly.

7.6 Muscle and fat sampling

The mice were sacrificed by cervical dislocation. Immediately after, lower leg muscles: soleus, gastrocnemius, musculus quadriceps femoris (MQF), extensor digitorum longus (EDL) and tibialis anterior were removed, weighed, and frozen in liquid nitrogen. Epididymal fat pad was collected for the estimation of fat size. Muscle weights reported are always average weights of left and right legs.

7.7 RNA and DNA isolation

The gastrocnemius muscle was pulverized and homogenized in liquid nitrogen and exactly 70 mg of muscle powder was weighted into Trizol reagent (Invitrogen,

Carlsbad, CA, USA) and further mixed and homogenized utilizing FastPrep FP120 apparatus (MP Biomedicals, Illkrich, France). Total RNA and DNA fractions were extracted according to the manufacturer's guidelines. The RNA and DNA concentrations were analyzed with Nanodrop ND-1000 (Thermo Fisher Scientific Inc., Waltham MA, USA) in duplicate. An OD260/OD280 ratio of 1.9 to 2.0 and gel electrophoresis showed that our RNA extraction yielded DNA-free and un-degraded RNA, respectively. For microarray analysis possible DNA was however, further degraded using DNAse according to manufacturer's instructions (TURBO DNA-free™ Kit, Applied Biosystems by Life Technologies, USA).

7.8 RNA amplifications and hybridizations

Generation of cRNA, hybridizations of the arrays and quality control of the raw data were performed by the Finnish Microarray and Sequencing Centre at Turku Center for Biotechnology. For the amplifications we used the total RNA of 5 mice per each mdx group and 4 control mice. More specifically, 300 ng of total-RNA was amplificated by Illumina® TotalPrep™ RNA Amplification Kit (Applied Biosystems cat # AMIL1791). The in vitro-transcripted (IVT)-reaction lasted overnight, over which cRNA was biotinylized. The concentrations of RNA and biotinylized cRNA were measured with Nanodrop ND-2000 (Thermo Fischer Scientific Inc. Waltham, MA, USA) and quality was checked with Agilentin Bioanalyzer – electrophoresis device (Agilent Technologies).

Hybridization was performed following Illumina Whole-Genome Gene Expression Direct Hybridization Assay Guide protocol (part #11322355, rev.A). 750 ng of each cRNA-sample was hybridized to Illumina Sentrix MouseRef-8 v2 Expression Bead Chip –microchip at 58 C° overnight. Hybridizations were detected by Cyanine3-streptavidin. Chips were scanned with Illumina Bead Array Reader –device (Factor=1, PMT=520, Filter=100%). Genome Studio v. 2011.1 (Gene Expression Module v. 1.9.0) software produced numerical values as non-normalized and without reducing background.

7.9 Data processing

Data were quantile normalized and checked using Chipster software (IT Center for Science, Espoo, Finland) (Kallio et al. 2011). The normalized gene expression data were imported into Excel spreadsheets (Microsoft Corp., Redmond, WA, USA) for downstream data analysis.

An elimination process was performed, in order to overcome the issue of genes with multiple probes which could affect the analysis. The probes with the highest fold-change ratio were chosen to represent a given gene. After the elimination process, 19186 genes were left from a total of 25697 genes. In addition, an annotation chip file was created using the Illumina annotation file: MouseRef-8_V2_0_R3_11278551_A (http://www.switchtoi.com/annotationfiles.ilmn).

Fold-change ratio (or intensity ratio) was used to create different ranking lists, in which the genes were ranked according to their expression differences between two groups per list. More specifically, the genes were ranked in order from the highest fold-change ratio (the ratio of the mean expression value of a gene of one group of mice divided by the mean expression value of the same gene in another group) to the lowest and including the negative values (for the determination of the direction of the changes). The ranking lists were then used in GSEA preranked analysis.

7.10 Gene clustering by Gene Set Enrichment Analysis (GSEA)

Enrichment for functionally related genes to the gene sets of three different collections was performed using Gene Set Enrichment Analysis software (GSEA; Version 2.0) (Subramanian et al., 2005). The collections were: the Gene Ontology biological processes collection (825 gene sets, C5:BP, version 3.0), the Canonical Pathways collection (1452 gene sets, C2:CP, version 3.0) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) collection (186 gene sets, C2:CP:KEGG, version 3.0) and can be found on the internet (http://www.broadinstitute.org/gsea/msigdb/collections.jsp).

More specifically, gene set enrichment analysis on a preranked gene list was performed, using the preranked gene lists created with Excel, as described in the data processing section. The number of permutations was set to 1000 and gene sets with at least five and no more than 500 genes were taken into account in each analysis. Each analysis was carried out at least five times and all the results are the mean values of those. The level of significance was set at False Discovery Rate (FDR) < 0.05 (for the identification of the most influenced pathways), but also FDR < 0.001 and FDR < 0.25 were used to show the differentiation between the profiles.

8 RESULTS

This section presents the results of the present thesis divided into two sub-sections, the background information and the gene expression profiling. The first describes the differences in body mass, muscle mass and fat mass and presents the activity of each group during the experimental period. The second sub-section provides a total overview of the large data set and focuses on selected pathways.

8.1 Background information

The effects of sActRIIB-Fc administration, voluntary running and the combination of those were investigated in modestly dystrophic mdx muscle in 7-week experiment. Body mass increased rapidly during the first 2-3 weeks after sActRIIB-Fc, but thereafter tended to stabilize to the rate of growth in the PBS group (figure 11).

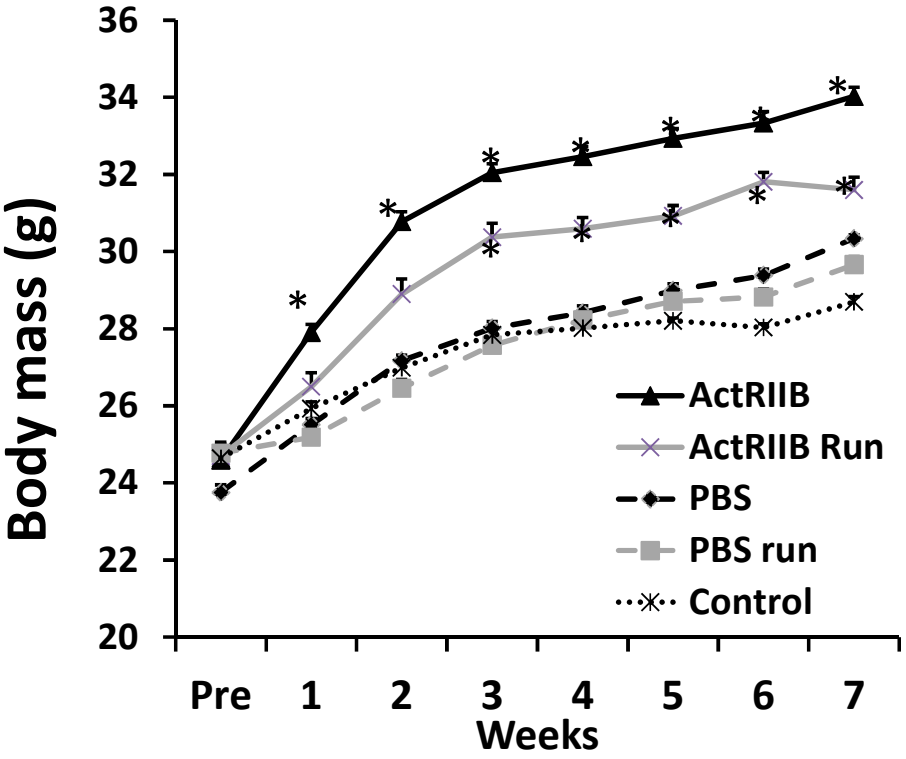

FIGURE 11. Changes in body mass after 7 wk sActRIIB-Fc (ActRIIB) administration with or without physical exercise (Run). The symbol * denotes difference ($P<0.05$) compared to PBS.

Voluntary running for 7 weeks attenuated the sActRIIB-Fc-induced increase in body mass when compared to PBS, mainly by decreasing fat mass (figures 12 and 13). In addition, we found decreased epididymal body fat (figure 12) and a trend (P=0.10) for a reduced retroperitoneal fat (figure13) by sActRIIB-Fc when these variables were normalized to body weight. This is even though the mice administered sActRIIB-Fc consumed more food than their controls (not shown).

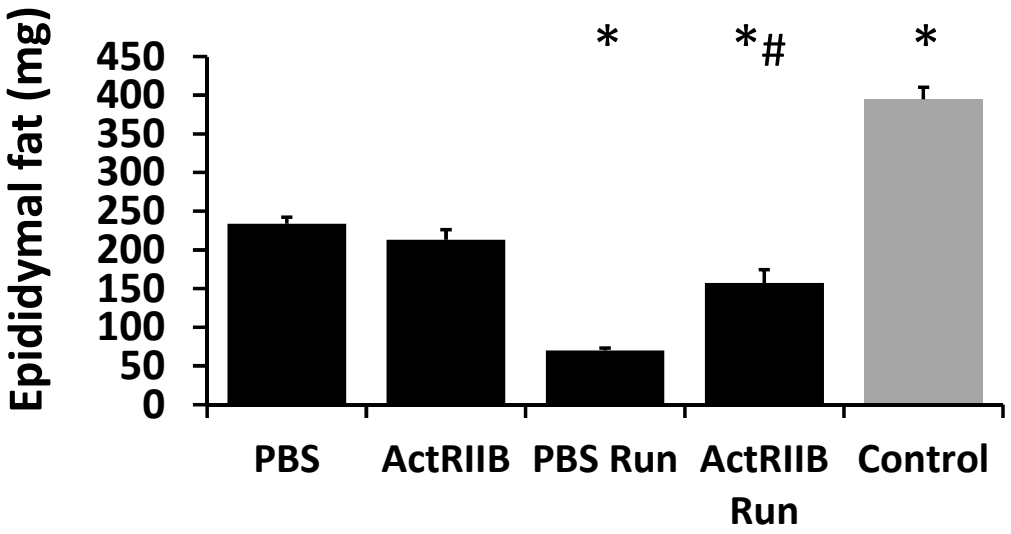

FIGURE 12. Changes in epididymal fat masses after 7 wk sActRIIB-Fc (ActRIIB) administration with or without physical exercise (Run). The symbol * denotes difference ($P<0.05$) and the # difference between sActRIIB-Fc and sActRIIB-Fc run.

Running also decreased the sActRIIB-Fc-induced increase in absolute muscle mass, but significantly only in gastrocnemius muscle (figure 14), but not in other muscles (data published in: Hulmi et al. 2013b). The results of voluntary wheel running (for the exercised groups: ActRIIB-Fc running and PBS running) are shown in figure 15.

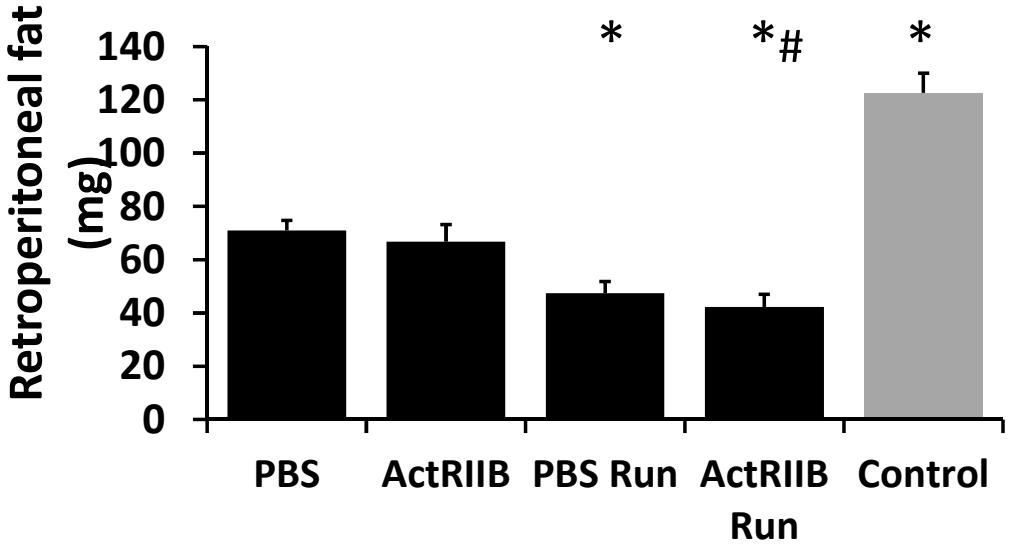

FIGURE 13. Changes in Retroperitoneal fat masses after 7 wk sActRIIB-Fc (ActRIIB) administration with or without physical exercise (Run). The symbol * denotes difference ($P<0.05$) and the # difference between sActRIIB-Fc and sActRIIB-Fc run.

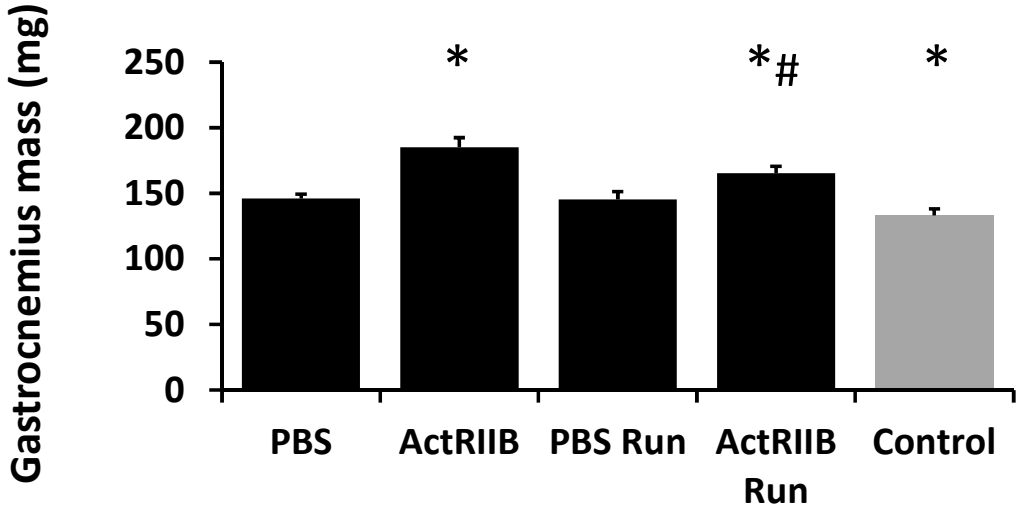

FIGURE 14. Changes in gastrocnemius muscle mass after 7 wk sActRIIB-Fc (ActRIIB) administration with or without physical exercise (Run). The symbol * denotes difference ($P<0.05$) compared to PBS and the # difference between sActRIIB-Fc and sActRIIB-Fc run.

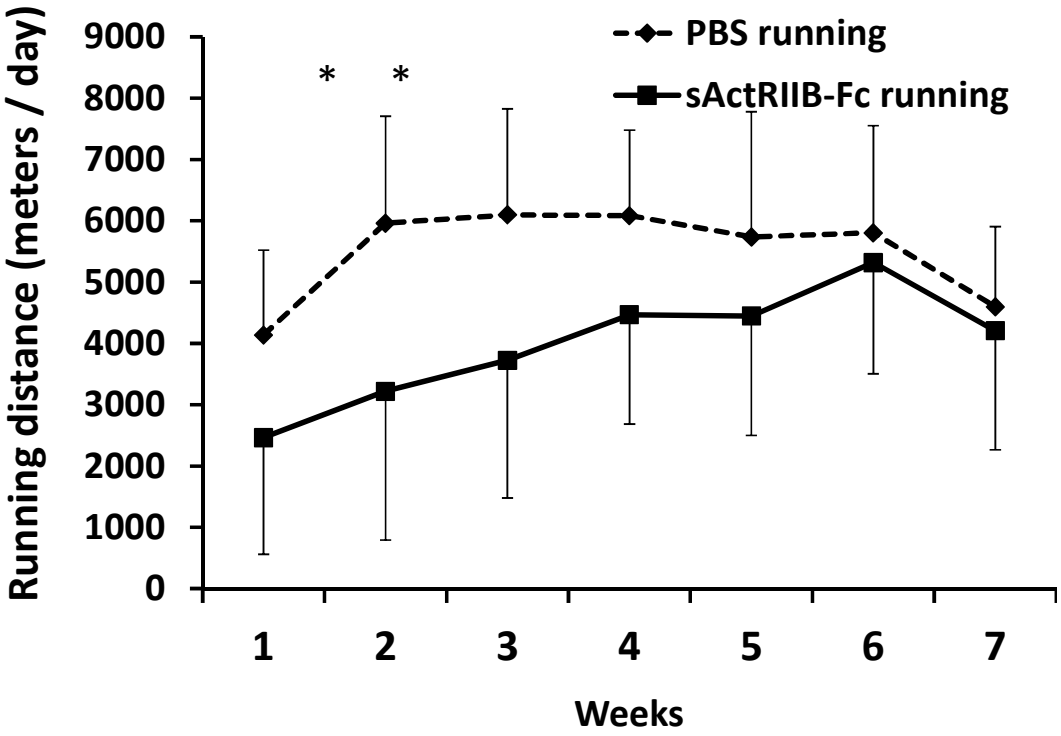

FIGURE 15. Weekly voluntary running in meters per day. * = statistical significance (P<0.05).

8.2 Gene expression profiling

This section presents a general overview of genetic expression responses to our intervention and emphasizes on the most influenced pathways (FDR < 0.25) affected by the treatment with myostatin/activin blocker sActRIIB-Fc with or without exercise in dystrophic mdx muscle. The study also includes healthy controls. Gene Set Enrichment analysis (GSEA) was used for gene expression profiling analysis and more specifically the canonical pathways (CP) gene set collection. These results are summarized in tables 1 and 2 and selected results are presented in table 3 and figure 16. The complete tables of the most influenced genes ($P < 0.05$) and the pathways (FDR < 0.25) of the three gene set collections are presented in the appendices 1 and 2 (the result of KEGG collection are included to the CP results). In addition, enrichment plots for some selected gene expression sets for each comparison are shown in the appendix 3 and the leading-edge genes for selected pathways in appendix 4.

TABLE 1. Summary of the most influenced genes in 6 comparisons.

	A vs P		PR vs P		AR vs P		AR vs PR		AR vs A		P vs C	
	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up	Down	Up
P<0.0001	1	3	1	3	0	1	0	0	0	0	872	478
P<0.001	5	9	6	9	5	6	5	3	5	10	1249	939
P<0.01	96	99	68	93	54	80	26	26	84	91	1756	1685
P<0.05	498	495	405	405	351	385	243	231	438	529	2356	2708

A = sActRIIB-Fc, P = PBS, AR = sActRIIB-Fc Running, PR = PBS Running and C = Healthy Control

TABLE 2. Summary of the most influenced pathways in the 3 gene set collections. FDR < 0.05 (in parenthesis FDR < 0.25). A = sActRIIB-Fc, P = PBS, AR = sActRIIB-Fc Running, PR = PBS Running and C = Healthy Control

Comparisons	GENE SET COLLECTIONS and PATHWAYS REGULATION					
	Biological Processes		Canonical Pathways		KEGG	
	Down	Up	Down	Up	Down	Up
A vs P	0 (0)	0 (7)	11 (26)	1 (4)	3 (9)	1 (10)
PR vs P	0 (7)	2 (11)	17 (42)	19 (50)	0 (6)	12 (25)
AR vs P	0 (0)	7 (17)	1 (6)	52 (104)	4 (5)	17 (24)
AR vs PR	0 (0)	0 (6)	0 (24)	92 (202)	0 (12)	10 (30)
AR vs A	0 (4)	9 (27)	1 (88)	35 (184)	0 (11)	21 (58)
P vs C	8 (35)	80 (265)	24 (60)	212 (360)	10 (24)	54 (79)

The treatment with sActRIIB-Fc activated 92 canonical cell processes in exercised muscles (sActRIIB-Fc running vs. PBS running), but only 1 when used in sedentary muscle (sActRIIB-Fc vs. PBS) (FDR < 0.05) (table 2). In contrast, sActRIIB-Fc did not decrease any canonical cell processes in active muscles (sActRIIB-Fc running vs. PBS

running) (FDR < 0.05), but decreased 11 processes when sActRIIB-Fc was used on sedentary muscle (sActRIIB-Fc vs. PBS) (table 2).

Exercise activated 19 canonical cell processes in dystrophic muscles (PBS running vs. PBS) and 35 when used combined with sActRIIB-Fc administration (sActRIIB-Fc running vs. sActRIIB-Fc) (FDR<0.05) (table 2). On the other hand, exercise decreased 17 canonical cell processes in active muscles (sActRIIB-Fc running vs. PBS running) (FDR < 0.05), but decreased only 1 process when combined with sActRIIB-Fc (sActRIIB-Fc running vs. sActRIIB-Fc) (table 2).

Interestingly, aerobic metabolism pathways (e.g. electron transport chain, oxidative phosphorylation, citrate cycle, lipids and lipoproteins metabolism and branched chain amino acid degradation) were of the most downregulated in dystrophic muscle (FDR < 0.05) (table 3, PBS vs. Control). sActRIIB-Fc treatment alone decreased part of these (electron transport chain and oxidative phosphorylation) even further (sActRIIB-Fc vs. PBS FDR < 0.05). On the other hand, all of these were upregulated by aerobic exercise (FDR < 0.05) in PBS mice and in exercised sActRIIB-Fc mice except citrate cycle (FDR < 0.30). These results are depicted in table 3 and suggest that running modulates aerobic gene expression profile in dystrophic skeletal muscle towards a healthier profile.

Many of the effects of exercise were similar between treated and untreated mice (increased BCAA degradation, oxidative phosphorylation, electron transport chain and fatty acid oxidation), but also differences were observed. Exercise alone (PBS running) increased significantly (FDR = 0.003) tricarboxylic acid (TCA) cycle pathway when compared to PBS group, whereas exercise with sActRIIB-Fc (sActRIIB-Fc running) did not increase TCA when compared to sActRIIB-Fc (FDR = 0.59) (Table 3).

Apoptosis, glutathione metabolism and conjugation, as well as purine and pyrimidine metabolism were all activated by the combination of sActRIIB-Fc and exercise, but not by each treatment alone (sActRIIB-Fc + Running vs. PBS, sActRIIB-Fc + Running vs. PBS + Running and sActRIIB-Fc + Running vs. sActRIIB-Fc in comparison with sActRIIB-Fc vs. PBS and PBS + Running vs. PBS, as shown in table 3). Furthermore, transcriptional responses of metabolism of xenobiotics by cytochrome P450, drug metabolism cytochrome P450 and metabolism of amino acids were all increased by

exercise with or without administration of sActRIIB-Fc, but decreased when dystrophic muscle compared to healthy sedentary muscle (PBS vs. C). Therefore, running in combination with sActRIIB-Fc modulated these pathways in mdx mice towards the profile of the healthy muscle.

TABLE 3. Regulation of selected pathways in the Canonical processes database of GSEA in the six comparisons.

The order of the pathways is based on their significancy in: 1) sActRIIB-Fc vs PBS, 2) PBS running vs PBS and 3) sActRIIB-Fc running vs PBS.

Pathway name	Comparisons					
	A vs P	PR vs P	AR vs P	AR vs PR	AR vs A	P vs C
KEGG_OXIDATIVE_PHOSPHORYLATION	↓**	↑**		↓*	↑**	↓**
REACTOME_DIABETES_PATHWAYS	↓**				↑*	
REACTOME_ELECTRON_TRANSPORT_CHAIN	↓**	↑***	↑**	↓*	↑**	↓***
REACTOME_GLUCOSE_REGULATION_OF_INSULIN_SECRETION	↓*			↓*	↑*	↓**
KEGG_PATHWAYS_IN_CANCER	↑*					↑***
REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS		↑***	↑**			↓***
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION		↑***	↑**		↑**	↓**
KEGG_CITRATE_CYCLE_TCA_CYCLE		↑**				
REACTOME_PYRUVATE_METABOLISM_AND_TCA_CYCLE		↑**	↑*			
KEGG_PEROXISOME		↑**	↑***		↑**	↓**
KEGG_FATTY_ACID_METABOLISM		↑**	↑**		↑**	
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM		↑**				
REACTOME_CITRIC_ACID_CYCLE/BIOCARTA_KREB_PATHWAY		↑**				
REACTOME_MITOCHONDRIAL_FATTY_ACID_BETA_OXIDATION		↑**	↑**		↑**	↓**
REACTOME_METABOLISM_OF_AMINO_ACIDS		↑**	↑**	↑**	↑**	↓*
KEGG_PPAR_SIGNALING_PATHWAY		↑*	↑**		↑*	↓***
KEGG_DRUG_METABOLISM_CYTOCHROME_P450		↑*	↑***	↑*	↑**	↓**
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM		↑*	↑**		↑*	↓**
KEGG_ARGININE_AND_PROLINE_METABOLISM		↑*				
REACTOME_REGULATION_OF_LIPID_METABOLISM_BY_PPAR_ALPHA		↑*	↑*			↓**
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450		↑*	↑***	↑**	↑**	↓**

KEGG_NITROGEN_METABOLISM	↑*	↑**			↓*
KEGG_GLUTATHIONE_METABOLISM		↑**	↑**	↑*	↓*
KEGG_PYRIMIDINE_METABOLISM		↑**	↑**	↑**	
KEGG_PURINE_METABOLISM		↑**	↑*	↑*	
REACTOME_GLUTATHIONE_CONJUGATION		↑**		↑*	↓*
REACTOME_APOPTOSIS		↑**	↑***	↑*	

***** = FDR < 0.001, ** = FDR < 0.05, * = FDR < 0.25**

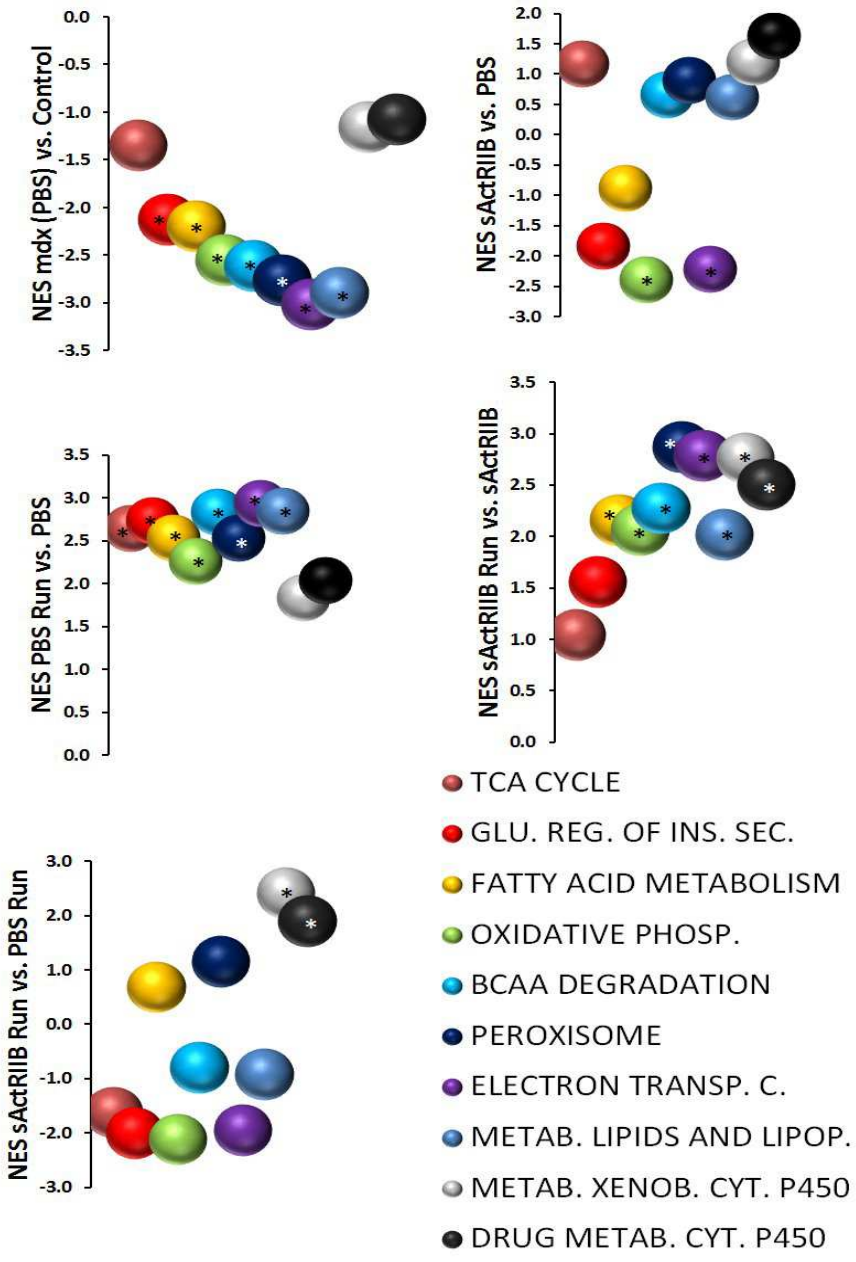

FIGURE 16. Aerobic metabolism and drug metabolism are regulated by exercise (Run) and/or sActRIIB-Fc (sActRIIB). Gene Set Enrichment Analysis (GSEA) results from microarray of gastrocnemius muscle. TCA cycle = Tricarboxylic acid / Krebs / citric acid cycle, Glu. Reg. of Ins. Sec = Glucose regulation of insulin secretion, Phosp = phosphorylation, transp. chain = transport chain, metab. lipids and lipop. = metabolism of lipids and lipoproteins, metab. xenob. cyt. P450 = metabolism of zenobiotics of cytochrome P450, drug metab. cyt. P450 = drug metabolism of cytochrome P450. NES = normalized enrichment score in GSEA (Subramanian *et al.* 2005). NES > 0 indicates upregulation and NES < 0 indicates downregulation. The order of the pathway balls is based on a FDR result in mdx vs. control comparison. * = FDR < 0.05.

9 DISCUSSION

The principal finding of the present thesis was that exercise modulates gene expression profiles of dystrophic muscle of mdx mice towards better aerobic metabolism and, as a consequence, towards a healthier profile. Higher expression could be seen in a wide range of pathways from lipid and BCAA metabolism to TCA cycle and electron transport chain. Furthermore, the blocking of myostatin/activins affects differently the transcription-wide gene expression profile of exercised compare to non-exercised muscle. On the other hand, the effects of exercise were also shown to be either dependent or independent of co-treatment with sActRIIB-Fc. This chapter discusses the most significant results of our interventions in between the comparisons and is divided into the corresponding sections: aerobic profile, oxidative damage, branched-chain amino acids (BCAAs) and other selected pathways. In addition, the interaction effect of exercise and myostatin/activin blocking is discussed in a separate section. The last section summarizes the conclusions of the present thesis.

For the first hypothesis, table 3 shows the opposite responses of sActRIIB-Fc treatment and exercise on important pathways of the aerobic metabolism, like electron transport chain and oxidative phosphorylation. Moreover, the pathways of aerobic metabolism are activated by exercise, in contrast to the decreases in the expression of the same pathways in dystrophic compared to the healthy muscle, confirming the exercise-induced changes towards the aerobic profile of the healthy muscle.

For the next two hypotheses, the differentiation can be determined by: 1) the total number of genes and pathways changed, 2) the direction of the changes of the genes and the pathways and 3) the differences in statistical significance of the changes. The number of affected genes and pathways are shown in table 1 and 2. For the second hypothesis (b), we can examine the comparisons sActRIIB-Fc vs. PBS and sActRIIB-Fc + Running vs. PBS + Running and for the third hypothesis (c) the comparisons PBS + Running vs.PBS and sActRIIB-Fc + Running vs. sActRIIB-Fc. Therefore, all of the hypotheses were correct. However, it is of interest to examine these differences on specific pathways and processes.

9.1 Aerobic profile

This section discusses the findings of our study related to aerobic-oxidative metabolism and aerobic energy production. Treatment with sActRIIB-Fc has been reported to suppress the expression of aerobic metabolism-related genes (Rahimov 2011). On the other hand, endurance exercise induces gene expression of aerobic metabolism (Timmons et al. 2005; Mahoney et al. 2005) and activities of aerobic enzymes (Hulmi et al. 2013b). More specifically, a previous article of the same study reports exercise-induced increases in mdx mice to healthy control levels on: PGC1-α protein content, cytochrome C, citrate synthase (CS) activity and succinate dehydrogenase (SDH) (Hulmi et al. 2013b).

The results indicate an exercised-induced shift in aerobic gene expression profile of mdx mice, even when treated with sActRIIB-Fc. The expression of pathways related to aerobic metabolism have increased with exercise (oxidative phosphorylation, electron transport chain, mitochondrial fatty acid beta oxidation, TCA cycle), even with sActRIIB-Fc treatment (electron transport chain and mitochondrial fatty acid beta oxidation). Moreover, this shift is shown in lipid metabolism expression profile, as the expression of several pathways related to lipid metabolism were increased (metabolism of lipid and lipoproteins, peroxisome, fatty acid metabolism, mitochondrial fatty acid beta oxidation, PPAR signaling pathway and regulation of lipid metabolism by PPAR-α pathways), even when sActRIIB-Fc was administrated. In addition, the increases in lipid and fatty acid metabolism were associated with increased levels of PDK4 protein (data not shown), a regulator of glucose-fat switch for the use of energy (Sugden and Holness 2003). PDK4 has been recently reported to be increased by voluntary running (Rinnankoski-Tuikka et al. 2012). Therefore, sActRIIB-Fc did not prevent the positive responses of exercise in most of the aerobic pathways.

The different responses to sActRIIB-Fc in sedentary and exercised muscle may be explained by a stronger effect of exercise alone in comparison when co-treated with sActRIIB-Fc. For example, TCA cycle gene set was significantly increased by exercise (independently of sActrIIB-Fc administration), but the effect was more significant in exercise alone than in combination. Thus, in comparison of the exercised sActRIIB-Fc treated muscle with the exercised PBS-treated muscle the direction of the change is the

opposite (appendix 2). In addition, the activity of citrate synthase and succinate dehydrogenase in the skeletal muscle of these mice was increased (Hulmi et al. 2013b). Further research is needed to reveal if different modes or volume of exercise could have even more beneficial effects on aerobic metabolism.

9.2 Oxidative damage

Recent studies report increased oxidative stress and decreased antioxidant markers in DMD patients and mdx mice indicating oxidative stress as a candidate mechanism mediating between pathology and dystrophin deficiency (Kim et al. 2013; Renjini et al. 2012; Kaczor et al. 2007). Oxidative damage in DMD is thought to be mainly caused by depletion of the antioxidant glutathione (GSH), which was attributed to lower the activity of gamma-glutamyl cysteine ligase, in a recent study in vitro (Renjini et al. 2012). Furthermore, treatment with N-acetylcysteine (NAC) has reported to have beneficial effects in mdx mice and reducing TNF-α levels, myonecrosis (de Senzi Moraes Pinto et al. 2013) and oxidation of glutathione and protein thiols (Terrill et al. 2012). Here, we report a decreased expression of glutathione (GSH) metabolism and conjugation pathways in dystrophic compared to healthy (PBS vs. C) muscle. In contrast, the combination of exercise with sActRIIB-Fc treatment increased this expression when compared to both PBS and sActRIIB-Fc treated mice and to exercised mice. This supports, in theory, the beneficial role of this combination of the two treatments for DMD muscle. In addition with this finding, we report increased expression of metabolism of xenobiotics by cytochrome P-450 pathway (and also drug metabolism of cytochrome P-450) in all exercised muscles (with or without treatment). Combining those results, there is indication for a possible role for cytochrome P-450 and at least one related toxic substance in DMD pathology.

N-acetyl-p-benzoquinone imine (NAPQI) is a toxic substance that is produced during xenobiotics metabolism of acetaminophen (paracetamol) by cytochrome P450 in liver and is detoxified by GSH conjugation (James et al. 2003). In skeletal muscle, it has been suggested that acetaminophen could be metabolized into myotoxic compound(s), by ethanol induction of cytochrome P450 and cause ethanol-associated rabdomyolysis (Riggs et al 1996, Smith et al. 2000). Therefore, it is logical to assume that the possible existence of a toxic substance, which has some similar

properties with NAPQI (formation catalysed by the P-450 enzymes, detoxification by GSH conjugation or NAC), could presumably connect increased GSH conjugation and metabolism of xenobiotics by P450 in muscle. However, in our study, there was no administration of acetaminophen, no oxidative stress markers were measured and the tissue examined was skeletal muscle, not liver. In conclusion, further investigation is required to reveal the aspects of the oxidative stress and cytochrome P-450 possible mechanisms in DMD pathology.

9.3 Branched-chain amino acids (BCAAs)

BCAA degradation is induced by exercise and it has been recently associated to increased fatty acid oxidation and better metabolic health (Shimomura et al. 2004; Kainulainen et al. 2013). Moreover, BCAA degradation also seems to be increased in twin pairs having enhanced habitual physical activity compared with their inactive co-twins (Leskinen et al. 2010) and is associated with lower serum BCAA concentration (Kujala et al. 2013). In addition, high aerobic capacity has been reported to be associated with expression of BCAA degradation gene set (Kivela et al. 2010).

The results of the present thesis showed that BCAA degradation pathway expression was decreased in dystrophic muscle and exercise reversed this decrease independently of sActRIIB-Fc treatment. It has been reported that mdx mice have higher protein turnover than healthy controls (MacLennan and Edwards 1990). Most likely, these results reflect enhanced mitochondrial energy production and BCAAs act as substrates for a small proportion of total aerobic energy production (Rennie et al. 2006) and they may also participate to mitochondrial fatty acid oxidation as it has been recently proposed in our laboratory (Kainulainen et al. 2013).

9.4 Other selected pathways

The overexpression of genes and pathways related to extracellular matrix (ECM), inflammation, cell adhesion, muscle structure/regeneration and TGF and IGF have been reported to be overexpressed in dystrophic muscle (Chen et al. 2000; Porter et al. 2002; Haslett et al. 2003; Marotta et al. 2009). The results of the present thesis are in

accordance with literature. Moreover, the expressions of growth factors TGFβ, IGF1 IGFMTOR and IGF1R pathways did not showed significant activity (increase or decrease, FDR > 0.275), although they responded differently in treatments. Calcineurin and ubiquitin-proteasome pathways have been related to dystrophic muscle (Bassel-Duby and Olson 2006; Franch and Price 2005), but there were not varied significantly between the comparisons in our study. Another interesting issue is that exercise seems to eliminate the expression of cancer pathways for mdx muscle, although the importance of this finding is controversial due to the possible multiple roles of the involved genes. Therefore, further investigation is needed to reveal the importance of exercise effects on cancer-related gene expression profile in dystrophic muscle.

9.5 The interaction effect of exercise and myostatin/activin blocking

The beneficial effects of myostatin/activin blocking using the sActRIIB-Fc have reported by recent studies (Lee et al. 2005, Pistilli et al. 2010, Rahimov et al. 2011), but these effects have not been studied in combination with exercise in dystrophic mdx mouse. The importance of exercise in pathological conditions and their treatments is crucial, since the state (exercised or sedentary) of the animal has a significant impact on its biology and physiology (Booth and Laye 2009). This impact is so important that habitually states of high physical activity have been recently proposed to be “the appropriate biological control condition reflecting the genetic and biological norm” (Booth and Laye 2009). The size of the difference between the two states is reflected on the numbers of genes and pathways up- or down-regulated (tables 1 and 2) by sActRIIB-Fc or PBS administration in sedentary and exercised muscle (sActRIIB-Fc vs. PBS compared to sActRIIB-Fc running vs. PBS running). It is a paradox, however, that although a larger number (almost double) of genes was influenced in sedentary than exercised muscle, the number of influenced pathways for exercised muscle was overwhelmingly larger than sedentary (only the up-regulated pathways had a 30- to 90-fold difference, 1 to 92 and 6 to 203 FDR > 0.05 and FDR > 0.25 respectively). This paradox indicates that individual genes in sedentary muscle may act contrary to each other, eliminating a targeted response. In addition, the differences between exercised and sedentary muscle may be partly explained by differences in “response plasticity” (Coffey et al. 2006) between the treated and the untreated mice. Treatment with sActRIIB-Fc might act as a resistance training mimetic (Pistilli 2011), making aerobic

exercise a stronger stimulus (Coffey et al. 2006) to treated than untreated mice (compare sActRIIB-Fc running vs. sActRIIB-Fc to PBS running vs. PBS in tables 1 and 2).

Exercise has beneficial effects in both myostatin deficient mice (Matsakas et al. 2012) and mdx mice (Hayes and Williams 1996), but these effects have not been studied in mdx mouse after postnatal myostatin inhibition. The results of the present thesis show that the effects of exercise vary between the comparisons showing both similar and different responses in: 1) the number of influenced genes (table 1), 2) the number of influenced pathways (table 2) and 3) the direction and significance of the influence in selected pathways (table 3 and figure 16). The combination of exercise with myostatin/activin blocking activated (upregulated) almost double number of pathways (52 and 104, FDR < 0.05 and FDR < 0.25 respectively) comparing to exercise alone (19 and 50, FDR < 0.05 and FDR < 0.25 respectively), while showed minimized downregulation in the same comparison (1 to 17 for FDR < 0.05 and 6 to 42 for FDR < 0.25) (compare sActRIIB-Fc running vs. PBS to PBS running vs. PBS in table 2). In addition, this combination activated (upregulated) many more pathways (52 and 104, FDR < 0.05 and FDR < 0.25 respectively) than myostatin/activin blocking alone (1 and 6, FDR < 0.05 and FDR < 0.25 respectively), while showed minimized downregulation in the same comparison (1 to 11 for FDR < 0.05 and 6 to 27 for FDR < 0.25) (compare sActRIIB-Fc running vs. PBS to sActRIIB-Fc vs. PBS in table 2). These results in combination with the results of physical measurements (figures 11, 12, 13, 14 and 15, description in 8.1) and some important pathways (table 3, figure 16 and description in section 8.2) show the beneficial character of the combination of exercise with sActRIIB-Fc administration compared to each treatment alone. Furthermore, the administration of sActRIIB-Fc does not seem to prevent many of the beneficial effects of exercise in mdx mice (sActRIIB-Fc + Running vs. PBS compared to PBS + Running vs. PBS and both compared to PBS vs. Control), in contrast with the effects of simvastatin (treatment for cardiovascular disease risk in patients with metabolic syndrome), which has been reported to attenuated exercise training adaptations (Mikus et al. 2013). In total, the combination of exercise with myoastatin/activin inhibition resulted in improvements in: body mass, skeletal muscle mass and fat mass (by sActRIIB-Fc administration), physical activity levels and oxidative capacity (by exercise, as reported in a previous article of the same study, Hulmi et al. 2013b) and gene expression profile (as partly can be seen in table 3 by comparing the responses of

the selected pathways in each intervention alone or in combination with the comparison of dystrophic to healthy control muscle). In addition, the combination of the treatments seems to reverse or attenuate the negative effects of myostatin/activin blocking alone, at least to a number of important pathways related to aerobic metabolism and some other processes (table 3), without any obvious side effects.

9.6 Conclusions

As far as we are aware, a series of novelties are presented in the present thesis: the description of gene expression profiles of exercised mdx mice, the description of gene expression profiles of sActRIIB-Fc-administrated mdx mice, the comparison between those two profiles and the comparison between them and the healthy muscle profile.

The main finding of the present thesis was that the beneficial effects of exercise in dystrophic muscle induce transcription responses towards the gene expression profile of the healthy muscle. Furthermore, these benefits were observed even with the administration of sActRIIB-Fc. The most profound changes were observed in the aerobic profile pathways, where the shift in gene expression is presented in many different pathways related to aerobic metabolism and aerobic energy production.

Moreover, the elevated expressions in glutathione and drug metabolism by cytochrome 450 pathways indicate a possible role for oxidative damage in DMD pathology. These findings are opening the door to future research for studying the role of cytochrome P450 in oxidative damage and DMD pathology. Furthermore, exercise-increases in BCAAs degradation and lipid metabolism confirm a possible connection between them, as was recently suggested (Kainulainen et al. 2013), but further research is needed to explore this possible connection.

In conclusion, the combination of exercise with myostatin/activin inhibition combines the benefits of each intervention alone resulting in improvements in: body mass, skeletal muscle mass and fat mass (by sActRIIB-Fc administration), physical activity levels and oxidative capacity (by exercise) and gene expression responses. In addition, the combination of the treatments seems to reverse or attenuate the negative effects of myostatin/activin blocking alone, at least to a number of important pathways related to

aerobic metabolism and some other processes (table 3), without any obvious side effects.

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APPENDIX 1: Results Tables – Biological Processes gene sets (FDR < 0.25)

sActRIIB-Fc vs PBS Down-regulated pathways

None significant at FDR < 0.25

sActRIIB-Fc vs PBS Up-regulated pathways

None significant at FDR < 0.25

TABLE 4. PBS + Running vs PBS Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
SECRETORY_PATHWAY	73	-0.2527	-2.5246	0.0000	0.0557	0.0504
SECRETION_BY_CELL	99	-0.2044	-2.3799	0.0000	0.0826	0.1430
TISSUE_DEVELOPMENT	119	-0.1827	-2.3218	0.0000	0.0847	0.2110
ENZYME_LINKED_RECEPTOR_PROTEIN_SIGNALING_PATHWAY	125	-0.1681	-2.1810	0.0017	0.1659	0.4704
EXOCYTOSIS	20	-0.3866	-2.1045	0.0024	0.1899	0.6502
CELLULAR_POLYSACCHARIDE_METABOLIC_PROCESS	12	-0.4899	-2.0954	0.0043	0.2102	0.6724
VACUOLE_ORGANIZATION_AND_BIOGENESIS	12	-0.4841	-2.0429	0.0064	0.2293	0.7910

TABLE 5. PBS + Running vs PBS Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
ORGANIC_ACID_METABOLIC_PROCESS	154	0.1796	2.5973	0.0000	0.0272	0.0286
CARBOXYLIC_ACID_METABOLIC_PROCESS	152	0.1716	2.4641	0.0000	0.0395	0.0796
AMINO_ACID_CATABOLIC_PROCESS	21	0.4216	2.3130	0.0027	0.0654	0.2176
AEROBIC_RESPIRATION	12	0.5355	2.2445	0.0004	0.0788	0.338
NITROGEN_COMPOUND_CATABOLIC_PROCESS	25	0.3874	2.2943	0.0008	0.0809	0.2468
ELECTRON_TRANSPORT_GO_0006118	42	0.2806	2.1529	0.0012	0.1225	0.5434
AMINE_CATABOLIC_PROCESS	23	0.3595	2.0986	0.0029	0.1464	0.672
BILE_ACID_METABOLIC_PROCESS	9	0.5600	2.0768	0.0045	0.1552	0.7186
GENERATION_OF_PRECURSOR_METABOLITES_AND_ENERGY	107	0.1638	1.9788	0.0056	0.2172	0.9024
NEGATIVE_REGULATION_OF_MAP_KINASE_ACTIVITY	17	0.3986	1.9936	0.0054	0.2192	0.8808
INACTIVATION_OF_MAPK_ACTIVITY	14	0.4322	1.9583	0.0106	0.2361	0.9194

sActRIIB-Fc + Running vs PBS Down-regulated pathways

None significant at FDR < 0.25

TABLE 6. sActRIIB-Fc + Running vs PBS Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
LIPID_METABOLIC_PROCESS	269	0.1309	2.5111	0.0000	0.0150	0.0516
CARBOXYLIC_ACID_METABOLIC_PROCESS	152	0.1744	2.4958	0.0004	0.0166	0.0564
ORGANIC_ACID_METABOLIC_PROCESS	154	0.1756	2.5573	0.0000	0.0215	0.0346
CELLULAR_LIPID_METABOLIC_PROCESS	213	0.1528	2.5899	0.0004	0.0218	0.0260
COENZYME_METABOLIC_PROCESS	34	0.3327	2.3171	0.0000	0.0432	0.217
MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	73	0.2340	2.3096	0.0008	0.0488	0.2356
ELECTRON_TRANSPORT_GO_0006118	42	0.2806	2.1508	0.0028	0.1076	0.5434
AROMATIC_COMPOUND_METABOLIC_PROCESS	21	0.3874	2.1391	0.0020	0.1078	0.5738
BILE_ACID_METABOLIC_PROCESS	9	0.5714	2.0953	0.0028	0.1218	0.6766
STEROID_METABOLIC_PROCESS	55	0.2339	2.0386	0.0061	0.1347	0.8028
REGULATION_OF_MITOTIC_CELL_CYCLE	20	0.3843	2.0594	0.0047	0.1357	0.7594
G2_M_TRANSITION_OF_MITOTIC_CELL_CYCLE	11	0.5088	2.0376	0.0024	0.1412	0.7970
TRIACYLGLYCEROL_METABOLIC_PROCESS	8	0.5758	2.0084	0.0050	0.1468	0.8544
NEGATIVE_REGULATION_OF_SECRETION	10	0.5206	1.9975	0.0043	0.1503	0.8718
COFACTOR_METABOLIC_PROCESS	49	0.2367	1.9775	0.0060	0.1555	0.9016
NEGATIVE_REGULATION_OF_CATALYTIC_ACTIVITY	58	0.2170	1.9448	0.0079	0.1787	0.9360

sActRIIB-Fc + Running vs PBS + Running Down-regulated pathways

None significant at FDR < 0.25

TABLE 7. sActRIIB-Fc + Running vs PBS + Running Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
CELL_CYCLE_PHASE	150	0.1513	2.1339	0.0016	0.1771	0.5800
MITOTIC_CELL_CYCLE	133	0.1561	2.1280	0.0016	0.1772	0.5882
REGULATION_OF_CELL_PROLIFERATION	262	0.1126	2.1068	0.0028	0.1870	0.6404
DNA_DEPENDENT_DNA_REPLICATION	46	0.2736	2.1819	0.0008	0.2027	0.4700
CELL_CYCLE_PROCESS	168	0.1442	2.1827	0.0020	0.2057	0.4664
CELL_PROLIFERATION_GO_0008283	437	0.0920	2.2288	0.0008	0.2403	0.3768

TABLE 8. sActRIIB-Fc + Running vs sActRIIB-Fc Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
CELLULAR_LOCALIZATION	316	-0.1109	-2.2632	0.0008	0.1266	0.3122
ESTABLISHMENT_OF_CELLULAR_LOCALIZATION	300	-0.1165	-2.3157	0.0008	0.1385	0.2342
INTRACELLULAR_TRANSPORT	241	-0.1308	-2.3342	0.0000	0.1882	0.208
POST_GOLGI_VESICLE_MEDIATED_TRANSPORT	13	-0.4364	-1.8971	0.0073	0.2364	0.9732

TABLE 9. sActRIIB-Fc + Running vs sActRIIB-Fc Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
CARBOXYLIC_ACID_METABOLIC_PROCESS	152	0.2058	2.9545	0.0000	0.0006	0.0012
ORGANIC_ACID_METABOLIC_PROCESS	154	0.2119	3.0457	0.0000	0.0008	0.0008
NEGATIVE_REGULATION_OF_SECRETION	10	0.7362	2.8475	0.0000	0.0012	0.0036
MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	73	0.2729	2.7293	0.0004	0.0022	0.0082
LIPID_METABOLIC_PROCESS	269	0.1386	2.6148	0.0000	0.0044	0.0224
CELLULAR_LIPID_METABOLIC_PROCESS	213	0.1533	2.5769	0.0000	0.0056	0.0300
TRIACYLGLYCEROL_METABOLIC_PROCESS	8	0.6706	2.3264	0.0004	0.0333	0.2096
NITROGEN_COMPOUND_CATABOLIC_PROCESS	25	0.3855	2.2922	0.0020	0.0374	0.2620
COENZYME_METABOLIC_PROCESS	34	0.3288	2.2805	0.0008	0.0404	0.2784

MITOCHONDRION_ORGANIZATION_AND_BIOGENESIS	42	0.2873	2.1999	0.0020	0.0579	0.4306
CELLULAR_BIOSYNTHETIC_PROCESS	265	0.1144	2.1403	0.0029	0.0720	0.5690
AMINO_ACID_CATABOLIC_PROCESS	21	0.3873	2.1245	0.0041	0.0749	0.5990
COFACTOR_METABOLIC_PROCESS	49	0.2605	2.1288	0.0016	0.0754	0.5916
AMINE_CATABOLIC_PROCESS	23	0.3646	2.0973	0.0016	0.0809	0.6678
FATTY_ACID_METABOLIC_PROCESS	52	0.2466	2.0663	0.0040	0.0881	0.7364
RESPONSE_TO_VIRUS	40	0.2762	2.0649	0.0028	0.0936	0.7408
ELECTRON_TRANSPORT_GO_0006118	42	0.2651	2.0486	0.0035	0.0977	0.7716
RESPONSE_TO_ABIOTIC_STIMULUS	83	0.1911	2.0235	0.0062	0.1061	0.8210
REGULATION_OF_MITOTIC_CELL_CYCLE	20	0.3644	1.9394	0.0062	0.1586	0.9468
AROMATIC_COMPOUND_METABOLIC_PROCESS	21	0.3535	1.9158	0.0085	0.1771	0.9628
RESPONSE_TO_WOUNDING	165	0.1251	1.8587	0.0101	0.2058	0.9880
IMMUNE_EFFECTOR_PROCESS	30	0.2883	1.8680	0.0133	0.2128	0.9858
M_PHASE_OF_MITOTIC_CELL_CYCLE	76	0.1793	1.8333	0.0117	0.2396	0.9918
CASPASE_ACTIVATION	24	0.3118	1.8185	0.0132	0.2442	0.9950
RESPONSE_TO_OTHER_ORGANISM	62	0.1941	1.8042	0.0179	0.2463	0.9964
ISOPRENOID_METABOLIC_PROCESS	9	0.4948	1.8121	0.0184	0.2473	0.9954
REGULATION_OF_PROTEIN_SECRETION	17	0.3656	1.8090	0.0177	0.2493	0.9962

TABLE 10. PBS vs Control Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
CARBOXYLIC_ACID_METABOLIC_PROCESS	152	-0.2267	-3.2340	0.0000	0.0000	0.0000
ORGANIC_ACID_METABOLIC_PROCESS	154	-0.2350	-3.3734	0.0000	0.0000	0.0000
MONOCARBOXYLIC_ACID_METABOLIC_PROCESS	73	-0.2594	-2.6309	0.0000	0.0045	0.0218
LIPID_METABOLIC_PROCESS	269	-0.1303	-2.4969	0.0000	0.0099	0.0618
CATABOLIC_PROCESS	194	-0.1444	-2.3375	0.0004	0.0220	0.1862
CELLULAR_CATABOLIC_PROCESS	186	-0.1495	-2.3494	0.0000	0.0228	0.1752
NITROGEN_COMPOUND_CATABOLIC_PROCESS	25	-0.3768	-2.2275	0.0004	0.0373	0.3728
POSITIVE_REGULATION_OF_MAPKKK_CASCADE	8	-0.6335	-2.2178	0.0008	0.0416	0.3910
REGULATION_OF_CHROMOSOME_ORGANIZATION_AND_BIOGENESIS	10	-0.5615	-2.1555	0.0012	0.0511	0.5260
FATTY_ACID_METABOLIC_PROCESS	52	-0.2466	-2.1095	0.0028	0.0623	0.6336
CELLULAR_LIPID_METABOLIC_PROCESS	213	-0.1207	-2.0389	0.0052	0.0825	0.7928
FATTY_ACID_OXIDATION	18	-0.4001	-2.0234	0.0048	0.0840	0.8234
RESPONSE_TO_TEMPERATURE_STIMULUS	14	-0.4427	-2.0105	0.0072	0.0896	0.8456
AMINE_CATABOLIC_PROCESS	23	-0.3489	-2.0002	0.0053	0.0926	0.8534
AROMATIC_COMPOUND_METABOLIC_PROCESS	21	-0.3577	-1.9713	0.0084	0.0934	0.9100
LIPID_CATABOLIC_PROCESS	35	-0.2797	-1.9704	0.0045	0.0938	0.9054
AMINO_ACID_METABOLIC_PROCESS	68	-0.1984	-1.9478	0.0107	0.0972	0.9354
ENERGY_DERIVATION_BY_OXIDATION_OF_ORGANIC_COMPOUNDS	33	-0.2850	-1.9472	0.0094	0.0999	0.9352
RESPONSE_TO_ENDOGENOUS_STIMULUS	173	-0.1245	-1.9232	0.0079	0.1096	0.9544
LIPID_HOMEOSTASIS	12	-0.4403	-1.8736	0.0095	0.1289	0.9856
BILE_ACID_METABOLIC_PROCESS	9	-0.5011	-1.8582	0.0119	0.1338	0.9904
GLUCOSE_METABOLIC_PROCESS	24	-0.3109	-1.8295	0.0161	0.1486	0.9960
GLUCAN_METABOLIC_PROCESS	8	-0.5185	-1.8201	0.0159	0.1554	0.9978

RESPONSE_TO_CARBOHYDRATE_STIMULUS	10	-0.4685	-1.7886	0.0162	0.1649	0.9994
COENZYME_BIOSYNTHETIC_PROCESS	9	-0.4877	-1.7964	0.0150	0.1659	0.9986
FATTY_ACID_BETA_OXIDATION	11	-0.4454	-1.7834	0.0153	0.1668	0.9994
POLYSACCHARIDE_METABOLIC_PROCESS	14	-0.3794	-1.7321	0.0230	0.1950	1.0000
AMINO_ACID_CATABOLIC_PROCESS	21	-0.3158	-1.7352	0.0260	0.1951	1.0000
ACUTE_INFLAMMATORY_RESPONSE	11	-0.4309	-1.7243	0.0203	0.2005	1.0000
CELLULAR_LIPID_CATABOLIC_PROCESS	32	-0.2555	-1.7106	0.0311	0.2051	0.9998
AMINO_ACID_AND_DERIVATIVE_METABOLIC_PROCESS	87	-0.1569	-1.7071	0.0279	0.2112	1.0000
CELLULAR_POLYSACCHARIDE_METABOLIC_PROCESS	12	-0.4032	-1.6937	0.0297	0.2145	1.0000
RESPONSE_TO_HEAT	8	-0.4804	-1.6659	0.0303	0.2297	1.0000
CHROMATIN_MODIFICATION	44	-0.2104	-1.6611	0.0303	0.2414	1.0000
SODIUM_ION_TRANSPORT	17	-0.3316	-1.6497	0.0305	0.2460	1.0000

TABLE 11. PBS vs Control Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
ACTIN_CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	90	0.3104	3.5066	0.0000	0.0000	0.0000
ACTIN_FILAMENT_BASED_PROCESS	98	0.3137	3.6267	0.0000	0.0000	0.0000
CELL_PROLIFERATION_GO_0008283	437	0.1405	3.3302	0.0000	0.0000	0.0000
CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	176	0.2421	3.7247	0.0000	0.0000	0.0000
I_KAPPAB_KINASE_NF_KAPPAB_CASCADE	95	0.3013	3.4248	0.0000	0.0000	0.0000
IMMUNE_SYSTEM_PROCESS	266	0.1862	3.5081	0.0000	0.0000	0.0000

POSITIVE_REGULATION_OF_I_KAPPAB_KINASE_NF_KAPPAB_CASCADE	73	0.3517	3.5306	0.0000	0.0000	0.0000
REGULATION_OF_I_KAPPAB_KINASE_NF_KAPPAB_CASCADE	76	0.3324	3.3935	0.0000	0.0000	0.0000
SMALL_GTPASE_MEDIATED_SIGNAL_TRANSDUCTION	76	0.3480	3.5347	0.0000	0.0000	0.0000
PROTEIN_KINASE_CASCADE	247	0.1718	3.1207	0.0000	0.0000	0.0002
REGULATION_OF_SIGNAL_TRANSDUCTION	185	0.1935	3.0651	0.0000	0.0000	0.0006
REGULATION_OF_CELL_PROLIFERATION	262	0.1572	2.9526	0.0000	0.0000	0.0014
APOPTOSIS_GO	378	0.1332	2.9705	0.0000	0.0000	0.0012
PROGRAMMED_CELL_DEATH	379	0.1321	2.9653	0.0000	0.0000	0.0014
MEMBRANE_ORGANIZATION_AND_BIOGENESIS	119	0.2375	3.0398	0.0000	0.0000	0.0006
IMMUNE_RESPONSE	191	0.1826	2.8862	0.0000	0.0002	0.0034
DEFENSE_RESPONSE	206	0.1725	2.8871	0.0000	0.0002	0.0028
REGULATION_OF_APOPTOSIS	300	0.1420	2.8380	0.0000	0.0002	0.0050
REGULATION_OF_PROGRAMMED_CELL_DEATH	301	0.1406	2.8258	0.0000	0.0004	0.0060
REGULATION_OF_DEVELOPMENTAL_PROCESS	385	0.1262	2.8262	0.0000	0.0004	0.0058
RAS_PROTEIN_SIGNAL_TRANSDUCTION	59	0.3090	2.7751	0.0000	0.0006	0.0088
ACTIN_POLYMERIZATION_AND_OR_DEPOLYMERIZATION	20	0.4983	2.6762	0.0000	0.0010	0.0186
POSITIVE_REGULATION_OF_SIGNAL_TRANSDUCTION	106	0.2256	2.7156	0.0000	0.0010	0.0132
ANATOMICAL_STRUCTURE_MORPHOGENESIS	338	0.1278	2.6981	0.0000	0.0010	0.0148
CELL_MIGRATION	79	0.2475	2.5886	0.0004	0.0021	0.0366
PROTEOLYSIS	164	0.1702	2.5503	0.0000	0.0027	0.0504
RESPONSE_TO_EXTERNAL_STIMULUS	269	0.1334	2.5328	0.0008	0.0030	0.0564
REGULATION_OF_CELLULAR_COMPONENT_SIZE	11	0.6173	2.5010	0.0000	0.0034	0.0702
REGULATION_OF_ACTIN_POLYMERIZATION_AND_OR_DEPOLYMERIZATION	9	0.6778	2.4631	0.0000	0.0042	0.0896
VESICLE_MEDIATED_TRANSPORT	163	0.1665	2.4621	0.0000	0.0043	0.0916
NERVOUS_SYSTEM_DEVELOPMENT	342	0.1146	2.4530	0.0000	0.0044	0.0968

POSITIVE_REGULATION_OF_CELL_PROLIFERATION	125	0.1858	2.4512	0.0004	0.0046	0.0984
ORGANELLE_ORGANIZATION_AND_BIOGENESIS	399	0.1062	2.4349	0.0004	0.0049	0.1080
ESTABLISHMENT_OF_CELLULAR_LOCALIZATION	300	0.1207	2.4240	0.0004	0.0052	0.1164
CELLULAR_LOCALIZATION	316	0.1172	2.3989	0.0000	0.0058	0.1382
RHO_PROTEIN_SIGNAL_TRANSDUCTION	33	0.3492	2.3912	0.0000	0.0064	0.1528
NEGATIVE_REGULATION_OF_APOPTOSIS	133	0.1756	2.3811	0.0008	0.0065	0.1554
INTRACELLULAR_TRANSPORT	241	0.1313	2.3788	0.0012	0.0069	0.1688
CELL_MATRIX_ADHESION	34	0.3376	2.3463	0.0004	0.0077	0.1926
NEGATIVE_REGULATION_OF_PROGRAMMED_CELL_DEATH	134	0.1722	2.3282	0.0004	0.0086	0.2146
NEGATIVE_REGULATION_OF_DEVELOPMENTAL_PROCESS	175	0.1509	2.3126	0.0000	0.0092	0.2338
REGULATION_OF_ACTIN_FILAMENT_LENGTH	10	0.6000	2.3136	0.0008	0.0093	0.2372
GLYCOPROTEIN_METABOLIC_PROCESS	81	0.2143	2.2629	0.0016	0.0124	0.3242
CATION_HOMEOSTASIS	93	0.1965	2.2245	0.0004	0.0153	0.3934
CELL_SUBSTRATE_ADHESION	35	0.3165	2.2161	0.0008	0.0159	0.4096
REGULATION_OF_HEART_CONTRACTION	21	0.3996	2.2078	0.0020	0.0165	0.4266
INFLAMMATORY_RESPONSE	109	0.1800	2.2075	0.0012	0.0167	0.4312
VASCULATURE_DEVELOPMENT	52	0.2579	2.2062	0.0020	0.0168	0.4284
APOPTOTIC_MITOCHONDRIAL_CHANGES	10	0.5657	2.1804	0.0004	0.0187	0.4828
ORGAN_MORPHOGENESIS	131	0.1607	2.1418	0.0016	0.0233	0.5764
MITOTIC_CELL_CYCLE	133	0.1602	2.1343	0.0037	0.0241	0.5938
PROTEIN_AMINO_ACID_PHOSPHORYLATION	228	0.1212	2.1177	0.0021	0.0258	0.6310
CELLULAR_CATION_HOMEOSTASIS	90	0.1881	2.1149	0.0028	0.0260	0.6406
PHOSPHORYLATION	256	0.1132	2.1121	0.0032	0.0263	0.6448
RESPONSE_TO_WOUNDING	165	0.1413	2.1093	0.0016	0.0264	0.6516
CELL_PROJECTION_BIOGENESIS	23	0.3670	2.1089	0.0044	0.0266	0.6524

LOCOMOTORY_BEHAVIOR	73	0.2092	2.1065	0.0012	0.0272	0.6594
REGULATION_OF_TRANSCRIPTION	469	0.0847	2.0949	0.0020	0.0284	0.6876
CYTOKINESIS	13	0.4748	2.0802	0.0020	0.0294	0.7180
ACTIN_FILAMENT_BASED_MOVEMENT	8	0.6023	2.0680	0.0043	0.0312	0.7460
ANATOMICAL_STRUCTURE_FORMATION	51	0.2454	2.0658	0.0033	0.0312	0.7552
ACTIN_FILAMENT_POLYMERIZATION	11	0.5071	2.0500	0.0028	0.0331	0.7832
REGULATION_OF_PROTEIN_POLYMERIZATION	8	0.5880	2.0493	0.0041	0.0334	0.7862
CELL_CYCLE_GO_0007049	269	0.1075	2.0442	0.0080	0.0335	0.7962
POSITIVE_REGULATION_OF_CELL_CYCLE	16	0.4256	2.0405	0.0040	0.0342	0.7996
REGULATION_OF_PROTEIN_METABOLIC_PROCESS	148	0.1437	2.0425	0.0036	0.0342	0.7962
RECEPTOR_MEDIATED_ENDOCYTOSIS	31	0.3072	2.0401	0.0052	0.0345	0.7968
POSITIVE_REGULATION_OF_CELLULAR_PROTEIN_METABOLIC_PROCESS	63	0.2158	2.0345	0.0028	0.0351	0.8118
RESPONSE_TO_OTHER_ORGANISM	62	0.2201	2.0167	0.0028	0.0374	0.8462
CELL_CYCLE_PHASE	150	0.1413	2.0170	0.0040	0.0382	0.8344
REGULATION_OF_ANATOMICAL_STRUCTURE_MORPHOGENESIS	22	0.3551	2.0011	0.0061	0.0399	0.8736
POSITIVE_REGULATION_OF_CATALYTIC_ACTIVITY	139	0.1452	1.9785	0.0075	0.0433	0.9062
ANTI_APOPTOSIS	101	0.1696	1.9749	0.0041	0.0441	0.9112
CELL_CELL_ADHESION	78	0.1908	1.9726	0.0095	0.0443	0.9118
NEURON_DEVELOPMENT	55	0.2275	1.9762	0.0051	0.0445	0.9082
ANGIOGENESIS	45	0.2480	1.9731	0.0044	0.0448	0.9024
GENERATION_OF_NEURONS	76	0.1936	1.9724	0.0080	0.0457	0.9084
REGULATION_OF_BIOLOGICAL_QUALITY	356	0.0907	1.9696	0.0056	0.0458	0.9156
CELL_CYCLE_PROCESS	168	0.1298	1.9605	0.0092	0.0464	0.9292
REGULATION_OF_PHOSPHORYLATION	44	0.2482	1.9458	0.0076	0.0499	0.9374
CASPASE_ACTIVATION	24	0.3322	1.9398	0.0117	0.0510	0.9470

CYTOKINE_BIOSYNTHETIC_PROCESS	36	0.2709	1.9275	0.0056	0.0529	0.9566
REGULATION_OF_ANGIOGENESIS	24	0.3278	1.9224	0.0107	0.0530	0.9614
POSITIVE_REGULATION_OF_JNK_ACTIVITY	12	0.4613	1.9151	0.0103	0.0544	0.9658
ESTABLISHMENT_OF_ORGANELLE_LOCALIZATION	14	0.4237	1.9171	0.0084	0.0551	0.9654
CHEMICAL_HOMEOSTASIS	133	0.1416	1.9114	0.0065	0.0552	0.9680
POST_TRANSLATIONAL_PROTEIN_MODIFICATION	403	0.0829	1.9125	0.0075	0.0555	0.9688
SKELETAL_DEVELOPMENT	89	0.1725	1.9128	0.0093	0.0555	0.9696
ION_HOMEOSTASIS	111	0.1554	1.9068	0.0119	0.0566	0.9702
RESPONSE_TO_BIOTIC_STIMULUS	91	0.1692	1.9016	0.0118	0.0568	0.9740
POSITIVE_REGULATION_OF_PROTEIN_METABOLIC_PROCESS	65	0.1997	1.8940	0.0102	0.0578	0.9796
LEUKOCYTE_CHEMOTAXIS	9	0.5163	1.8886	0.0093	0.0590	0.9810
LEUKOCYTE_ACTIVATION	54	0.2174	1.8864	0.0125	0.0599	0.9814
PROTEIN_POLYMERIZATION	15	0.3981	1.8798	0.0110	0.0604	0.9840
POST_GOLGI_VESICLE_MEDIATED_TRANSPORT	13	0.4303	1.8776	0.0133	0.0606	0.9844
LEUKOCYTE_MIGRATION	11	0.4658	1.8780	0.0102	0.0610	0.9844
REGULATION_OF_MOLECULAR_FUNCTION	278	0.0973	1.8816	0.0077	0.0611	0.9832
POSITIVE_REGULATION_OF_TRANSFERASE_ACTIVITY	71	0.1893	1.8728	0.0133	0.0620	0.9852
CALCIUM_MEDIATED_SIGNALING	13	0.4283	1.8683	0.0134	0.0638	0.9868
LEUKOCYTE_DIFFERENTIATION	31	0.2794	1.8580	0.0096	0.0647	0.9908
APOPTOTIC_PROGRAM	53	0.2153	1.8513	0.0094	0.0661	0.9898
G2_M_TRANSITION_OF_MITOTIC_CELL_CYCLE	11	0.4543	1.8358	0.0172	0.0684	0.9956
REGULATION_OF_CELL_CELL_ADHESION	9	0.5055	1.8376	0.0121	0.0690	0.9934
CYTOKINE_METABOLIC_PROCESS	37	0.2513	1.8350	0.0103	0.0696	0.9962
FOCAL_ADHESION_FORMATION	9	0.4997	1.8327	0.0132	0.0698	0.9960
GLYCOPROTEIN_CATABOLIC_PROCESS	11	0.4585	1.8331	0.0140	0.0705	0.9962

SPHINGOID_METABOLIC_PROCESS	11	0.4510	1.8313	0.0138	0.0712	0.9976
REGULATION_OF_CELLULAR_PROTEIN_METABOLIC_PROCESS	139	0.1328	1.8198	0.0112	0.0722	0.9960
GLYCOPROTEIN_BIOSYNTHETIC_PROCESS	67	0.1888	1.8195	0.0148	0.0726	0.9972
INTERPHASE	58	0.2012	1.8213	0.0158	0.0730	0.9970
MYELOID_LEUKOCYTE_DIFFERENTIATION	13	0.4192	1.8158	0.0111	0.0737	0.9990
PROTEIN_AMINO_ACID_N_LINKED_GLYCOSYLATION	28	0.2862	1.8135	0.0143	0.0743	0.9988
CELLULAR_RESPONSE_TO_EXTRACELLULAR_STIMULUS	11	0.4478	1.8086	0.0141	0.0743	0.9986
NEURON_DIFFERENTIATION	70	0.1847	1.8080	0.0170	0.0750	0.9986
CELL_DIVISION	15	0.3927	1.8105	0.0144	0.0751	0.9964
INTERFERON_GAMMA_BIOSYNTHETIC_PROCESS	11	0.4479	1.8047	0.0107	0.0761	0.9994
DNA_MODIFICATION	9	0.4903	1.8002	0.0150	0.0765	0.9974
POSITIVE_REGULATION_OF_LYMPHOCYTE_ACTIVATION	19	0.3439	1.7979	0.0146	0.0766	0.9986
AMINE_TRANSPORT	34	0.2611	1.7986	0.0176	0.0769	0.9984
REGULATION_OF_CELL_MORPHOGENESIS	11	0.4459	1.8035	0.0155	0.0773	0.9990
NEGATIVE_REGULATION_OF_CELL_PROLIFERATION	132	0.1338	1.7923	0.0183	0.0783	0.9992
REGULATION_OF_HYDROLASE_ACTIVITY	67	0.1865	1.7917	0.0140	0.0789	0.9998
MICROTUBULE_BASED_PROCESS	67	0.1878	1.7953	0.0184	0.0791	0.9992
RESPONSE_TO_VIRUS	40	0.2413	1.7893	0.0129	0.0793	0.9994
T_CELL_ACTIVATION	35	0.2564	1.7865	0.0188	0.0798	0.9996
NEGATIVE_REGULATION_OF_GROWTH	33	0.2642	1.7854	0.0194	0.0803	0.9994
POSITIVE_REGULATION_OF_T_CELL_ACTIVATION	17	0.3532	1.7520	0.0213	0.0901	1.0000
IMMUNE_SYSTEM_DEVELOPMENT	66	0.1830	1.7499	0.0190	0.0905	0.9998
PEPTIDYL_TYROSINE_MODIFICATION	25	0.2902	1.7517	0.0204	0.0906	0.9998
REGULATION_OF_TRANSCRIPTIONDNA_DEPENDENT	374	0.0773	1.7399	0.0267	0.0947	1.0000
HEMOPOIETIC_OR_LYMPHOID_ORGAN_DEVELOPMENT	63	0.1851	1.7330	0.0215	0.0971	1.0000

ICOSANOID_METABOLIC_PROCESS	10	0.4467	1.7305	0.0236	0.0987	0.9996
LYMPHOCYTE_ACTIVATION	49	0.2090	1.7217	0.0233	0.1011	1.0000
DETECTION_OF_EXTERNAL_STIMULUS	20	0.3174	1.7177	0.0251	0.1026	1.0000
MEIOTIC_CELL_CYCLE	33	0.2515	1.7098	0.0301	0.1052	1.0000
CELLULAR_DEFENSE_RESPONSE	40	0.2291	1.7092	0.0211	0.1053	1.0000
CELL_ACTIVATION	60	0.1875	1.7115	0.0264	0.1054	1.0000
POSITIVE_REGULATION_OF_PHOSPHATE_METABOLIC_PROCESS	25	0.2853	1.7004	0.0276	0.1091	1.0000
POSITIVE_REGULATION_OF_CASPASE_ACTIVITY	27	0.2720	1.6938	0.0217	0.1107	1.0000
PROTEIN_COMPLEX_ASSEMBLY	146	0.1190	1.6913	0.0267	0.1120	1.0000
CHROMOSOME_SEGREGATION	25	0.2816	1.6894	0.0304	0.1128	1.0000
REGULATION_OF_CYTOKINE_BIOSYNTHETIC_PROCESS	33	0.2479	1.6863	0.0274	0.1140	1.0000
REGULATION_OF_T_CELL_ACTIVATION	23	0.2973	1.6834	0.0298	0.1148	1.0000
REGULATION_OF_GTPASE_ACTIVITY	13	0.3833	1.6814	0.0287	0.1152	1.0000
LYSOSOME_ORGANIZATION_AND_BIOGENESIS	11	0.4128	1.6723	0.0331	0.1190	1.0000
NEURITE_DEVELOPMENT	48	0.2048	1.6712	0.0335	0.1193	1.0000
REGULATION_OF_PH	12	0.3955	1.6646	0.0312	0.1217	1.0000
POSITIVE_REGULATION_OF_PHOSPHORYLATION	23	0.2905	1.6661	0.0371	0.1222	1.0000
INTERPHASE_OF_MITOTIC_CELL_CYCLE	53	0.1927	1.6586	0.0357	0.1234	1.0000
HEMOPOIESIS	61	0.1812	1.6543	0.0314	0.1253	1.0000
REGULATION_OF_CELL_CYCLE	155	0.1140	1.6544	0.0333	0.1254	1.0000
RESPONSE_TO_LIGHT_STIMULUS	45	0.2080	1.6444	0.0373	0.1289	1.0000
REGULATION_OF_RNA_METABOLIC_PROCESS	379	0.0734	1.6419	0.0365	0.1298	1.0000
REGULATION_OF_CELL_SHAPE	10	0.4276	1.6415	0.0412	0.1301	1.0000
CELL_CYCLE_ARREST_GO_0007050	49	0.1979	1.6373	0.0423	0.1305	1.0000
NEUROGENESIS	84	0.1515	1.6380	0.0394	0.1307	1.0000

SISTER_CHROMATID_SEGREGATION	12	0.3880	1.6303	0.0387	0.1326	1.0000
HOMEOSTATIC_PROCESS	178	0.1068	1.6352	0.0418	0.1326	1.0000
DETECTION_OF_STIMULUS	40	0.2173	1.6309	0.0394	0.1332	1.0000
MITOTIC_SISTER_CHROMATID_SEGREGATION	12	0.3880	1.6274	0.0390	0.1347	1.0000
POSITIVE_REGULATION_OF_DEVELOPMENTAL_PROCESS	194	0.1002	1.6226	0.0403	0.1365	1.0000
REGULATION_OF_CELL_GROWTH	36	0.2266	1.6210	0.0383	0.1373	1.0000
REGULATION_OF_CELL_MIGRATION	26	0.2663	1.6183	0.0397	0.1378	1.0000
REGULATION_OF_CYTOSKELETON_ORGANIZATION_AND_BIOGENESIS	23	0.2792	1.6168	0.0361	0.1384	1.0000
RESPONSE_TO_STRESS	436	0.0669	1.6124	0.0406	0.1392	1.0000
REGULATION_OF_TRANSCRIPTION_FROM_RNA_POLYMERASE_II_PROMOTER	235	0.0904	1.6126	0.0436	0.1399	1.0000
REGULATION_OF_JNK_ACTIVITY	14	0.3541	1.6054	0.0426	0.1418	1.0000
OLIGOSACCHARIDE_METABOLIC_PROCESS	11	0.3987	1.6038	0.0410	0.1427	1.0000
REGULATION_OF_PROTEIN_AMINO_ACID_PHOSPHORYLATION	26	0.2638	1.6021	0.0423	0.1433	1.0000
ACTIVATION_OF_JNK_ACTIVITY	10	0.4112	1.5935	0.0417	0.1461	1.0000
VACUOLE_ORGANIZATION_AND_BIOGENESIS	12	0.3783	1.5909	0.0511	0.1470	1.0000
MITOSIS	75	0.1563	1.5900	0.0455	0.1471	1.0000
DETECTION_OF_STIMULUS_INVOLVED_IN_SENSORY_PERCEPTION	18	0.3118	1.5898	0.0561	0.1475	1.0000
POSITIVE_REGULATION_OF_HYDROLASE_ACTIVITY	47	0.1948	1.5884	0.0454	0.1480	1.0000
MACROMOLECULE_LOCALIZATION	202	0.0950	1.5871	0.0488	0.1485	1.0000
POSITIVE_REGULATION_OF_PROTEIN_AMINO_ACID_PHOSPHORYLATION	17	0.3134	1.5862	0.0468	0.1490	1.0000
AMYLOID_PRECURSOR_PROTEIN_METABOLIC_PROCESS	10	0.4134	1.5827	0.0456	0.1494	1.0000
INTERFERON_GAMMA_PRODUCTION	13	0.3640	1.5804	0.0494	0.1501	1.0000
MEIOSIS_I	19	0.3028	1.5761	0.0576	0.1517	1.0000
BRAIN_DEVELOPMENT	44	0.1999	1.5742	0.0503	0.1527	1.0000
REGULATION_OF_LYMPHOCYTE_ACTIVATION	28	0.2491	1.5718	0.0533	0.1528	1.0000

REGULATION_OF_TRANSPORT	57	0.1774	1.5663	0.0520	0.1542	1.0000
POSITIVE_REGULATION_OF_EPITHELIAL_CELL_PROLIFERATION	9	0.4282	1.5660	0.0498	0.1545	1.0000
REGULATION_OF_KINASE_ACTIVITY	131	0.1170	1.5642	0.0510	0.1555	1.0000
REGULATION_OF_PROTEIN_KINASE_ACTIVITY	129	0.1185	1.5624	0.0527	0.1557	1.0000
REGULATION_OF_CATALYTIC_ACTIVITY	232	0.0888	1.5614	0.0456	0.1560	1.0000
NEGATIVE_REGULATION_OF_ANGIOGENESIS	12	0.3693	1.5603	0.0547	0.1561	1.0000
PROTEIN_LOCALIZATION	183	0.0985	1.5603	0.0597	0.1567	1.0000
CELL_CELL_SIGNALING	348	0.0725	1.5587	0.0503	0.1568	1.0000
POSITIVE_REGULATION_OF_TRANSLATION	29	0.2434	1.5614	0.0592	0.1574	1.0000
REGULATION_OF_TRANSFERASE_ACTIVITY	135	0.1142	1.5554	0.0576	0.1577	1.0000
CERAMIDE_METABOLIC_PROCESS	10	0.4055	1.5557	0.0542	0.1578	1.0000
MUSCLE_DEVELOPMENT	86	0.1445	1.5539	0.0498	0.1583	1.0000
MITOTIC_CELL_CYCLE_CHECKPOINT	18	0.3018	1.5529	0.0595	0.1587	1.0000
GOLGI_VESICLE_TRANSPORT	44	0.1986	1.5512	0.0501	0.1587	1.0000
M_PHASE_OF_MITOTIC_CELL_CYCLE	76	0.1533	1.5511	0.0550	0.1598	1.0000
REGULATION_OF_INTERFERON_GAMMA_BIOSYNTHETIC_PROCESS	10	0.4024	1.5444	0.0573	0.1606	1.0000
CENTRAL_NERVOUS_SYSTEM_DEVELOPMENT	109	0.1276	1.5486	0.0546	0.1608	1.0000
PEPTIDYL_AMINO_ACID_MODIFICATION	50	0.1854	1.5442	0.0548	0.1610	1.0000
POSITIVE_REGULATION_OF_CELLULAR_METABOLIC_PROCESS	191	0.0959	1.5399	0.0569	0.1620	1.0000
SPHINGOLIPID_METABOLIC_PROCESS	27	0.2494	1.5423	0.0609	0.1622	1.0000
PEPTIDYL_TYROSINE_PHOSPHORYLATION	23	0.2666	1.5435	0.0573	0.1626	1.0000
LYSOSOMAL_TRANSPORT	9	0.4139	1.5392	0.0626	0.1626	1.0000
PROTEOGLYCAN_METABOLIC_PROCESS	17	0.3131	1.5424	0.0738	0.1631	1.0000
TISSUE_DEVELOPMENT	119	0.1216	1.5404	0.0521	0.1633	1.0000
BEHAVIOR	124	0.1174	1.5346	0.0612	0.1641	1.0000

HOMOPHILIC_CELL_ADHESION	15	0.3279	1.5323	0.0602	0.1644	1.0000
REGULATION_OF_T_CELL_PROLIFERATION	13	0.3507	1.5277	0.0645	0.1664	1.0000
REGULATION_OF_GROWTH	47	0.1879	1.5248	0.0619	0.1669	1.0000
REGULATION_OF_ENDOTHELIAL_CELL_PROLIFERATION	9	0.4098	1.5199	0.0702	0.1709	1.0000
MESODERM_DEVELOPMENT	17	0.3086	1.5205	0.0624	0.1711	1.0000
ORGANELLE_LOCALIZATION	20	0.2826	1.5112	0.0660	0.1743	1.0000
INTERLEUKIN_8_BIOSYNTHETIC_PROCESS	9	0.4156	1.5083	0.0689	0.1758	1.0000
REGULATION_OF_DNA_BINDING	43	0.1919	1.5032	0.0656	0.1772	1.0000
CELLULAR_HOMEOSTASIS	126	0.1148	1.5022	0.0688	0.1772	1.0000
HUMORAL_IMMUNE_RESPONSE	29	0.2325	1.5008	0.0712	0.1788	1.0000
MONOVALENT_INORGANIC_CATION_HOMEOSTASIS	13	0.3448	1.4974	0.0843	0.1803	1.0000
PEPTIDE_METABOLIC_PROCESS	8	0.4371	1.4953	0.0750	0.1818	1.0000
POSITIVE_REGULATION_OF_CYTOKINE_BIOSYNTHETIC_PROCESS	22	0.2674	1.4916	0.0764	0.1819	1.0000
REGULATION_OF_CELL_ADHESION	30	0.2309	1.4955	0.0770	0.1827	1.0000
G1_PHASE	13	0.3389	1.4900	0.0711	0.1847	1.0000
NEGATIVE_REGULATION_OF_TRANSCRIPTION	151	0.1039	1.4845	0.0670	0.1871	1.0000
REGULATION_OF_NEUROGENESIS	14	0.3284	1.4826	0.0757	0.1873	1.0000
VIRAL_REPRODUCTIVE_PROCESS	27	0.2409	1.4826	0.0758	0.1878	1.0000
ESTABLISHMENT_OF_PROTEIN_LOCALIZATION	163	0.1002	1.4801	0.0799	0.1880	1.0000
POSITIVE_REGULATION_OF_MAP_KINASE_ACTIVITY	37	0.2062	1.4794	0.0772	0.1891	1.0000
LYMPHOCYTE_DIFFERENTIATION	21	0.2700	1.4796	0.0726	0.1900	1.0000
DETECTION_OF_BIOTIC_STIMULUS	7	0.4502	1.4744	0.0834	0.1923	1.0000
VIRAL_INFECTIOUS_CYCLE	23	0.2554	1.4739	0.0842	0.1923	1.0000
PHOSPHOINOSITIDE_BIOSYNTHETIC_PROCESS	20	0.2750	1.4711	0.0816	0.1944	1.0000
CYTOSKELETON_DEPENDENT_INTRACELLULAR_TRANSPORT	21	0.2668	1.4577	0.0883	0.2006	1.0000

REGULATION_OF_MITOSIS	37	0.2011	1.4557	0.0866	0.2023	1.0000
REGULATION_OF_CELLULAR_PH	9	0.3954	1.4532	0.0857	0.2041	1.0000
MEIOTIC_RECOMBINATION	17	0.2904	1.4527	0.0870	0.2043	1.0000
REGULATION_OF_TRANSCRIPTION_FACTOR_ACTIVITY	37	0.2011	1.4492	0.0828	0.2056	1.0000
ESTABLISHMENT_OF_VESICLE_LOCALIZATION	8	0.4140	1.4460	0.0922	0.2064	1.0000
SYSTEM_PROCESS	490	0.0576	1.4455	0.0908	0.2074	1.0000
MYELOID_CELL_DIFFERENTIATION	30	0.2226	1.4396	0.0869	0.2094	1.0000
G1_S_TRANSITION_OF_MITOTIC_CELL_CYCLE	24	0.2467	1.4424	0.0965	0.2102	1.0000
VITAMIN_TRANSPORT	9	0.3874	1.4405	0.0879	0.2102	1.0000
REGULATION_OF_VIRAL_REPRODUCTION	8	0.4138	1.4342	0.0908	0.2141	1.0000
NEUTRAL_AMINO_ACID_TRANSPORT	8	0.4114	1.4332	0.1051	0.2142	1.0000
G_PROTEIN_COUPLED_RECEPTOR_PROTEIN_SIGNALING_PATHWAY	284	0.0725	1.4305	0.0881	0.2160	1.0000
NEGATIVE_REGULATION_OF_METABOLIC_PROCESS	211	0.0857	1.4277	0.0993	0.2177	1.0000
RIBONUCLEOTIDE_METABOLIC_PROCESS	14	0.3168	1.4192	0.1089	0.2231	1.0000
MULTI_ORGANISM_PROCESS	120	0.1112	1.4198	0.1073	0.2232	1.0000
COFACTOR_TRANSPORT	9	0.3874	1.4194	0.1080	0.2233	1.0000
JAK_STAT_CASCADE	28	0.2246	1.4164	0.1016	0.2242	1.0000
M_PHASE	103	0.1180	1.4154	0.0993	0.2248	1.0000
POSITIVE_REGULATION_OF_T_CELL_PROLIFERATION	10	0.3648	1.4102	0.1044	0.2282	1.0000
AMINO_ACID_TRANSPORT	22	0.2498	1.4095	0.1014	0.2292	1.0000
NEGATIVE_REGULATION_OF_CELL_ADHESION	16	0.2902	1.4071	0.1051	0.2302	1.0000
NUCLEAR_IMPORT	42	0.1828	1.4064	0.1049	0.2312	1.0000
NEGATIVE_REGULATION_OF_CELLULAR_METABOLIC_PROCESS	209	0.0839	1.4031	0.1115	0.2326	1.0000
CELL_CYCLE_CHECKPOINT_GO_0000075	39	0.1873	1.3977	0.1030	0.2358	1.0000
DETECTION_OF_ABIOTIC_STIMULUS	19	0.2685	1.3963	0.1096	0.2374	1.0000

REGULATION_OF_MAP_KINASE_ACTIVITY	57	0.1551	1.3885	0.1129	0.2419	1.0000
TISSUE_MORPHOGENESIS	12	0.3287	1.3869	0.1118	0.2426	1.0000
ECTODERM_DEVELOPMENT	70	0.1391	1.3839	0.1150	0.2457	1.0000
RESPONSE_TO_CHEMICAL_STIMULUS	252	0.0752	1.3835	0.1209	0.2461	1.0000
POSITIVE_REGULATION_OF_METABOLIC_PROCESS	197	0.0843	1.3801	0.1175	0.2478	1.0000
REGULATION_OF_MUSCLE_CONTRACTION	14	0.3056	1.3747	0.1242	0.2498	1.0000

APPENDIX 2: Results Tables – Canonical Pathways gene sets (FDR < 0.25)

TABLE 12. sActRIIB-Fc vs PBS Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_PACKAGING_OF_TELOMERE_ENDS	35	-0.4595	-3.2516	0.0000	0.0000	0
REACTOME_RNA_POLYMERASE_I_PROMOTER_CLEARANCE	60	-0.3445	-3.1547	0.0000	0.0000	0
REACTOME_RNA_POLYMERASE_I_PROMOTER_OPENING	41	-0.4142	-3.1606	0.0000	0.0000	0
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	82	-0.2681	-2.8521	0.0000	0.0000	0.0018
KEGG_OXIDATIVE_PHOSPHORYLATION	101	-0.2042	-2.3800	0.0004	0.0181	0.1538
REACTOME_RNA_POLYMERASE_I_III_AND_MITOCHONDRIAL_TRANSCRIPTION	95	-0.2088	-2.3672	0.0000	0.0184	0.1768
BIOCARTA_ERYTH_PATHWAY	14	-0.5299	-2.3916	0.0000	0.0189	0.1542
REACTOME_TELOMERE_MAINTENANCE	63	-0.2528	-2.3529	0.0012	0.0201	0.1892
KEGG_GLYCEROLIPID_METABOLISM	42	-0.2958	-2.2605	0.0012	0.0305	0.3408
REACTOME_DIABETES_PATHWAYS	310	-0.1096	-2.2347	0.0012	0.0329	0.3924
REACTOME_ELECTRON_TRANSPORT_CHAIN	58	-0.2457	-2.2067	0.0012	0.0384	0.4516
KEGG_HUNTINGTONS_DISEASE	142	-0.1428	-1.9723	0.0053	0.1434	0.9260
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	249	-0.1055	-1.9278	0.0064	0.1606	0.965
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	71	-0.1925	-1.9185	0.0068	0.1661	0.9736
REACTOME_REGULATION_OF_INSULIN_SECRETION	177	-0.1252	-1.9363	0.0084	0.1692	0.9582

BIOCARTA_STEM_PATHWAY	13	-0.4332	-1.8917	0.0086	0.1771	0.9864
REACTOME_FORMATION_OF_A_POOL_OF_FREE_40S_SUBUNITS	66	-0.1943	-1.8700	0.0111	0.1818	0.991
KEGG_RIBOSOME	61	-0.1983	-1.8323	0.0142	0.2000	0.9968
REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT	86	-0.1702	-1.8385	0.0156	0.2023	0.9958
REACTOME_GLUCOSE_REGULATION_OF_INSULIN_SECRETION	130	-0.1360	-1.8356	0.0109	0.2039	0.9958
KEGG_PARKINSONS_DISEASE	101	-0.1534	-1.8053	0.0152	0.2063	0.9988
KEGG_HEMATOPOIETIC_CELL_LINEAGE	63	-0.1925	-1.7962	0.0149	0.2067	0.999
REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1_ALPHA	9	-0.4973	-1.8089	0.0179	0.2087	0.9988
KEGG_ALZHEIMERS_DISEASE	139	-0.1298	-1.7996	0.0172	0.2094	0.9992
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	45	-0.2247	-1.7843	0.0204	0.2105	1
REACTOME_VIRAL_MRNA_TRANSLATION	59	-0.1932	-1.7397	0.0236	0.2454	1

TABLE 13. sActRIIB-Fc vs PBS Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
KEGG_PATHWAYS_IN_CANCER	293	0.1322	2.6280	0.0000	0.0357	0.0264
REACTOME_DOWNSTREAM_SIGNALING_OF_ACTIVATED_FGFR	40	0.2625	1.9664	0.0061	0.2359	0.9354
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	109	0.1633	1.9703	0.0041	0.2398	0.9310
WNT_SIGNALING	86	0.1764	1.9288	0.0090	0.2474	0.9674

TABLE 14. PBS + Running vs PBS Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_INSULIN_SYNTHESIS_AND_SECRETION	101	-0.2742	-3.2554	0.0000	0.0000	0.0000
REACTOME_FORMATION_OF_A_POOL_OF_FREE_40S_SUBUNITS	66	-0.3185	-3.0450	0.0000	0.0002	0.0006
KEGG_RIBOSOME	61	-0.3149	-2.9012	0.0000	0.0002	0.0020
REACTOME_GTP_HYDROLYSIS_AND_JOINING_OF_THE_60S_RIBOSOMAL_SUBUNIT	76	-0.2846	-2.8820	0.0000	0.0004	0.0020
REACTOME_VIRAL_MRNA_TRANSLATION	59	-0.3235	-2.9309	0.0000	0.0004	0.0018
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	71	-0.3074	-3.0433	0.0000	0.0006	0.0008
REACTOME_PEPTIDE_CHAIN_ELONGATION	59	-0.3235	-2.8830	0.0000	0.0006	0.0026
REACTOME_TRANSLATION	89	-0.2577	-2.8402	0.0000	0.0006	0.0032
REACTOME_METABOLISM_OF_PROTEINS	170	-0.1680	-2.5400	0.0004	0.0061	0.0510
REACTOME_PACKAGING_OF_TELOMERE_ENDS	35	-0.3497	-2.4318	0.0008	0.0121	0.1172
REACTOME_INFLUENZA_LIFE_CYCLE	104	-0.2024	-2.4235	0.0000	0.0128	0.1242
REACTOME_TELOMERE_MAINTENANCE	63	-0.2578	-2.3860	0.0000	0.0154	0.1614
REACTOME_RNA_POLYMERASE_I_PROMOTER_OPENING	41	-0.3144	-2.3794	0.0004	0.0159	0.1672
REACTOME_MITOTIC_M_M_G1_PHASES	143	-0.1599	-2.2536	0.0008	0.0323	0.3572
REACTOME_REGULATION_OF_GENE_EXPRESSION_IN_BETA_CELLS	74	-0.2228	-2.2448	0.0016	0.0349	0.3820
REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1_ALPHA	9	-0.5960	-2.1971	0.0012	0.0429	0.4802
REACTOME_CELL_CYCLE_MITOTIC	277	-0.1139	-2.1716	0.0012	0.0467	0.5352
REACTOME_MITOTIC_PROMETAPHASE	79	-0.2063	-2.1460	0.0008	0.0534	0.5960
KEGG_LYSOSOME	111	-0.1704	-2.1108	0.0020	0.0644	0.6740
BIOCARTA_PROTEASOME_PATHWAY	19	-0.3968	-2.0899	0.0020	0.0699	0.7202
REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT	86	-0.1886	-2.0656	0.0020	0.0746	0.7762
KEGG_PENTOSE_AND_GLUCURONATE_INTERCONVERSIONS	10	-0.5350	-2.0559	0.0029	0.0779	0.7950

REACTOME_RNA_POLYMERASE_I_PROMOTER_CLEARANCE	60	-0.2208	-2.0398	0.0036	0.0837	0.8284
REACTOME_TRANSLATION_INITIATION_COMPLEX_FORMATION	43	-0.2580	-2.0258	0.0040	0.0886	0.8488
KEGG_PROTEASOME	41	-0.2618	-1.9860	0.0045	0.1070	0.9166
KEGG_NON_SMALL_CELL_LUNG_CANCER	47	-0.2374	-1.9480	0.0048	0.1205	0.9526
REACTOME_AXON_GUIDANCE	131	-0.1464	-1.9447	0.0089	0.1246	0.9590
BIOCARTA_MPR_PATHWAY	29	-0.3007	-1.9320	0.0104	0.1310	0.9646
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	82	-0.1793	-1.9097	0.0090	0.1414	0.9766
ST_B_CELL_ANTIGEN_RECEPTOR	33	-0.2780	-1.8831	0.0149	0.1572	0.9854
REACTOME_SIGNALING_BY_WNT	55	-0.2139	-1.8719	0.0103	0.1621	0.9916
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	55	-0.2139	-1.8562	0.0142	0.1710	0.9942
REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	36	-0.2592	-1.8601	0.0145	0.1732	0.9920
REACTOME_P53_INDEPENDENT_DNA_DAMAGE_RESPONSE	42	-0.2402	-1.8429	0.0135	0.1771	0.9962
REACTOME_CDC20_PHOSPHO_APC_MEDIATED_DEGRADATION_OF_CYCLIN_A	62	-0.1981	-1.8356	0.0126	0.1829	0.9958
REACTOME_SCF_SKP2_MEDIATED_DEGRADATION_OF_P27_P21	50	-0.2193	-1.8143	0.0130	0.1930	0.9982
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	45	-0.2260	-1.7915	0.0175	0.2100	0.9988
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	44	-0.2263	-1.7803	0.0227	0.2163	0.9992
REACTOME_UNFOLDED_PROTEIN_RESPONSE	16	-0.3703	-1.7776	0.0192	0.2211	0.9992
REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE	45	-0.2260	-1.7756	0.0174	0.2217	0.9994
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1_APC	56	-0.1986	-1.7456	0.0212	0.2459	0.9994
REACTOME_RNA_POL_II_CTD_PHOSPHORYLATION_AND_INTERACTION_WITH_CE	21	-0.3195	-1.7385	0.0207	0.2462	1.0000

TABLE 15. PBS + Running vs PBS Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	60	0.3456	3.1492	0.0000	0.0002	0.0002
REACTOME_ELECTRON_TRANSPORT_CHAIN	58	0.3313	2.9380	0.0000	0.0004	0.0014
REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS	195	0.1768	2.8489	0.0000	0.0010	0.0034
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	39	0.3837	2.8398	0.0000	0.0010	0.0036
REACTOME_GLUCOSE_REGULATION_OF_INSULIN_SECRETION	130	0.2042	2.7347	0.0000	0.0016	0.009
KEGG_CITRATE_CYCLE_TCA_CYCLE	27	0.4261	2.6459	0.0000	0.0033	0.0214
REACTOME_PYRUVATE_METABOLISM_AND_TCA_CYCLE	32	0.3818	2.5808	0.0000	0.0044	0.0382
REACTOME_INTEGRATION_OF_ENERGY_METABOLISM	193	0.1599	2.5782	0.0000	0.0045	0.0378
KEGG_BUTANOATE_METABOLISM	30	0.3882	2.5281	0.0000	0.0051	0.0552
KEGG_PEROXISOME	73	0.2525	2.5348	0.0000	0.0051	0.0548
KEGG_FATTY_ACID_METABOLISM	34	0.3612	2.5359	0.0000	0.0052	0.0532
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	29	0.3935	2.5177	0.0000	0.0055	0.0618
REACTOME_CITRIC_ACID_CYCLE	17	0.4937	2.4633	0.0004	0.0073	0.0896
REACTOME_MITOCHONDRIAL_FATTY_ACID_BETA_OXIDATION	9	0.6236	2.3011	0.0000	0.0211	0.2756
KEGG_OXIDATIVE_PHOSPHORYLATION	101	0.1921	2.2665	0.0004	0.0244	0.3408
BIOCARTA_KREB_PATHWAY	6	0.7367	2.2413	0.0020	0.0283	0.3946
REACTOME_STEROID_METABOLISM	51	0.2667	2.2395	0.0008	0.0285	0.3946
REACTOME_INTRINSIC_PATHWAY	15	0.4738	2.2044	0.0028	0.0329	0.4698
REACTOME_METABOLISM_OF_AMINO_ACIDS	143	0.1543	2.1559	0.0012	0.0427	0.5814
KEGG_PPAR_SIGNALING_PATHWAY	60	0.2328	2.1185	0.0015	0.0512	0.6706
REACTOME_BIOLOGICAL_OXIDATIONS	74	0.2078	2.1043	0.0040	0.0533	0.7084
KEGG_PARKINSONS_DISEASE	101	0.1784	2.1007	0.0024	0.0549	0.717

BIOCARTA_FIBRINOLYSIS_PATHWAY	12	0.4928	2.0625	0.0051	0.0623	0.7994
BIOCARTA_INTRINSIC_PATHWAY	23	0.3566	2.0453	0.0052	0.0654	0.8288
REACTOME_ETHANOL_OXIDATION	6	0.6730	2.0456	0.0028	0.0662	0.8306
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	38	0.2803	2.0428	0.0044	0.0666	0.8326
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	16	0.4220	2.0304	0.0063	0.0700	0.8554
KEGG_PROPANOATE_METABOLISM	27	0.3214	1.9878	0.0056	0.0792	0.9152
KEGG_ARGININE_AND_PROLINE_METABOLISM	45	0.2468	1.9856	0.0043	0.0803	0.914
REACTOME_PHASE_1_FUNCTIONALIZATION_OF_COMPOUNDS	39	0.2661	1.9700	0.0082	0.0869	0.937
REACTOME_PHOSPHOLIPASE_CMEDIATED_CASCADE	22	0.3519	1.9601	0.0069	0.0891	0.945
KEGG_MATURITY_ONSET_DIABETES_OF_THE_YOUNG	22	0.3456	1.9302	0.0096	0.0998	0.9706
BIOCARTA_LECTIN_PATHWAY	10	0.4968	1.9113	0.0068	0.1078	0.9784
KEGG_ALZHEIMERS_DISEASE	139	0.1392	1.9114	0.0075	0.1080	0.9792
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	29	0.2921	1.8938	0.0084	0.1092	0.9864
REACTOME_ENDOGENOUS_STEROLS	12	0.4517	1.8947	0.0124	0.1100	0.9862
REACTOME_NUCLEAR_RECEPTOR_TRANSCRIPTION_PATHWAY	48	0.2335	1.8995	0.0077	0.1102	0.9866
KEGG_HUNTINGTONS_DISEASE	142	0.1344	1.8749	0.0105	0.1174	0.9928
REACTOME_REGULATION_OF_LIPID_METABOLISM_BY_PEROXISOME_PROLIFERATOR_ACTIVATED_RECEPTOR_ALPHA	50	0.2231	1.8744	0.0126	0.1185	0.9926
BIOCARTA_VDR_PATHWAY	10	0.4827	1.8600	0.0112	0.1271	0.9944
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	35	0.2614	1.8388	0.0114	0.1331	0.9968
REACTOME_REGULATION_OF_INSULIN_SECRETION	177	0.1167	1.8014	0.0153	0.1559	0.9992
KEGG_NITROGEN_METABOLISM	8	0.5188	1.7932	0.0227	0.1622	0.9996
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	15	0.3821	1.7846	0.0179	0.1646	0.9996
REACTOME_METABOLISM_OF_BILE_ACIDS_AND_BILE_SALTS	23	0.3124	1.7859	0.0143	0.1652	0.9998
REACTOME_SIGNALING_BY_VEGF	11	0.4387	1.7702	0.0204	0.1729	0.9998
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_24_HYDROXYCHOLESTEROL	9	0.4794	1.7475	0.0245	0.1887	0.9998

KEGG_CYSTEINE_AND_METHIONINE_METABOLISM	27	0.2761	1.7363	0.0214	0.1962	1
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS	18	0.3377	1.7295	0.0223	0.2046	1
REACTOME_FRS2MEDIATED_CASCADE	26	0.2819	1.7050	0.0305	0.2239	1

TABLE 16. sActRIIB-Fc + Running vs PBS Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	70	-0.2627	-2.5822	0.0000	0.0387	0.0396
KEGG_INOSITOL_PHOSPHATE_METABOLISM	52	-0.2866	-2.4138	0.0004	0.0696	0.1336
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	63	-0.2428	-2.2581	0.0004	0.1261	0.3568
REACTOME_MTOR_SIGNALLING	26	-0.3620	-2.2266	0.0016	0.1303	0.4162
KEGG_INSULIN_SIGNALING_PATHWAY	124	-0.1723	-2.2075	0.0008	0.1305	0.4592
ST_WNT_CA2_CYCLIC_GMP_PATHWAY	18	-0.3972	-2.0433	0.0047	0.2496	0.8214

TABLE 17. sActRIIB-Fc +Running vs PBS Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	60	0.3817	3.4652	0.0000	0.0000	0
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	38	0.4396	3.1935	0.0000	0.0000	0
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	35	0.4667	3.2942	0.0000	0.0000	0
KEGG_PEROXISOME	73	0.3023	3.0343	0.0000	0.0000	0.0002
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	39	0.3711	2.7494	0.0000	0.0022	0.0114
REACTOME_COMPLEMENT_CASCADE	17	0.5481	2.7068	0.0000	0.0024	0.0138
REACTOME_BIOLOGICAL_OXIDATIONS	74	0.2596	2.6078	0.0000	0.0045	0.0316

REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	46	0.3151	2.5431	0.0000	0.0063	0.0496
REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS	195	0.1544	2.5042	0.0012	0.0073	0.067
REACTOME_METABOLISM_OF_AMINO_ACIDS	143	0.1783	2.4820	0.0004	0.0078	0.0788
REACTOME_SCF_SKP2_MEDIATED_DEGRADATION_OF_P27_P21	50	0.2960	2.4658	0.0004	0.0088	0.0906
KEGG_GLUTATHIONE_METABOLISM	46	0.3027	2.4704	0.0004	0.0089	0.0938
REACTOME_MITOCHONDRIAL_FATTY_ACID_BETA_OXIDATION	9	0.6586	2.4304	0.0012	0.0098	0.1168
BIOCARTA_COMP_PATHWAY	16	0.5013	2.3907	0.0008	0.0098	0.1514
KEGG_PROTEASOME	41	0.3153	2.3831	0.0000	0.0107	0.1604
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	29	0.3684	2.3723	0.0012	0.0107	0.1726
KEGG_PYRIMIDINE_METABOLISM	84	0.2235	2.3804	0.0008	0.0108	0.1642
KEGG_PURINE_METABOLISM	136	0.1745	2.3800	0.0000	0.0108	0.1644
KEGG_FATTY_ACID_METABOLISM	34	0.3437	2.3719	0.0004	0.0112	0.1718
REACTOME_STABILIZATION_OF_P53	44	0.3032	2.3741	0.0000	0.0112	0.1726
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	55	0.2669	2.3245	0.0004	0.0133	0.2330
KEGG_BUTANOATE_METABOLISM	30	0.3490	2.2720	0.0008	0.0168	0.3140
KEGG_P53_SIGNALING_PATHWAY	61	0.2461	2.2579	0.0004	0.0184	0.3404
REACTOME_SYNTHESIS_OF_DNA	86	0.2048	2.2257	0.0012	0.0203	0.4170
REACTOME_S_PHASE	97	0.1918	2.2154	0.0012	0.0212	0.4306
REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX	51	0.2622	2.2063	0.0008	0.0212	0.4550
REACTOME_P53_INDEPENDENT_DNA_DAMAGE_RESPONSE	42	0.2901	2.2098	0.0012	0.0212	0.4502
BIOCARTA_FIBRINOLYSIS_PATHWAY	12	0.5265	2.2099	0.0012	0.0220	0.4440
KEGG_TRYPTOPHAN_METABOLISM	36	0.3118	2.2011	0.0004	0.0221	0.4678
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	62	0.2373	2.1981	0.0028	0.0223	0.4752
KEGG_RNA_POLYMERASE	20	0.4065	2.1847	0.0029	0.0228	0.4980
REACTOME_COMMON_PATHWAY	13	0.4938	2.1620	0.0032	0.0241	0.5522

REACTOME_ELECTRON_TRANSPORT_CHAIN	58	0.2425	2.1616	0.0016	0.0242	0.5490
BIOCARTA_LECTIN_PATHWAY	10	0.5519	2.1570	0.0036	0.0246	0.5616
REACTOME_GLUTATHIONE_CONJUGATION	16	0.4464	2.1669	0.0028	0.0247	0.5410
REACTOME_REMOVAL_OF_DNA_PATCH_CONTAINING_ABASIC_RESIDUE	15	0.4556	2.1566	0.0020	0.0254	0.5670
REACTOME_G1_S_TRANSITION	98	0.1861	2.1424	0.0024	0.0256	0.5912
KEGG_PPAR_SIGNALING_PATHWAY	60	0.2316	2.1390	0.0012	0.0257	0.6022
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	16	0.4452	2.1339	0.0028	0.0266	0.6160
BIOCARTA_P53_PATHWAY	14	0.4635	2.0969	0.0048	0.0305	0.7074
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	45	0.2647	2.0971	0.0019	0.0311	0.7050
REACTOME_EARLY_PHASE_OF_HIV_LIFE_CYCLE	13	0.4731	2.0833	0.0029	0.0319	0.7344
REACTOME_REGULATION_OF_APC_ACTIVATORS_BETWEEN_G1_S_AND_EARLY_ANAPHASE	69	0.2137	2.0792	0.0012	0.0324	0.7448
REACTOME_RNA_POLYMERASE_III_TRANSCRIPTION_INITIATION_FROM_TYPE_3_PROMOTER	18	0.4066	2.0730	0.0024	0.0337	0.7620
REACTOME_APOPTOSIS	120	0.1596	2.0563	0.0029	0.0356	0.7920
KEGG_NITROGEN_METABOLISM	8	0.5911	2.0546	0.0025	0.0362	0.7980
BIOCARTA_INTRINSIC_PATHWAY	23	0.3562	2.0394	0.0039	0.0377	0.8292
BIOCARTA_CLASSIC_PATHWAY	11	0.5006	2.0390	0.0024	0.0378	0.8310
REACTOME_HIV_INFECTION	163	0.1371	2.0143	0.0024	0.0425	0.8710
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_7ALPHA_HYDROXYCHOLESTEROL	14	0.4451	2.0042	0.0059	0.0455	0.8856
REACTOME_RNA_POLYMERASE_III_CHAIN_ELONGATION	10	0.5207	1.9996	0.0054	0.0469	0.8890
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	23	0.3374	1.9470	0.0089	0.0584	0.9522
REACTOME_PHASE_II_CONJUGATION	35	0.2770	1.9385	0.0093	0.0613	0.9610
REACTOME_CELL_CYCLE_MITOTIC	277	0.1005	1.9384	0.0069	0.0618	0.9602
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	15	0.4087	1.9245	0.0093	0.0625	0.9678
REACTOME_STEROID_METABOLISM	51	0.2285	1.9272	0.0060	0.0626	0.9636
REACTOME_INTRINSIC_PATHWAY	15	0.4141	1.9304	0.0087	0.0628	0.9666

REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE	45	0.2424	1.9172	0.0084	0.0653	0.9754
REACTOME_M_G1_TRANSITION	60	0.2092	1.9116	0.0091	0.0656	0.9762
REACTOME_MITOTIC_M_M_G1_PHASES	143	0.1374	1.9124	0.0090	0.0663	0.9758
REACTOME_BASE_FREE_SUGAR_PHOSPHATE_REMOVAL_ VIA_THE_SINGLE_NUCLEOTIDE_REPLACEMENT_PATHWAY	10	0.4888	1.8838	0.0094	0.0733	0.9860
REACTOME_METABOLISM_OF_BILE_ACIDS_AND_BILE_SALTS	23	0.3271	1.8806	0.0075	0.0736	0.9886
REACTOME_BASE_EXCISION_REPAIR	18	0.3667	1.8825	0.0126	0.0746	0.9860
REACTOME_PHASE_1_FUNCTIONALIZATION_OF_COMPOUNDS	39	0.2544	1.8728	0.0116	0.0763	0.9838
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS	18	0.3657	1.8732	0.0102	0.0767	0.9882
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	31	0.2793	1.8601	0.0083	0.0796	0.9934
BIOCARTA_G2_PATHWAY	22	0.3305	1.8522	0.0119	0.0812	0.9960
REACTOME_DNA_REPLICATION_PRE_INITIATION	74	0.1840	1.8575	0.0109	0.0812	0.9922
REACTOME_SIGNALING_BY_WNT	55	0.2122	1.8511	0.0107	0.0815	0.9954
BIOCARTA_EXTRINSIC_PATHWAY	13	0.4272	1.8358	0.0117	0.0870	0.9982
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1_APC	56	0.2064	1.8233	0.0125	0.0916	0.9980
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	12	0.4324	1.8130	0.0151	0.0936	0.9994
REACTOME_REGULATION_OF_GENE_EXPRESSION_IN_BETA_CELLS	74	0.1770	1.8049	0.0170	0.0979	0.9988
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	29	0.2790	1.7922	0.0214	0.1024	0.9994
BIOCARTA_P27_PATHWAY	12	0.4232	1.7883	0.0164	0.1047	0.9990
REACTOME_CELL_CYCLE_CHECKPOINTS	106	0.1478	1.7778	0.0178	0.1078	0.9994
REACTOME_CDC20_PHOSPHO_APC_MEDIATED_DEGRADATION_OF_CYCLIN_A	62	0.1909	1.7749	0.0166	0.1082	0.9994
REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT	86	0.1622	1.7568	0.0236	0.1167	0.9994
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_1	24	0.3001	1.7446	0.0199	0.1211	0.9994
SA_PROGRAMMED_CELL_DEATH	12	0.4147	1.7424	0.0191	0.1228	0.9996
BIOCARTA_CTL_PATHWAY	10	0.4510	1.7404	0.0251	0.1238	0.9996
REACTOME_ENDOGENOUS_STEROLS	12	0.4080	1.7311	0.0320	0.1284	0.9996

BIOCARTA_AMI_PATHWAY	19	0.3318	1.7338	0.0255	0.1291	0.9996
REACTOME_HIV_LIFE_CYCLE	92	0.1544	1.7279	0.0203	0.1302	0.9996
REACTOME_POST_TRANSLATIONAL_PROTEIN_MODIFICATION	35	0.2429	1.7143	0.0255	0.1370	0.9998
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	71	0.1718	1.6989	0.0252	0.1438	0.9998
KEGG_ARACHIDONIC_ACID_METABOLISM	44	0.2146	1.6765	0.0289	0.1579	1.0000
REACTOME_GAMMA_CARBOXYLATION_TRANSPORT_AND_AMINO_TERMINAL_CLEAVAGE_OF_PROTEINS	10	0.4371	1.6715	0.0332	0.1611	1.0000
REACTOME_STEROID_HORMONE_BIOSYNTHESIS	13	0.3838	1.6702	0.0304	0.1621	1.0000
BIOCARTA_RB_PATHWAY	11	0.4096	1.6608	0.0332	0.1667	1.0000
REACTOME_HIV1_TRANSCRIPTION_INITIATION	31	0.2497	1.6609	0.0323	0.1668	1.0000
KEGG_TAURINE_AND_HYPOTAURINE_METABOLISM	10	0.4285	1.6596	0.0292	0.1673	1.0000
BIOCARTA_MITOCHONDRIA_PATHWAY	20	0.3046	1.6245	0.0405	0.1910	1.0000
REACTOME_REGULATION_OF_LIPID_METABOLISM_ BY_PEROXISOME_PROLIFERATOR_ACTIVATED_RECEPTOR_ALPHA	50	0.1936	1.6097	0.0445	0.2034	1.0000
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_24_HYDROXYCHOLESTEROL	9	0.4348	1.6014	0.0458	0.2077	1.0000
KEGG_STEROID_HORMONE_BIOSYNTHESIS	29	0.2494	1.5995	0.0435	0.2093	1.0000
REACTOME_PYRUVATE_METABOLISM_AND_TCA_CYCLE	32	0.2402	1.5918	0.0487	0.2122	1.0000
REACTOME_GAP_JUNCTION_ASSEMBLY	17	0.3182	1.5899	0.0476	0.2160	1.0000
BIOCARTA_ARF_PATHWAY	15	0.3412	1.5868	0.0484	0.2175	1.0000
REACTOME_INTEGRATION_OF_ENERGY_METABOLISM	193	0.0966	1.5857	0.0468	0.2201	1.0000
REACTOME_PEROXISOMAL_LIPID_METABOLISM	19	0.3032	1.5833	0.0412	0.2214	1.0000
REACTOME_G2_M_CHECKPOINTS	41	0.2058	1.5619	0.0591	0.2353	1.0000
REACTOME_INTRINSIC_PATHWAY_FOR_APOPTOSIS	29	0.2443	1.5584	0.0572	0.2382	1.0000
REACTOME_RNA_POLYMERASE_III_TRANSCRIPTION_INITIATION	25	0.2608	1.5567	0.0582	0.2391	1.0000

TABLE 18. sActRIIB-Fc + Running vs PBS + Running Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_PLC_GAMMA1_SIGNALLING	32	-0.3436	-2.2981	0.0004	0.0751	0.2670
REACTOME_TRNA_AMINOACYLATION	38	-0.3132	-2.2874	0.0020	0.0751	0.2856
REACTOME_CAM_PATHWAY	24	-0.4060	-2.3566	0.0016	0.0816	0.1828
REACTOME_PLC_BETA_MEDIATED_EVENTS	36	-0.3089	-2.1835	0.0024	0.0848	0.4962
REACTOME_REGULATION_OF_AMPK_ACTIVITY_VIA_LKB1	13	-0.4896	-2.1561	0.0024	0.0885	0.5672
REACTOME_INTEGRATION_OF_ENERGY_METABOLISM	193	-0.1358	-2.1868	0.0012	0.0911	0.4938
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_HEPARAN_SULFATE	24	-0.3731	-2.1948	0.0012	0.0932	0.4756
KEGG_OXIDATIVE_PHOSPHORYLATION	101	-0.1783	-2.1067	0.0028	0.1038	0.6858
REACTOME_ACTIVATED_AMPK_STIMULATES_FATTY_ACID_OXIDATION_IN_MUSCLE	15	-0.5102	-2.3893	0.0000	0.1303	0.1486
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	39	-0.2686	-1.9866	0.0053	0.1690	0.9044
REACTOME_GLUCOSE_REGULATION_OF_INSULIN_SECRETION	130	-0.1514	-1.9953	0.0048	0.1694	0.8944
BIOCARTA_LEPTIN_PATHWAY	10	-0.5000	-1.9540	0.0072	0.1717	0.9440
KEGG_ALZHEIMERS_DISEASE	139	-0.1381	-1.9127	0.0080	0.1729	0.9766
BIOCARTA_CELL2CELL_PATHWAY	14	-0.4234	-1.9104	0.0088	0.1747	0.9782
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	19	-0.3618	-1.8971	0.0112	0.1751	0.9858
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	98	-0.1672	-1.9331	0.0077	0.1769	0.9622
REACTOME_ELECTRON_TRANSPORT_CHAIN	58	-0.2182	-1.9450	0.0052	0.1803	0.9544
BIOCARTA_INTEGRIN_PATHWAY	33	-0.2904	-1.9780	0.0055	0.1825	0.9234
REACTOME_ENERGY_DEPENDENT_REGULATION_OF_MTOR_BY_LKB1_AMPK	16	-0.3935	-1.9012	0.0097	0.1853	0.9752
REACTOME_EFFECTS_OF_PIP2_HYDROLYSIS	15	-0.4003	-1.8683	0.0084	0.1857	0.9896
REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	23	-0.3339	-1.9199	0.0093	0.1872	0.9686

REACTOME_PKA_ACTIVATION	15	-0.3975	-1.8484	0.0117	0.1964	0.9946
BIOCARTA_CHREBP2_PATHWAY	37	-0.2546	-1.8418	0.0111	0.2059	0.9946
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	70	-0.1826	-1.8225	0.0172	0.2149	0.9960

TABLE 19. sActRIIB-Fc + Running vs PBS + Running Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_CELL_CYCLE_MITOTIC	277	0.2006	3.8729	0.0000	0.0000	0.0000
REACTOME_DNA_REPLICATION_PRE_INITIATION	74	0.3116	3.1290	0.0000	0.0000	0.0000
REACTOME_G1_S_TRANSITION	98	0.3227	3.7747	0.0000	0.0000	0.0000
REACTOME_MITOTIC_M_M_G1_PHASES	143	0.2417	3.3501	0.0000	0.0000	0.0000
REACTOME_S_PHASE	97	0.3216	3.7320	0.0000	0.0000	0.0000
REACTOME_SYNTHESIS_OF_DNA	86	0.3183	3.4460	0.0000	0.0000	0.0000
REACTOME_SCF_SKP2_MEDIATED_DEGRADATION_OF_P27_P21	50	0.3671	3.0988	0.0000	0.0000	0.0002
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	55	0.3453	3.0124	0.0000	0.0000	0.0006
REACTOME_APOPTOSIS	120	0.2326	2.9865	0.0000	0.0000	0.0016
REACTOME_CELL_CYCLE_CHECKPOINTS	106	0.2496	2.9659	0.0000	0.0000	0.001
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	62	0.3175	2.9402	0.0000	0.0000	0.0014
REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	46	0.3531	2.8683	0.0000	0.0002	0.0036
REACTOME_M_G1_TRANSITION	60	0.3105	2.8321	0.0000	0.0004	0.0052
REACTOME_P53_INDEPENDENT_DNA_DAMAGE_RESPONSE	42	0.3602	2.7706	0.0000	0.0006	0.0076
REACTOME_STABILIZATION_OF_P53	44	0.3451	2.7260	0.0000	0.0008	0.0126
BIOCARTA_CDC42RAC_PATHWAY	15	0.5849	2.7341	0.0000	0.0008	0.0112
REACTOME_FORMATION_OF_A_POOL_OF_FREE_40S_SUBUNITS	66	0.2777	2.6486	0.0000	0.0014	0.0204

REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX	51	0.3094	2.6356	0.0000	0.0016	0.0224
KEGG_DNA_REPLICATION	33	0.3825	2.6046	0.0000	0.0018	0.029
REACTOME_PEPTIDE_CHAIN_ELONGATION	59	0.2871	2.5834	0.0000	0.0021	0.0354
KEGG_RIBOSOME	61	0.2766	2.5451	0.0000	0.0027	0.0486
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	71	0.2504	2.5131	0.0000	0.0034	0.0636
KEGG_PROTEASOME	41	0.3317	2.4963	0.0004	0.0035	0.0706
REACTOME_REGULATION_OF_ORNITHINE_DECARBOXYLASE	45	0.3123	2.4884	0.0000	0.0035	0.0742
REACTOME_METABOLISM_OF_PROTEINS	170	0.1645	2.5001	0.0000	0.0036	0.0728
REACTOME_G_PROTEIN_BETA_GAMMA_SIGNALLING	27	0.4045	2.4810	0.0000	0.0039	0.0796
REACTOME_G_BETA_GAMMA_SIGNALLING_THROUGH_PI3KGAMMA	24	0.4276	2.4648	0.0000	0.0042	0.0902
REACTOME_VIRAL_MRNA_TRANSLATION	59	0.2701	2.4462	0.0004	0.0046	0.1082
REACTOME_HIV_INFECTION	163	0.1634	2.4285	0.0000	0.0046	0.115
REACTOME_GTP_HYDROLYSIS_AND_JOINING_OF_THE_60S_RIBOSOMAL_SUBUNIT	76	0.2375	2.4348	0.0000	0.0047	0.1108
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	35	0.3465	2.4225	0.0004	0.0048	0.1212
REACTOME_TRANSLATION	89	0.2194	2.4121	0.0004	0.0052	0.1306
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	45	0.3046	2.3927	0.0000	0.0053	0.1464
REACTOME_INSULIN_SYNTHESIS_AND_SECRETION	101	0.2028	2.3994	0.0000	0.0054	0.145
REACTOME_TRANSCRIPTION_COUPLED_NER	39	0.3225	2.3863	0.0004	0.0055	0.1524
REACTOME_DNA_STRAND_ELONGATION	29	0.3698	2.3861	0.0008	0.0055	0.1516
REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	29	0.3744	2.3767	0.0016	0.0058	0.162
REACTOME_HIV1_TRANSCRIPTION_INITIATION	31	0.3531	2.3634	0.0008	0.0059	0.1768
REACTOME_SIGNALING_BY_WNT	55	0.2723	2.3635	0.0008	0.0060	0.1766
BIOCARTA_P53_PATHWAY	14	0.5205	2.3500	0.0004	0.0063	0.195
REACTOME_MITOTIC_PROMETAPHASE	79	0.2255	2.3445	0.0008	0.0065	0.2044
ST_PHOSPHOINOSITIDE_3_KINASE_PATHWAY	31	0.3539	2.3382	0.0012	0.0067	0.2074

KEGG_PYRIMIDINE_METABOLISM	84	0.2144	2.3281	0.0004	0.0068	0.2222
REACTOME_REGULATION_OF_APC_ACTIVATORS_BETWEEN_G1_S_AND_EARLY_ANAPHASE	69	0.2371	2.3205	0.0004	0.0072	0.2358
REACTOME_METABLISM_OF_NUCLEOTIDES	63	0.2493	2.3123	0.0008	0.0078	0.254
REACTOME_G2_M_CHECKPOINTS	41	0.3028	2.3003	0.0004	0.0080	0.268
REACTOME_GLUCAGON_TYPE_LIGAND_RECEPTORS	30	0.3478	2.2870	0.0008	0.0085	0.2946
REACTOME_G_BETA_GAMMA_SIGNALLING_THROUGH_PLC_BETA	19	0.4318	2.2529	0.0008	0.0102	0.3544
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	23	0.3908	2.2533	0.0008	0.0102	0.3522
KEGG_P53_SIGNALING_PATHWAY	61	0.2425	2.2469	0.0000	0.0106	0.3638
BIOCARTA_CELLCYCLE_PATHWAY	21	0.4073	2.2349	0.0024	0.0114	0.3898
KEGG_CELL_CYCLE	116	0.1768	2.2231	0.0012	0.0120	0.4164
REACTOME_INHIBITION_OF_INSULIN_SECRETION_BY_ADRENALINE_NORADRENALINE	26	0.3626	2.2172	0.0012	0.0122	0.425
KEGG_GLUTATHIONE_METABOLISM	46	0.2782	2.2160	0.0004	0.0123	0.4264
REACTOME_LAGGING_STRAND_SYNTHESIS	20	0.4110	2.2104	0.0016	0.0124	0.436
REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS	35	0.3154	2.2090	0.0008	0.0126	0.4416
REACTOME_NUCLEOTIDE_EXCISION_REPAIR	44	0.2772	2.2011	0.0015	0.0134	0.4632
KEGG_LYSOSOME	111	0.1764	2.1872	0.0012	0.0147	0.4942
REACTOME_PLATELET_ACTIVATION_TRIGGERS	54	0.2467	2.1570	0.0008	0.0167	0.5618
REACTOME_MRNA_PROCESSING	26	0.3493	2.1328	0.0028	0.0189	0.628
BIOCARTA_ARF_PATHWAY	15	0.4593	2.1337	0.0031	0.0193	0.6208
REACTOME_CDC20_PHOSPHO_APC_MEDIATED_DEGRADATION_OF_CYCLIN_A	62	0.2301	2.1216	0.0028	0.0201	0.6522
REACTOME_DNA_REPAIR	91	0.1904	2.1219	0.0021	0.0202	0.647
REACTOME_METABOLISM_OF_AMINO_ACIDS	143	0.1498	2.1214	0.0032	0.0205	0.6518
REACTOME_METABOLISM_OF_RNA	87	0.1921	2.1084	0.0028	0.0217	0.6802
REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	36	0.2919	2.0841	0.0039	0.0250	0.7408
REACTOME_COMMON_PATHWAY	13	0.4731	2.0689	0.0027	0.0267	0.776

REACTOME_THROMBOXANE_SIGNALLING_THROUGH_TP_RECEPTOR	22	0.3661	2.0468	0.0048	0.0300	0.8212
REACTOME_G1_PHASE	14	0.4475	2.0365	0.0068	0.0311	0.8374
REACTOME_METABOLISM_OF_MRNA	41	0.2676	2.0307	0.0041	0.0319	0.8484
REACTOME_GLOBAL_GENOMIC_NER	32	0.2998	2.0139	0.0052	0.0346	0.8812
REACTOME_INFLUENZA_LIFE_CYCLE	104	0.1680	2.0038	0.0072	0.0359	0.8888
BIOCARTA_CTCF_PATHWAY	20	0.3708	1.9955	0.0067	0.0372	0.903
REACTOME_INTRINSIC_PATHWAY_FOR_APOPTOSIS	29	0.3120	1.9936	0.0080	0.0380	0.8992
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_12	19	0.3791	1.9830	0.0068	0.0388	0.9204
REACTOME_AUTODEGRADATION_OF_CDH1_BY_CDH1_APC	56	0.2232	1.9790	0.0035	0.0401	0.9212
KEGG_MISMATCH_REPAIR	22	0.3524	1.9783	0.0062	0.0402	0.9262
REACTOME_G_PROTEIN_ACTIVATION	26	0.3240	1.9736	0.0072	0.0405	0.9304
REACTOME_REPAIR_SYNTHESIS_OF_PATCH_27_30_BASES_LONG_BY_DNA_POLYMERASE	15	0.4257	1.9700	0.0086	0.0414	0.93
REACTOME_TRANSCRIPTION	155	0.1348	1.9671	0.0085	0.0417	0.9384
BIOCARTA_SALMONELLA_PATHWAY	12	0.4681	1.9617	0.0080	0.0419	0.9424
KEGG_HOMOLOGOUS_RECOMBINATION	26	0.3206	1.9578	0.0054	0.0424	0.9452
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	82	0.1852	1.9595	0.0065	0.0426	0.942
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS	109	0.1601	1.9540	0.0064	0.0430	0.9486
BIOCARTA_CASPASE_PATHWAY	22	0.3426	1.9406	0.0068	0.0450	0.9564
REACTOME_REGULATION_OF_GENE_EXPRESSION_IN_BETA_CELLS	74	0.1923	1.9405	0.0073	0.0453	0.9576
KEGG_PENTOSE_AND_GLUCURONATE_INTERCONVERSIONS	10	0.5023	1.9404	0.0071	0.0462	0.957
BIOCARTA_TFF_PATHWAY	19	0.3669	1.9321	0.0076	0.0465	0.9634
REACTOME_PROSTANOID_HORMONES	10	0.4908	1.9240	0.0065	0.0475	0.97
REACTOME_PYRIMIDINE_METABOLISM	19	0.3661	1.9204	0.0088	0.0479	0.9726
BIOCARTA_DNAFRAGMENT_PATHWAY	9	0.5224	1.9201	0.0091	0.0481	0.9702
REACTOME_FORMATION_OF_THE_EARLY_ELONGATION_COMPLEX	27	0.3071	1.9193	0.0060	0.0488	0.9704

BIOCARTA_RHO_PATHWAY	27	0.3089	1.9083	0.0087	0.0505	0.9748
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	38	0.2595	1.9057	0.0081	0.0512	0.9792
REACTOME_POLYMERASE_SWITCHING	14	0.4161	1.8945	0.0076	0.0527	0.9834
REACTOME_E2F_TRANSCRIPTIONAL_TARGETS_AT_G1_S	22	0.3361	1.8940	0.0094	0.0530	0.9808
REACTOME_TRANSLATION_INITIATION_COMPLEX_FORMATION	43	0.2441	1.8863	0.0066	0.0555	0.9834
REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	33	0.2729	1.8734	0.0111	0.0575	0.9898
REACTOME_REMOVAL_OF_DNA_PATCH_CONTAINING_ABASIC_RESIDUE	15	0.4007	1.8699	0.0149	0.0583	0.9904
SA_G1_AND_S_PHASES	12	0.4478	1.8608	0.0146	0.0602	0.993
KEGG_NUCLEOTIDE_EXCISION_REPAIR	42	0.2403	1.8543	0.0098	0.0626	0.9942
REACTOME_PHASE_II_CONJUGATION	35	0.2628	1.8504	0.0129	0.0633	0.9942
BIOCARTA_CHEMICAL_PATHWAY	21	0.3347	1.8473	0.0105	0.0642	0.995
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	21	0.3345	1.8386	0.0146	0.0662	0.996
REACTOME_RNA_POLYMERASE_I_CHAIN_ELONGATION	24	0.3159	1.8351	0.0173	0.0680	0.996
REACTOME_COLLAGEN_MEDIATED_ACTIVATION_CASCADE	20	0.3403	1.8343	0.0171	0.0681	0.9956
REACTOME_EXTENSION_OF_TELOMERES	28	0.2896	1.8270	0.0113	0.0696	0.9978
KEGG_PURINE_METABOLISM	136	0.1327	1.8151	0.0144	0.0737	0.9982
BIOCARTA_P27_PATHWAY	12	0.4302	1.8058	0.0179	0.0754	0.9988
REACTOME_REMOVAL_OF_THE_FLAP_INTERMEDIATE_FROM_THE_C_STRAND	10	0.4589	1.7860	0.0176	0.0815	0.9996
BIOCARTA_MPR_PATHWAY	29	0.2790	1.7772	0.0165	0.0842	0.9994
BIOCARTA_MCM_PATHWAY	17	0.3564	1.7746	0.0175	0.0849	0.9998
REACTOME_SYNTHESIS_AND_INTERCONVERSION_OF_NUCLEOTIDE_DI_AND_TRIPHOSPHATES	16	0.3679	1.7786	0.0152	0.0851	0.9998
SA_FAS_SIGNALING	6	0.5848	1.7753	0.0187	0.0852	0.9994
SIG_PIP3_SIGNALING_IN_B_LYMPHOCYTES	29	0.2747	1.7676	0.0147	0.0873	1
BIOCARTA_RACCYCD_PATHWAY	24	0.3015	1.7610	0.0230	0.0905	0.9998
BIOCARTA_G2_PATHWAY	22	0.3113	1.7570	0.0215	0.0911	1

KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	31	0.2639	1.7518	0.0202	0.0927	1
REACTOME_NUCLEOTIDE_LIKE_PURINERGIC_RECEPTORS	14	0.3847	1.7501	0.0181	0.0930	1
REACTOME_HIV_LIFE_CYCLE	92	0.1531	1.7367	0.0167	0.0973	1
REACTOME_SIGNALING_IN_IMMUNE_SYSTEM	268	0.0921	1.7360	0.0179	0.0980	1
BIOCARTA_TH1TH2_PATHWAY	15	0.3697	1.7317	0.0205	0.0989	1
BIOCARTA_EXTRINSIC_PATHWAY	13	0.3961	1.7296	0.0245	0.0998	1
REACTOME_P2Y_RECEPTORS	10	0.4461	1.7267	0.0203	0.1000	1
KEGG_ARACHIDONIC_ACID_METABOLISM	44	0.2185	1.7269	0.0264	0.1010	1
BIOCARTA_G1_PATHWAY	23	0.2967	1.7250	0.0229	0.1013	1
REACTOME_RNA_POLYMERASE_II_TRANSCRIPTION	72	0.1739	1.7231	0.0247	0.1019	1
REACTOME_RNA_POLYMERASE_III_TRANSCRIPTION_INITIATION_FROM_TYPE_3_PROMOTER	18	0.3366	1.7187	0.0224	0.1024	1
REACTOME_REGULATION_OF_INSULIN_SECRETION_BY_GLUCAGON_LIKE_PEPTIDE_1	51	0.2020	1.7196	0.0300	0.1033	1
SA_PROGRAMMED_CELL_DEATH	12	0.4021	1.7067	0.0269	0.1073	1
KEGG_ALLOGRAFT_REJECTION	15	0.3609	1.6946	0.0273	0.1102	1
KEGG_RNA_POLYMERASE	20	0.3154	1.6854	0.0304	0.1136	1
REACTOME_GS_ALPHA_MEDIATED_EVENTS_IN_GLUCAGON_SIGNALLING	25	0.2823	1.6862	0.0317	0.1137	1
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_1	24	0.2879	1.6815	0.0324	0.1144	1
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	12	0.4014	1.6838	0.0303	0.1145	1
REACTOME_THROMBIN_SIGNALLING_THROUGH_PROTEINASE_ACTIVATED_RECEPTORS	26	0.2749	1.6783	0.0301	0.1148	1
REACTOME_GENE_EXPRESSION	357	0.0770	1.6815	0.0279	0.1150	1
REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT	86	0.1541	1.6819	0.0346	0.1150	1
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	32	0.2487	1.6751	0.0362	0.1184	1
REACTOME_MRNA_DECAY_BY_5_TO_3_EXORIBONUCLEASE	11	0.4121	1.6675	0.0303	0.1187	1
BIOCARTA_ACTINY_PATHWAY	18	0.3292	1.6717	0.0272	0.1188	1
BIOCARTA_NKT_PATHWAY	26	0.2749	1.6669	0.0342	0.1193	1

REACTOME_RNA_POL_II_CTD_PHOSPHORYLATION_AND_INTERACTION_WITH_CE	21	0.3039	1.6632	0.0316	0.1206	1
BIOCARTA_PTC1_PATHWAY	10	0.4277	1.6575	0.0370	0.1218	1
REACTOME_TRANSCRIPTION_OF_THE_HIV_GENOME	50	0.1974	1.6586	0.0318	0.1218	1
REACTOME_HOMOLOGOUS_RECOMBINATION_REPAIR	13	0.3791	1.6604	0.0332	0.1220	1
REACTOME_CYCLIN_A1_ASSOCIATED_EVENTS_DURING_G2_M_TRANSITION	14	0.3652	1.6543	0.0397	0.1235	1
BIOCARTA_DEATH_PATHWAY	29	0.2545	1.6450	0.0440	0.1261	1
REACTOME_EARLY_PHASE_OF_HIV_LIFE_CYCLE	13	0.3773	1.6447	0.0342	0.1270	1
REACTOME_RNA_POLYMERASE_I_III_AND_MITOCHONDRIAL_TRANSCRIPTION	95	0.1433	1.6438	0.0348	0.1271	1
BIOCARTA_COMP_PATHWAY	16	0.3389	1.6401	0.0388	0.1275	1
BIOCARTA_MITOCHONDRIA_PATHWAY	20	0.3061	1.6349	0.0369	0.1290	1
BIOCARTA_RAS_PATHWAY	21	0.2955	1.6262	0.0421	0.1329	1
REACTOME_RNA_POLYMERASE_I_PROMOTER_OPENING	41	0.2158	1.6279	0.0411	0.1329	1
REACTOME_RNA_POLYMERASE_I_TRANSCRIPTION_INITIATION	20	0.2977	1.6215	0.0397	0.1354	1
REACTOME_RNA_POLYMERASE_I_PROMOTER_CLEARANCE	60	0.1785	1.6210	0.0363	0.1370	1
SIG_CHEMOTAXIS	41	0.2132	1.6148	0.0362	0.1390	1
REACTOME_LYSOSOME_VESICLE_BIOGENESIS	21	0.2939	1.6102	0.0418	0.1412	1
REACTOME_SIGNAL_AMPLIFICATION	29	0.2518	1.6044	0.0423	0.1429	1
REACTOME_ACTIVATION_OF_KAINATE_RECEPTORS_UPON_GLUTAMATE_BINDING	30	0.2477	1.6038	0.0418	0.1437	1
REACTOME_BASE_EXCISION_REPAIR	18	0.3118	1.5990	0.0412	0.1463	1
REACTOME_TELOMERE_MAINTENANCE	63	0.1703	1.5958	0.0462	0.1467	1
SIG_PIP3_SIGNALING_IN_CARDIAC_MYOCTES	57	0.1794	1.5929	0.0485	0.1492	1
REACTOME_MRNA_DECAY_BY_3_TO_5_EXORIBONUCLEASE	11	0.3963	1.5885	0.0466	0.1506	1
KEGG_RNA_DEGRADATION	52	0.1884	1.5875	0.0471	0.1512	1
KEGG_COLORECTAL_CANCER	57	0.1777	1.5804	0.0501	0.1538	1
BIOCARTA_PROTEASOME_PATHWAY	19	0.2992	1.5762	0.0483	0.1561	1

BIOCARTA_D4GDI_PATHWAY	12	0.3762	1.5765	0.0465	0.1565	1
REACTOME_CASPASE_MEDIATED_CLEAVAGE_OF_CYTOSKELETAL_PROTEINS	11	0.3885	1.5737	0.0485	0.1567	1
REACTOME_REMOVAL_OF_THE_FLAP_INTERMEDIATE	14	0.3446	1.5690	0.0542	0.1595	1
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	21	0.2849	1.5653	0.0480	0.1612	1
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY	74	0.1546	1.5537	0.0515	0.1671	1
REACTOME_RNA_POLYMERASE_III_CHAIN_ELONGATION	10	0.4032	1.5554	0.0524	0.1679	1
REACTOME_PURINE_METABOLISM	26	0.2564	1.5509	0.0481	0.1702	1
REACTOME_PYRIMIDINE_CATABOLISM	8	0.4448	1.5393	0.0575	0.1754	1
REACTOME_TOLL_LIKE_RECEPTOR_4_CASCADE	25	0.2560	1.5382	0.0550	0.1768	1
REACTOME_COMPLEMENT_CASCADE	17	0.3132	1.5382	0.0561	0.1774	1
REACTOME_G_ALPHA_Q_SIGNALLING_EVENTS	136	0.1124	1.5279	0.0625	0.1840	1
REACTOME_AMINE_LIGAND_BINDING_RECEPTORS	39	0.2061	1.5258	0.0595	0.1847	1
KEGG_PANCREATIC_CANCER	65	0.1598	1.5117	0.0676	0.1942	1
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	44	0.1922	1.5045	0.0747	0.2000	1
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_7ALPHA_HYDROXYCHOLESTEROL	14	0.3294	1.5018	0.0699	0.2016	1
REACTOME_GENES_INVOLVED_IN_APOPTOTIC_CLEAVAGE_OF_CELLULAR_PROTEINS	33	0.2189	1.4943	0.0640	0.2059	1
KEGG_SPHINGOLIPID_METABOLISM	36	0.2108	1.4904	0.0780	0.2089	1
BIOCARTA_ATRBRCA_PATHWAY	16	0.3066	1.4853	0.0744	0.2117	1
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	88	0.1343	1.4858	0.0754	0.2121	1
REACTOME_UNFOLDED_PROTEIN_RESPONSE	16	0.3082	1.4796	0.0741	0.2164	1
REACTOME_DUAL_INCISION_REACTION_IN_TC_NER	23	0.2542	1.4632	0.0857	0.2262	1
KEGG_STEROID_HORMONE_BIOSYNTHESIS	29	0.2254	1.4553	0.0849	0.2314	1
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	29	0.2268	1.4532	0.0851	0.2333	1
REACTOME_LATE_PHASE_OF_HIV_LIFE_CYCLE	79	0.1396	1.4538	0.0932	0.2341	1
REACTOME_RNA_POLYMERASE_I_TRANSCRIPTION_TERMINATION	17	0.2913	1.4530	0.0851	0.2349	1

KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	41	0.1904	1.4513	0.0820	0.2353	1
BIOCARTA_RB_PATHWAY	11	0.3579	1.4491	0.0878	0.2362	1
REACTOME_HEMOSTASIS	248	0.0790	1.4468	0.0859	0.2380	1
KEGG_TGF_BETA_SIGNALING_PATHWAY	80	0.1372	1.4465	0.0944	0.2386	1
BIOCARTA_CLASSIC_PATHWAY	11	0.3559	1.4440	0.0896	0.2397	1
REACTOME_RNA_POLYMERASE_I_PROMOTER_ESCAPE	16	0.2949	1.4439	0.0829	0.2404	1
REACTOME_VIRAL_MESSENGER_RNA_SYNTHESIS	10	0.3721	1.4401	0.0933	0.2417	1
KEGG_APOPTOSIS	74	0.1419	1.4377	0.0960	0.2436	1
KEGG_ASTHMA	15	0.3075	1.4303	0.1009	0.2492	1
REACTOME_SYNTHESIS_OF_GLYCOSYLPHOSPHATIDYLINOSITOL	16	0.2977	1.4291	0.1037	0.2499	1

TABLE 20. sActRIIB-Fc + Running vs sActRIIB-Fc Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_STRIATED_MUSCLE_CONTRACTION	28	-0.3935	-2.4859	0.0008	0.0468	0.0714
REACTOME_PLC_GAMMA1_SIGNALLING	32	-0.3710	-2.4869	0.0000	0.0568	0.0704
KEGG_INSULIN_SIGNALING_PATHWAY	124	-0.1722	-2.2403	0.0020	0.0627	0.369
BIOCARTA_PYK2_PATHWAY	27	-0.3589	-2.2448	0.0016	0.0677	0.3602
REACTOME_TRKA_SIGNALLING_FROM_THE_PLASMA_MEMBRANE	96	-0.1989	-2.2509	0.0004	0.0682	0.3516
REACTOME_PKA_ACTIVATION	15	-0.4904	-2.2835	0.0004	0.0736	0.2906
REACTOME_REGULATION_OF_AMPK_ACTIVITY_VIA_LKB1	13	-0.4742	-2.0819	0.0028	0.0752	0.739
BIOCARTA_GABA_PATHWAY	9	-0.5745	-2.1287	0.0031	0.0755	0.6224
BIOCARTA_MEF2D_PATHWAY	15	-0.4346	-2.0512	0.0039	0.0759	0.8002
BIOCARTA_NOS1_PATHWAY	20	-0.4265	-2.2790	0.0004	0.0764	0.3024

KEGG_FOCAL_ADHESION	174	-0.1380	-2.0948	0.0036	0.0765	0.708
BIOCARTA_GATA3_PATHWAY	15	-0.4693	-2.1724	0.0020	0.0770	0.5224
REACTOME_PLC_BETA_MEDIATED_EVENTS	36	-0.3020	-2.1326	0.0016	0.0777	0.6158
REACTOME_ACTIVATED_AMPK_STIMULATES_FATTY_ACID_OXIDATION_IN_MUSCLE	15	-0.4486	-2.0935	0.0016	0.0779	0.7062
BIOCARTA_AKAP13_PATHWAY	9	-0.5807	-2.1085	0.0012	0.0783	0.6786
REACTOME_MUSCLE_CONTRACTION	46	-0.2616	-2.0828	0.0012	0.0787	0.7332
REACTOME_GLUCOSE_METABOLISM	50	-0.2419	-2.0319	0.0044	0.0788	0.8324
ST_INTERLEUKIN_13_PATHWAY	6	-0.6691	-2.0437	0.0016	0.0790	0.8184
BIOCARTA_VIP_PATHWAY	24	-0.3574	-2.1115	0.0036	0.0800	0.6692
KEGG_MAPK_SIGNALING_PATHWAY	231	-0.1166	-2.0638	0.0033	0.0806	0.7688
REACTOME_GLYCOLYSIS	21	-0.3697	-2.0236	0.0033	0.0806	0.8506
BIOCARTA_CCR5_PATHWAY	17	-0.4067	-2.0010	0.0048	0.0830	0.888
REACTOME_IRS_RELATED_EVENTS	74	-0.2098	-2.1147	0.0024	0.0837	0.6624
ST_IL_13_PATHWAY	6	-0.6691	-2.0264	0.0057	0.0838	0.8476
BIOCARTA_ALK_PATHWAY	34	-0.2849	-1.9833	0.0028	0.0841	0.9112
REACTOME_MTOR_SIGNALLING	26	-0.3281	-1.9984	0.0050	0.0855	0.8912
ST_STAT3_PATHWAY	9	-0.5391	-1.9807	0.0076	0.0868	0.9194
BIOCARTA_AT1R_PATHWAY	28	-0.3158	-1.9789	0.0051	0.0872	0.9212
BIOCARTA_P35ALZHEIMERS_PATHWAY	10	-0.5114	-1.9615	0.0053	0.0885	0.9402
REACTOME_CAM_PATHWAY	24	-0.3917	-2.2918	0.0004	0.0890	0.28
REACTOME_ENERGY_DEPENDENT_REGULATION_OF_MTOR_BY_LKB1_AMPK	16	-0.4069	-1.9678	0.0054	0.0891	0.9282
WNT_SIGNALING	86	-0.1819	-1.9486	0.0044	0.0891	0.9524
KEGG_ECM_RECEPTOR_INTERACTION	74	-0.1901	-1.9510	0.0078	0.0896	0.9434
REACTOME_SIGNALLING_BY_NGF	190	-0.1192	-1.9351	0.0076	0.0928	0.962
BIOCARTA_INTEGRIN_PATHWAY	33	-0.2802	-1.9103	0.0118	0.1021	0.979

REACTOME_DARPP32_EVENTS	25	-0.3213	-1.9107	0.0063	0.1031	0.9784
BIOCARTA_TALL1_PATHWAY	14	-0.4215	-1.9071	0.0113	0.1055	0.978
ST_P38_MAPK_PATHWAY	32	-0.2804	-1.8825	0.0106	0.1083	0.9888
BIOCARTA_PLCE_PATHWAY	10	-0.4887	-1.8860	0.0057	0.1084	0.9866
BIOCARTA_CFTR_PATHWAY	10	-0.4887	-1.8827	0.0139	0.1092	0.9862
BIOCARTA_EGF_PATHWAY	29	-0.2915	-1.8633	0.0106	0.1116	0.9928
BIOCARTA_EGFR_SMRTE_PATHWAY	9	-0.5109	-1.8760	0.0093	0.1120	0.9898
KEGG_LONG_TERM_POTENTIATION	58	-0.2097	-1.8602	0.0093	0.1163	0.993
BIOCARTA_VITCB_PATHWAY	11	-0.4578	-1.8414	0.0100	0.1223	0.9954
BIOCARTA_IL6_PATHWAY	19	-0.3497	-1.8320	0.0138	0.1236	0.9962
BIOCARTA_IL2_PATHWAY	18	-0.3569	-1.8351	0.0076	0.1244	0.9964
BIOCARTA_ARAP_PATHWAY	13	-0.4192	-1.8380	0.0126	0.1250	0.995
BIOCARTA_NTHI_PATHWAY	22	-0.3207	-1.8116	0.0160	0.1315	0.9976
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM	70	-0.1841	-1.8060	0.0152	0.1357	0.998
REACTOME_SIGNALING_BY_NOTCH	16	-0.3650	-1.7817	0.0190	0.1446	0.9988
BIOCARTA_PDGF_PATHWAY	30	-0.2731	-1.7784	0.0202	0.1457	0.999
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	75	-0.1749	-1.7793	0.0166	0.1482	0.999
BIOCARTA_BIOPEPTIDES_PATHWAY	40	-0.2398	-1.7724	0.0155	0.1495	0.999
REACTOME_SIGNALING_BY_BMP	20	-0.3312	-1.7631	0.0159	0.1530	0.9992
BIOCARTA_KERATINOCYTE_PATHWAY	42	-0.2277	-1.7469	0.0217	0.1548	0.9994
BIOCARTA_GSK3_PATHWAY	25	-0.2947	-1.7470	0.0226	0.1559	0.9994
BIOCARTA_MCALPAIN_PATHWAY	21	-0.3191	-1.7547	0.0198	0.1559	0.9992
KEGG_WNT_SIGNALING_PATHWAY	135	-0.1273	-1.7398	0.0227	0.1590	1
KEGG_STARCH_AND_SUCROSE_METABOLISM	27	-0.2778	-1.7367	0.0227	0.1595	0.9998
BIOCARTA_MTOR_PATHWAY	22	-0.3091	-1.7278	0.0211	0.1600	0.9996

BIOCARTA_NO1_PATHWAY	29	-0.2738	-1.7310	0.0221	0.1621	0.9996
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	75	-0.1678	-1.7134	0.0255	0.1660	0.9998
KEGG_ADIPOCYTOKINE_SIGNALING_PATHWAY	63	-0.1833	-1.7072	0.0253	0.1673	1
REACTOME_FURTHER_PLATELET_RELEASATE	21	-0.3121	-1.7060	0.0276	0.1681	0.9998
REACTOME_GLYCOGEN_BREAKDOWN_GLYCOGENOLYSIS	13	-0.3951	-1.7157	0.0254	0.1689	0.9998
BIOCARTA_EIF_PATHWAY	13	-0.3941	-1.7059	0.0223	0.1714	1
BIOCARTA_SHH_PATHWAY	14	-0.3767	-1.7075	0.0249	0.1718	1
BIOCARTA_AGPCR_PATHWAY	12	-0.4025	-1.6935	0.0280	0.1761	1
BIOCARTA_WNT_PATHWAY	23	-0.2925	-1.6823	0.0384	0.1774	1
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	109	-0.1395	-1.6867	0.0263	0.1784	1
REACTOME_NF_KB_IS_ACTIVATED_AND_SIGNALS_SURVIVAL	10	-0.4387	-1.6927	0.0269	0.1795	1
REACTOME_POST_NMDA_RECEPTOR_ACTIVATION_EVENTS	29	-0.2627	-1.6813	0.0344	0.1819	1
REACTOME_METABOLISM_OF_CARBOHYDRATES	102	-0.1399	-1.6672	0.0285	0.1864	1
BIOCARTA_CARM1_PATHWAY	12	-0.4025	-1.6685	0.0335	0.1870	1
REACTOME_PLATELET_ACTIVATION	149	-0.1156	-1.6419	0.0374	0.2031	1
BIOCARTA_41BB_PATHWAY	15	-0.3458	-1.6342	0.0365	0.2069	1
REACTOME_G_ALPHA_Z_SIGNALLING_EVENTS	12	-0.3902	-1.6333	0.0413	0.2098	1
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	101	-0.1361	-1.6197	0.0376	0.2191	1
BIOCARTA_PS1_PATHWAY	12	-0.3903	-1.6150	0.0402	0.2214	1
KEGG_GNRH_SIGNALING_PATHWAY	90	-0.1443	-1.5976	0.0436	0.2354	1
BIOCARTA_CK1_PATHWAY	15	-0.3392	-1.5833	0.0481	0.2426	1
BIOCARTA_ARENRF2_PATHWAY	13	-0.3556	-1.5793	0.0450	0.2442	1
SIG_CHEMOTAXIS	41	-0.2068	-1.5734	0.0540	0.2443	1
BIOCARTA_ACE2_PATHWAY	11	-0.3877	-1.5713	0.0546	0.2450	1
BIOCARTA_GPCR_PATHWAY	30	-0.2376	-1.5708	0.0480	0.2462	1

REACTOME_SHCMEDIATED_CASCADE	21	-0.2848	-1.5699	0.0586	0.2469	1
KEGG_RENAL_CELL_CARCINOMA	65	-0.1650	-1.5697	0.0454	0.2471	1
REACTOME_P38MAPK_EVENTS	12	-0.3770	-1.5662	0.0571	0.2478	1

TABLE 21. sActRIIB-Fc + Running vs sActRIIB-Fc Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_MITOCHONDRIAL_FATTY_ACID_BETA_OXIDATION	9	0.7529	2.7412	0.0004	0.0014	0.0086
REACTOME_ELECTRON_TRANSPORT_CHAIN	58	0.3107	2.7866	0.0000	0.0014	0.0064
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	35	0.3910	2.7659	0.0000	0.0016	0.0076
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	23	0.4724	2.7510	0.0000	0.0016	0.0084
REACTOME_COMPLEMENT_CASCADE	17	0.5763	2.8673	0.0000	0.0018	0.0036
KEGG_PEROXISOME	73	0.2861	2.8759	0.0000	0.0022	0.0038
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	60	0.3147	2.8788	0.0000	0.0026	0.0032
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	82	0.2456	2.5928	0.0000	0.0040	0.0306
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	38	0.3437	2.5064	0.0000	0.0071	0.0632
BIOCARTA_COMP_PATHWAY	16	0.5027	2.4489	0.0000	0.0098	0.0958
REACTOME_SYNTHESIS_OF_BILE_ACIDS_ AND_BILE_SALTS_VIA_7ALPHA_HYDROXYCHOLESTEROL	14	0.5382	2.4404	0.0004	0.0106	0.1038
KEGG_PYRIMIDINE_METABOLISM	84	0.2242	2.4105	0.0004	0.0121	0.1276
REACTOME_METABOLISM_OF_AMINO_ACIDS	143	0.1696	2.3992	0.0000	0.0125	0.1416
KEGG_RETINOL_METABOLISM	28	0.3642	2.2796	0.0000	0.0255	0.3072
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	39	0.3048	2.2779	0.0000	0.0255	0.3100
KEGG_BUTANOATE_METABOLISM	30	0.3491	2.2675	0.0004	0.0263	0.3282

BIOCARTA_ERYTH_PATHWAY	14	0.4914	2.2143	0.0008	0.0305	0.4364
SA_PROGRAMMED_CELL_DEATH	12	0.5282	2.2051	0.0004	0.0339	0.4584
REACTOME_G_BETA_GAMMA_SIGNALLING_THROUGH_PLC_BETA	19	0.4184	2.1897	0.0012	0.0351	0.4892
KEGG_PRION_DISEASES	31	0.3305	2.1961	0.0008	0.0352	0.4738
KEGG_HUNTINGTONS_DISEASE	142	0.1565	2.1823	0.0032	0.0355	0.5036
REACTOME_HIV1_TRANSCRIPTION_INITIATION	31	0.3319	2.1837	0.0004	0.0360	0.5012
REACTOME_FORMATION_OF_FIBRIN_CLOT_CLOTTING_CASCADE	29	0.3325	2.1561	0.0023	0.0361	0.5658
KEGG_FATTY_ACID_METABOLISM	34	0.3065	2.1616	0.0012	0.0365	0.5554
REACTOME_INTRINSIC_PATHWAY_FOR_APOPTOSIS	29	0.3396	2.1520	0.0028	0.0372	0.5794
REACTOME_BIOLOGICAL_OXIDATIONS	74	0.2117	2.1323	0.0027	0.0395	0.6282
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	12	0.5026	2.1235	0.0016	0.0419	0.6490
REACTOME_BASE_FREE_SUGAR_PHOSPHATE_REMOVAL_ VIA_THE_SINGLE_NUCLEOTIDE_REPLACEMENT_PATHWAY	10	0.5421	2.0957	0.0051	0.0435	0.7124
KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS	11	0.5140	2.0815	0.0012	0.0456	0.7444
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_12	19	0.3957	2.0641	0.0039	0.0479	0.7854
BIOCARTA_PTC1_PATHWAY	10	0.5395	2.0741	0.0036	0.0480	0.7638
REACTOME_REMOVAL_OF_DNA_PATCH_CONTAINING_ABASIC_RESIDUE	15	0.4422	2.0620	0.0035	0.0481	0.7902
REACTOME_STEROID_METABOLISM	51	0.2444	2.0714	0.0028	0.0484	0.7702
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS	18	0.4032	2.0720	0.0024	0.0485	0.7642
KEGG_OXIDATIVE_PHOSPHORYLATION	101	0.1766	2.0652	0.0049	0.0490	0.7850
KEGG_NITROGEN_METABOLISM	8	0.5826	2.0349	0.0025	0.0523	0.8470
REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS	195	0.1232	2.0233	0.0059	0.0542	0.8602
KEGG_PPAR_SIGNALING_PATHWAY	60	0.2203	2.0139	0.0047	0.0562	0.8808
REACTOME_METABOLISM_OF_BILE_ACIDS_AND_BILE_SALTS	23	0.3492	2.0111	0.0051	0.0565	0.8858
REACTOME_HIV_INFECTION	163	0.1346	2.0131	0.0056	0.0568	0.8786
BIOCARTA_LECTIN_PATHWAY	10	0.5238	2.0024	0.0051	0.0568	0.9016

KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	29	0.3141	2.0036	0.0052	0.0581	0.8954
KEGG_GLUTATHIONE_METABOLISM	46	0.2476	1.9921	0.0067	0.0596	0.9068
KEGG_TRYPTOPHAN_METABOLISM	36	0.2798	1.9944	0.0051	0.0596	0.9064
BIOCARTA_CASPASE_PATHWAY	22	0.3457	1.9504	0.0045	0.0703	0.9532
REACTOME_G1_S_TRANSITION	98	0.1682	1.9411	0.0071	0.0726	0.9634
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	15	0.4143	1.9369	0.0097	0.0737	0.9650
REACTOME_THROMBOXANE_SIGNALLING_THROUGH_TP_RECEPTOR	22	0.3407	1.9116	0.0084	0.0828	0.9796
REACTOME_ACTIVATION_OF_KAINATE_RECEPTORS_UPON_GLUTAMATE_BINDING	30	0.2957	1.9021	0.0073	0.0868	0.9842
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_1	24	0.3255	1.8930	0.0104	0.0869	0.9856
REACTOME_FORMATION_OF_A_POOL_OF_FREE_40S_SUBUNITS	66	0.1949	1.8868	0.0096	0.0888	0.9892
REACTOME_G_PROTEIN_ACTIVATION	26	0.3127	1.8824	0.0110	0.0901	0.9916
KEGG_PROPANOATE_METABOLISM	27	0.3000	1.8640	0.0098	0.0951	0.9942
KEGG_PARKINSONS_DISEASE	101	0.1567	1.8617	0.0118	0.0957	0.9932
REACTOME_MITOTIC_M_M_G1_PHASES	143	0.1337	1.8586	0.0077	0.0962	0.9952
REACTOME_SCF_SKP2_MEDIATED_DEGRADATION_OF_P27_P21	50	0.2235	1.8586	0.0152	0.0969	0.9944
REACTOME_BASE_EXCISION_REPAIR	18	0.3644	1.8502	0.0136	0.0994	0.9950
REACTOME_TRANSCRIPTION_COUPLED_NER	39	0.2487	1.8375	0.0139	0.1022	0.9960
BIOCARTA_EXTRINSIC_PATHWAY	13	0.4225	1.8346	0.0160	0.1028	0.9974
BIOCARTA_CLASSIC_PATHWAY	11	0.4602	1.8375	0.0155	0.1031	0.9954
REACTOME_MRNA_PROCESSING	26	0.3010	1.8369	0.0137	0.1040	0.9964
REACTOME_DUAL_INCISION_REACTION_IN_TC_NER	23	0.3196	1.8221	0.0150	0.1068	0.9982
REACTOME_CELL_CYCLE_MITOTIC	277	0.0943	1.8167	0.0155	0.1088	0.9986
REACTOME_INHIBITION_OF_INSULIN_SECRETION_BY_ADRENALINE_NORADRENALINE	26	0.2998	1.8161	0.0132	0.1096	0.9990
KEGG_CELL_CYCLE	116	0.1437	1.8029	0.0160	0.1129	0.9990
KEGG_GRAFT_VERSUS_HOST_DISEASE	13	0.4143	1.8046	0.0148	0.1140	0.9990

REACTOME_THROMBIN_SIGNALLING_THROUGH_PROTEINASE_ACTIVATED_RECEPTORS	26	0.2953	1.7959	0.0138	0.1159	0.9996
KEGG_NUCLEOTIDE_EXCISION_REPAIR	42	0.2328	1.7851	0.0158	0.1189	0.9998
REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT	86	0.1639	1.7828	0.0151	0.1201	0.9994
REACTOME_DNA_REPAIR	91	0.1584	1.7698	0.0170	0.1238	0.9998
KEGG_ARACHIDONIC_ACID_METABOLISM	44	0.2233	1.7688	0.0189	0.1261	0.9998
KEGG_TYROSINE_METABOLISM	32	0.2591	1.7470	0.0220	0.1333	0.9998
REACTOME_CHYLOMICRON_MEDIATED_LIPID_TRANSPORT	16	0.3607	1.7405	0.0178	0.1363	1.0000
REACTOME_PYRIMIDINE_METABOLISM	19	0.3314	1.7222	0.0268	0.1383	0.9998
KEGG_BASAL_TRANSCRIPTION_FACTORS	29	0.2685	1.7333	0.0271	0.1385	1.0000
KEGG_BIOSYNTHESIS_OF_UNSATURATED_FATTY_ACIDS	18	0.3401	1.7284	0.0289	0.1398	1.0000
BIOCARTA_TH1TH2_PATHWAY	15	0.3681	1.7179	0.0256	0.1398	1.0000
REACTOME_COMMON_PATHWAY	13	0.3949	1.7275	0.0237	0.1399	1.0000
REACTOME_PEPTIDE_CHAIN_ELONGATION	59	0.1916	1.7230	0.0229	0.1401	1.0000
REACTOME_PHASE_II_CONJUGATION	35	0.2452	1.7138	0.0269	0.1421	1.0000
KEGG_RNA_POLYMERASE	20	0.3218	1.7206	0.0224	0.1422	1.0000
REACTOME_CYCLIN_E_ASSOCIATED_EVENTS_DURING_G1_S_TRANSITION_	55	0.1950	1.7140	0.0236	0.1423	1.0000
REACTOME_EARLY_PHASE_OF_HIV_LIFE_CYCLE	13	0.3876	1.7066	0.0256	0.1441	1.0000
KEGG_RIBOSOME	61	0.1857	1.7065	0.0222	0.1450	1.0000
REACTOME_APOPTOSIS	120	0.1362	1.7064	0.0244	0.1452	1.0000
KEGG_SNARE_INTERACTIONS_IN_VESICULAR_TRANSPORT	32	0.2522	1.6959	0.0318	0.1470	1.0000
KEGG_P53_SIGNALING_PATHWAY	61	0.1850	1.6941	0.0293	0.1474	1.0000
BIOCARTA_INTRINSIC_PATHWAY	23	0.2996	1.6968	0.0298	0.1483	1.0000
BIOCARTA_FIBRINOLYSIS_PATHWAY	12	0.4053	1.6956	0.0276	0.1486	1.0000
BIOCARTA_CHEMICAL_PATHWAY	21	0.3051	1.6794	0.0308	0.1490	1.0000
REACTOME_SYNTHESIS_OF_DNA	86	0.1541	1.6777	0.0307	0.1492	1.0000

REACTOME_HIV_LIFE_CYCLE	92	0.1514	1.6809	0.0330	0.1499	1.0000
REACTOME_TRANSCRIPTION	155	0.1156	1.6789	0.0257	0.1506	1.0000
BIOCARTA_AMI_PATHWAY	19	0.3199	1.6662	0.0354	0.1519	1.0000
REACTOME_MITOTIC_PROMETAPHASE	79	0.1580	1.6672	0.0241	0.1527	1.0000
REACTOME_S_PHASE	97	0.1463	1.6756	0.0270	0.1532	1.0000
KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM	26	0.2719	1.6649	0.0288	0.1537	1.0000
REACTOME_RESOLUTION_OF_AP_SITES_ VIA_THE_SINGLE_NUCLEOTIDE_REPLACEMENT_PATHWAY	12	0.3921	1.6547	0.0358	0.1537	1.0000
KEGG_NICOTINATE_AND_NICOTINAMIDE_METABOLISM	21	0.2993	1.6567	0.0300	0.1548	1.0000
REACTOME_RNA_POLYMERASE_I_TRANSCRIPTION_TERMINATION	17	0.3315	1.6580	0.0335	0.1562	1.0000
REACTOME_M_G1_TRANSITION	60	0.1808	1.6528	0.0325	0.1566	1.0000
BIOCARTA_P53_PATHWAY	14	0.3675	1.6438	0.0358	0.1582	1.0000
REACTOME_REGULATION_OF_GENE_EXPRESSION_IN_BETA_CELLS	74	0.1625	1.6465	0.0391	0.1584	1.0000
REACTOME_RNA_POLYMERASE_I_PROMOTER_ESCAPE	16	0.3397	1.6435	0.0408	0.1593	1.0000
REACTOME_INTRINSIC_PATHWAY	15	0.3519	1.6423	0.0368	0.1594	1.0000
KEGG_PROTEASOME	41	0.2161	1.6372	0.0382	0.1600	1.0000
REACTOME_CELL_CYCLE_CHECKPOINTS	106	0.1352	1.6352	0.0331	0.1600	1.0000
REACTOME_PEROXISOMAL_LIPID_METABOLISM	19	0.3125	1.6321	0.0437	0.1605	1.0000
KEGG_ALLOGRAFT_REJECTION	15	0.3486	1.6387	0.0368	0.1608	1.0000
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	31	0.2476	1.6307	0.0442	0.1625	1.0000
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	71	0.1654	1.6274	0.0441	0.1635	1.0000
REACTOME_LIPOPROTEIN_METABOLISM	23	0.2814	1.6143	0.0437	0.1659	1.0000
REACTOME_FORMATION_OF_THE_EARLY_ELONGATION_COMPLEX	27	0.2623	1.6185	0.0361	0.1663	1.0000
KEGG_PURINE_METABOLISM	136	0.1183	1.6165	0.0415	0.1665	1.0000
REACTOME_DNA_REPLICATION_PRE_INITIATION	74	0.1605	1.6130	0.0395	0.1672	1.0000

REACTOME_REGULATION_OF_APC_ACTIVATORS_BETWEEN_G1_S_AND_EARLY_ANAPHASE	69	0.1648	1.6078	0.0441	0.1672	1.0000
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	14	0.3565	1.6073	0.0497	0.1683	1.0000
BIOCARTA_P27_PATHWAY	12	0.3796	1.6064	0.0413	0.1686	1.0000
REACTOME_CYCLIN_A1_ASSOCIATED_EVENTS_DURING_G2_M_TRANSITION	14	0.3527	1.6075	0.0486	0.1690	1.0000
REACTOME_NUCLEOTIDE_EXCISION_REPAIR	44	0.2040	1.6039	0.0372	0.1693	1.0000
REACTOME_METABOLISM_OF_PROTEINS	170	0.1051	1.5976	0.0454	0.1705	1.0000
REACTOME_METABLISM_OF_NUCLEOTIDES	63	0.1705	1.6008	0.0409	0.1706	1.0000
REACTOME_RNA_POLYMERASE_I_PROMOTER_CLEARANCE	60	0.1735	1.5911	0.0480	0.1735	1.0000
REACTOME_SCF_BETA_TRCP_MEDIATED_DEGRADATION_OF_EMI1	46	0.1959	1.5826	0.0506	0.1738	1.0000
REACTOME_DIABETES_PATHWAYS	310	0.0781	1.5808	0.0551	0.1756	1.0000
REACTOME_RNA_POLYMERASE_I_PROMOTER_OPENING	41	0.2068	1.5861	0.0474	0.1757	1.0000
KEGG_LYSOSOME	111	0.1288	1.5735	0.0467	0.1773	1.0000
KEGG_STEROID_HORMONE_BIOSYNTHESIS	29	0.2446	1.5779	0.0536	0.1777	1.0000
BIOCARTA_G2_PATHWAY	22	0.2832	1.5788	0.0529	0.1779	1.0000
BIOCARTA_CYTOKINE_PATHWAY	19	0.3014	1.5729	0.0507	0.1783	1.0000
REACTOME_SIGNAL_AMPLIFICATION	29	0.2450	1.5728	0.0501	0.1791	1.0000
REACTOME_VIRAL_MRNA_TRANSLATION	59	0.1746	1.5685	0.0539	0.1793	1.0000
REACTOME_STABILIZATION_OF_P53	44	0.1989	1.5670	0.0491	0.1804	1.0000
REACTOME_GLUCOSE_REGULATION_OF_INSULIN_SECRETION	130	0.1175	1.5624	0.0551	0.1806	1.0000
REACTOME_REGULATION_OF_INSULIN_SECRETION	177	0.1010	1.5651	0.0518	0.1807	1.0000
REACTOME_FORMATION_OF_THE_TERNARY_COMPLEX_ AND_SUBSEQUENTLY_THE_43S_COMPLEX	36	0.2196	1.5598	0.0537	0.1834	1.0000
REACTOME_P53_INDEPENDENT_DNA_DAMAGE_RESPONSE	42	0.2021	1.5579	0.0511	0.1837	1.0000
REACTOME_CDC20_PHOSPHO_APC_MEDIATED_DEGRADATION_OF_CYCLIN_A	62	0.1673	1.5618	0.0503	0.1840	1.0000
BIOCARTA_ARF_PATHWAY	15	0.3339	1.5586	0.0517	0.1847	1.0000

REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	33	0.2316	1.5558	0.0626	0.1853	1.0000
REACTOME_PHASE_1_FUNCTIONALIZATION_OF_COMPOUNDS	39	0.2067	1.5342	0.0598	0.1947	1.0000
BIOCARTA_MITOCHONDRIA_PATHWAY	20	0.2862	1.5274	0.0611	0.1952	1.0000
REACTOME_TELOMERE_MAINTENANCE	63	0.1631	1.5278	0.0652	0.1957	1.0000
REACTOME_CYTOSOLIC_SULFONATION_OF_SMALL_MOLECULES	6	0.5006	1.5305	0.0652	0.1959	1.0000
REACTOME_CDT1_ASSOCIATION_WITH_THE_CDC6_ORC_ORIGIN_COMPLEX	51	0.1797	1.5246	0.0645	0.1960	1.0000
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	16	0.3145	1.5288	0.0636	0.1962	1.0000
REACTOME_GLUCAGON_TYPE_LIGAND_RECEPTORS	30	0.2351	1.5260	0.0669	0.1972	1.0000
BIOCARTA_TCAPOPTOSIS_PATHWAY	8	0.4368	1.5196	0.0605	0.1998	1.0000
REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	62	0.1630	1.5190	0.0657	0.2003	1.0000
REACTOME_GLUTATHIONE_CONJUGATION	16	0.3130	1.5150	0.0700	0.2007	1.0000
BIOCARTA_D4GDI_PATHWAY	12	0.3636	1.5146	0.0679	0.2010	1.0000
REACTOME_SNRNP_ASSEMBLY	46	0.1878	1.5107	0.0761	0.2028	1.0000
REACTOME_SYNTHESIS_AND_INTERCONVERSION_OF_NUCLEOTIDE_DI_AND_TRIPHOSPHATES	16	0.3142	1.5097	0.0677	0.2044	1.0000
KEGG_LYSINE_DEGRADATION	40	0.2010	1.5053	0.0721	0.2053	1.0000
REACTOME_POST_TRANSLATIONAL_PROTEIN_MODIFICATION	35	0.2115	1.5027	0.0669	0.2058	1.0000
REACTOME_RNA_POLYMERASE_I_CHAIN_ELONGATION	24	0.2564	1.5039	0.0714	0.2071	1.0000
KEGG_BASE_EXCISION_REPAIR	32	0.2242	1.5005	0.0697	0.2073	1.0000
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	221	0.0880	1.4996	0.0711	0.2085	1.0000
BIOCARTA_ATM_PATHWAY	17	0.3005	1.4883	0.0811	0.2128	1.0000
REACTOME_GAMMA_CARBOXYLATION_TRANSPORT_ AND_AMINO_TERMINAL_CLEAVAGE_OF_PROTEINS	10	0.3839	1.4886	0.0762	0.2140	1.0000
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	21	0.2701	1.4884	0.0724	0.2144	1.0000
REACTOME_DOUBLE_STRAND_BREAK_REPAIR	19	0.2822	1.4797	0.0796	0.2166	1.0000
REACTOME_VIF_MEDIATED_DEGRADATION_OF_APOBEC3G	45	0.1862	1.4796	0.0817	0.2171	1.0000
SA_REG_CASCADE_OF_CYCLIN_EXPR	12	0.3547	1.4831	0.0762	0.2177	1.0000

KEGG_ASCORBATE_AND_ALDARATE_METABOLISM	10	0.3850	1.4754	0.0888	0.2195	1.0000
KEGG_TAURINE_AND_HYPOTAURINE_METABOLISM	10	0.3842	1.4759	0.0793	0.2205	1.0000
REACTOME_PYRIMIDINE_CATABOLISM	8	0.4202	1.4642	0.0813	0.2244	1.0000
REACTOME_HOMOLOGOUS_RECOMBINATION_REPAIR	13	0.3318	1.4631	0.0818	0.2264	1.0000
REACTOME_RNA_POL_II_CTD_PHOSPHORYLATION_AND_INTERACTION_WITH_CE	21	0.2636	1.4613	0.0919	0.2267	1.0000
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	44	0.1844	1.4547	0.0849	0.2280	1.0000
REACTOME_REGULATION_OF_INSULIN_LIKE_GROWTH_FACTOR_ACTIVITY_ BY_INSULIN_LIKE_GROWTH_FACTOR_BINDING_PROTEINS	12	0.3478	1.4541	0.0894	0.2298	1.0000
REACTOME_RNA_POLYMERASE_II_TRANSCRIPTION	72	0.1456	1.4482	0.0873	0.2317	1.0000
KEGG_ALZHEIMERS_DISEASE	139	0.1060	1.4499	0.0858	0.2321	1.0000
REACTOME_INFLUENZA_LIFE_CYCLE	104	0.1210	1.4502	0.0903	0.2324	1.0000
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	41	0.1914	1.4502	0.0905	0.2334	1.0000
REACTOME_TRANSCRIPTION_OF_THE_HIV_GENOME	50	0.1732	1.4473	0.0782	0.2336	1.0000
REACTOME_MRNA_DECAY_BY_5_TO_3_EXORIBONUCLEASE	11	0.3590	1.4465	0.0919	0.2339	1.0000
REACTOME_PHASE_1_FUNCTIONALIZATION	11	0.3600	1.4424	0.0923	0.2366	1.0000
KEGG_HISTIDINE_METABOLISM	25	0.2456	1.4416	0.0892	0.2370	1.0000
REACTOME_RNA_POLYMERASE_I_TRANSCRIPTION_INITIATION	20	0.2668	1.4348	0.0916	0.2390	1.0000
BIOCARTA_RB_PATHWAY	11	0.3584	1.4347	0.1003	0.2405	1.0000
BIOCARTA_HSP27_PATHWAY	14	0.3133	1.4220	0.0958	0.2463	1.0000
BIOCARTA_CTL_PATHWAY	10	0.3676	1.4212	0.1042	0.2475	1.0000
REACTOME_G_BETA_GAMMA_SIGNALLING_THROUGH_PI3KGAMMA	24	0.2421	1.4166	0.1063	0.2483	1.0000

TABLE 22. PBS vs Control Down-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
REACTOME_ELECTRON_TRANSPORT_CHAIN	58	-0.3372	-3.0090	0.0000	0.0002	0.0002
REACTOME_METABOLISM_OF_LIPIDS_AND_LIPOPROTEINS	195	-0.1765	-2.8861	0.0000	0.0004	0.0024
KEGG_PPAR_SIGNALING_PATHWAY	60	-0.3145	-2.9166	0.0000	0.0004	0.0014
REACTOME_BIOLOGICAL_OXIDATIONS	74	-0.2782	-2.8024	0.0000	0.0010	0.0056
KEGG_PEROXISOME	73	-0.2739	-2.7613	0.0000	0.0012	0.0084
KEGG_VALINE_LEUCINE_AND_ISOLEUCINE_DEGRADATION	39	-0.3556	-2.6080	0.0000	0.0033	0.0304
KEGG_OXIDATIVE_PHOSPHORYLATION	101	-0.2156	-2.5443	0.0004	0.0046	0.049
REACTOME_REGULATION_OF_LIPID_METABOLISM_ BY_PEROXISOME_PROLIFERATOR_ACTIVATED_RECEPTOR_ALPHA	50	-0.3024	-2.4994	0.0000	0.0052	0.0686
KEGG_METABOLISM_OF_XENOBIOTICS_BY_CYTOCHROME_P450	35	-0.3505	-2.4809	0.0000	0.0061	0.0794
REACTOME_INTEGRATION_OF_ENERGY_METABOLISM	193	-0.1503	-2.4176	0.0000	0.0083	0.1288
REACTOME_MITOCHONDRIAL_FATTY_ACID_BETA_OXIDATION	9	-0.6570	-2.3936	0.0000	0.0099	0.1534
BIOCARTA_PGC1A_PATHWAY	21	-0.4227	-2.3172	0.0012	0.0141	0.2548
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	38	-0.3119	-2.2961	0.0012	0.0153	0.2826
BIOCARTA_ETC_PATHWAY	9	-0.6252	-2.2955	0.0008	0.0153	0.2976
KEGG_PARKINSONS_DISEASE	101	-0.1915	-2.2607	0.0004	0.0168	0.3456
KEGG_PROPANOATE_METABOLISM	27	-0.3588	-2.2536	0.0012	0.0172	0.3618
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS	18	-0.4457	-2.2534	0.0021	0.0174	0.3638
REACTOME_PHASE_II_CONJUGATION	35	-0.3186	-2.2320	0.0012	0.0179	0.4046
REACTOME_BRANCHED_CHAIN_AMINO_ACID_CATABOLISM	16	-0.4675	-2.2372	0.0012	0.0181	0.3922
KEGG_FATTY_ACID_METABOLISM	34	-0.3173	-2.1922	0.0016	0.0211	0.4912
REACTOME_SYNTHESIS_OF_BILE_ACIDS_AND_BILE_SALTS_VIA_7ALPHA_HYDROXYCHOLESTEROL	14	-0.4843	-2.1764	0.0008	0.0231	0.5282
REACTOME_GLUCOSE_REGULATION_OF_INSULIN_SECRETION	130	-0.1593	-2.1234	0.0023	0.0304	0.6518

REACTOME_TRNA_AMINOACYLATION	38	-0.2863	-2.1097	0.0032	0.0316	0.6784
REACTOME_PHASE_1_FUNCTIONALIZATION_OF_COMPOUNDS	39	-0.2794	-2.0514	0.0035	0.0444	0.7966
KEGG_PRIMARY_BILE_ACID_BIOSYNTHESIS	15	-0.4345	-2.0264	0.0036	0.0501	0.8502
REACTOME_TRIACYLGLYCERIDE_BIOSYNTHESIS	12	-0.4643	-1.9554	0.0051	0.0685	0.9472
REACTOME_NUCLEAR_RECEPTOR_TRANSCRIPTION_PATHWAY	48	-0.2384	-1.9520	0.0094	0.0699	0.9472
KEGG_GLUTATHIONE_METABOLISM	46	-0.2439	-1.9521	0.0040	0.0711	0.9432
REACTOME_ETHANOL_OXIDATION	6	-0.6376	-1.9400	0.0056	0.0733	0.9574
REACTOME_METABOLISM_OF_AMINO_ACIDS	143	-0.1369	-1.9079	0.0096	0.0840	0.9752
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	39	-0.2558	-1.8825	0.0099	0.0939	0.9884
KEGG_PYRUVATE_METABOLISM	34	-0.2633	-1.8350	0.0153	0.1157	0.9966
REACTOME_ACTIVATED_TAK1_MEDIATES_P38_MAPK_ACTIVATION	13	-0.4035	-1.7942	0.0208	0.1322	0.9992
KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_METABOLISM	29	-0.2781	-1.7980	0.0139	0.1337	0.9994
REACTOME_GLUCOSE_METABOLISM	50	-0.2140	-1.7865	0.0134	0.1380	0.9994
REACTOME_METABOLISM_OF_BILE_ACIDS_AND_BILE_SALTS	23	-0.3128	-1.7771	0.0161	0.1423	0.9994
REACTOME_VITAMIN_B5_(PANTOTHENATE)_METABOLISM	11	-0.4421	-1.7679	0.0227	0.1448	1.0000
REACTOME_REGULATION_OF_PYRUVATE_DEHYDROGENASE_COMPLEX	11	-0.4346	-1.7641	0.0244	0.1468	1.0000
REACTOME_FRS2MEDIATED_CASCADE	26	-0.2927	-1.7570	0.0212	0.1478	1.0000
REACTOME_MICRORNA_BIOGENESIS	14	-0.3889	-1.7551	0.0266	0.1496	1.0000
REACTOME_CYTOSOLIC_TRNA_AMINOACYLATION	23	-0.3031	-1.7285	0.0256	0.1579	1.0000
REACTOME_COMMON_PATHWAY	13	-0.3893	-1.7175	0.0224	0.1594	1.0000
KEGG_GLYCINE_SERINE_AND_THREONINE_METABOLISM	31	-0.2586	-1.7238	0.0248	0.1596	1.0000
REACTOME_PHOSPHOLIPASE_CMEDIATED_CASCADE	22	-0.3031	-1.7084	0.0239	0.1643	1.0000
BIOCARTA_NUCLEARRS_PATHWAY	13	-0.3846	-1.6893	0.0282	0.1689	1.0000
REACTOME_ACTIVATED_AMPK_STIMULATES_FATTY_ACID_OXIDATION_IN_MUSCLE	15	-0.3614	-1.6783	0.0281	0.1696	1.0000
KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS	11	-0.4232	-1.6902	0.0279	0.1713	1.0000

KEGG_RNA_POLYMERASE	20	-0.3178	-1.6867	0.0264	0.1720	1.0000
REACTOME_DOWNSTREAM_SIGNALING_OF_ACTIVATED_FGFR	40	-0.2251	-1.6808	0.0329	0.1720	1.0000
REACTOME_GLUTATHIONE_CONJUGATION	16	-0.3465	-1.6720	0.0311	0.1742	1.0000
KEGG_NITROGEN_METABOLISM	8	-0.4813	-1.6756	0.0278	0.1752	1.0000
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	60	-0.1857	-1.6747	0.0267	0.1753	1.0000
REACTOME_MITOCHONDRIAL_TRNA_AMINOACYLATION	18	-0.3236	-1.6575	0.0323	0.1820	1.0000
REACTOME_APOPTOSIS_INDUCED_DNA_FRAGMENTATION	11	-0.4028	-1.6393	0.0307	0.1910	1.0000
REACTOME_IRS_RELATED_EVENTS	74	-0.1618	-1.6336	0.0404	0.1928	1.0000
REACTOME_ENDOGENOUS_STEROLS	12	-0.3895	-1.6248	0.0367	0.1981	1.0000
REACTOME_GLYCOGEN_BREAKDOWN_GLYCOGENOLYSIS	13	-0.3668	-1.5969	0.0463	0.2181	1.0000
REACTOME_PI3K_CASCADE	35	-0.2236	-1.5778	0.0520	0.2302	1.0000
REACTOME_REGULATION_OF_INSULIN_SECRETION	177	-0.1021	-1.5794	0.0478	0.2343	1.0000
KEGG_SELENOAMINO_ACID_METABOLISM	24	-0.2664	-1.5599	0.0535	0.2489	1.0000

TABLE 23. PBS vs Control Up-regulated pathways

NAME	SIZE	ES	NES	NOM p-val	FDR q-val	FWER p-val
KEGG_CHEMOKINE_SIGNALING_PATHWAY	161	0.2836	4.1756	0.0000	0.0000	0
KEGG_FOCAL_ADHESION	174	0.2473	3.8080	0.0000	0.0000	0.0000
KEGG_LYSOSOME	111	0.3638	4.4622	0.0000	0.0000	0
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY	94	0.3077	3.5153	0.0000	0.0000	0.0000
REACTOME_AXON_GUIDANCE	131	0.3001	3.9898	0.0000	0.0000	0
REACTOME_FORMATION_OF_PLATELET_PLUG	168	0.2331	3.4957	0.0000	0.0000	0.0000
REACTOME_HEMOSTASIS	248	0.2243	4.0617	0.0000	0.0000	0

REACTOME_PLATELET_ACTIVATION	149	0.2534	3.5964	0.0000	0.0000	0.0000
REACTOME_SIGNALING_IN_IMMUNE_SYSTEM	268	0.2579	4.9065	0.0000	0.0000	0
REACTOME_TOLL_LIKE_RECEPTOR_4_CASCADE	25	0.6064	3.6090	0.0000	0.0000	0.0000
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	44	0.4051	3.1589	0.0000	0.0000	0.0004
REACTOME_TOLL_RECEPTOR_CASCADES	79	0.3003	3.1737	0.0000	0.0000	0.0002
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	186	0.2061	3.2706	0.0000	0.0000	0.0002
REACTOME_CELL_SURFACE_INTERACTIONS_AT_THE_VASCULAR_WALL	83	0.2890	3.0758	0.0000	0.0000	0.0006
KEGG_FC_GAMMA_R_MEDIATED_PHAGOCYTOSIS	85	0.2843	3.1158	0.0000	0.0000	0.0006
KEGG_ECM_RECEPTOR_INTERACTION	74	0.3158	3.1583	0.0000	0.0000	0.0004
REACTOME_ACTIVATED_TLR4_SIGNALLING	21	0.5605	3.0918	0.0000	0.0000	0.0008
REACTOME_INNATE_IMMUNITY_SIGNALING	96	0.2740	3.1485	0.0000	0.0000	0.0004
REACTOME_SIGNALING_BY_PDGF	60	0.3207	2.9055	0.0000	0.0000	0.0028
KEGG_PATHWAYS_IN_CANCER	293	0.1576	3.1015	0.0000	0.0000	0.0006
REACTOME_SEMAPHORIN_INTERACTIONS	57	0.3383	3.0016	0.0000	0.0000	0.001
KEGG_AXON_GUIDANCE	113	0.2195	2.7194	0.0004	0.0000	0.0104
REACTOME_NCAM_SIGNALING_FOR_NEURITE_OUT_GROWTH	56	0.3348	2.9515	0.0000	0.0000	0.0020
KEGG_SMALL_CELL_LUNG_CANCER	78	0.2743	2.8453	0.0000	0.0000	0.0040
KEGG_LEISHMANIA_INFECTION	51	0.3524	2.9713	0.0000	0.0000	0.0020
REACTOME_IMMUNOREGULATORY_INTERACTIONS_ BETWEEN_A_LYMPHOID_AND_A_NON_LYMPHOID_CELL	44	0.3814	2.9749	0.0000	0.0000	0.0020
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION	221	0.1686	2.8927	0.0000	0.0000	0.0026
KEGG_ENDOCYTOSIS	150	0.2061	2.9032	0.0000	0.0000	0.0028
REACTOME_RHO_GTPASE_CYCLE	99	0.2548	2.9785	0.0000	0.0000	0.0020
BIOCARTA_HIVNEF_PATHWAY	51	0.3459	2.9371	0.0000	0.0000	0.0024
KEGG_VIRAL_MYOCARDITIS	41	0.3827	2.8887	0.0000	0.0000	0.0030

REACTOME_NCAM1_INTERACTIONS	39	0.3920	2.8827	0.0000	0.0000	0.0026
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	101	0.2440	2.8694	0.0000	0.0000	0.0034
REACTOME_PLATELET_DEGRANULATION	77	0.2771	2.8666	0.0004	0.0000	0.0034
BIOCARTA_RHO_PATHWAY	27	0.4443	2.7590	0.0000	0.0000	0.0064
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	65	0.2913	2.7507	0.0000	0.0002	0.0096
KEGG_APOPTOSIS	74	0.2717	2.7399	0.0000	0.0002	0.0084
REACTOME_MYD88_CASCADE	16	0.5661	2.7078	0.0000	0.0002	0.0114
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	98	0.2336	2.6969	0.0000	0.0002	0.0128
ST_T_CELL_SIGNAL_TRANSDUCTION	41	0.3508	2.6733	0.0000	0.0004	0.0162
REACTOME_INTEGRIN_CELL_SURFACE_INTERACTIONS	75	0.2671	2.7193	0.0000	0.0004	0.0102
BIOCARTA_IL10_PATHWAY	17	0.5394	2.6710	0.0000	0.0006	0.0192
KEGG_SPHINGOLIPID_METABOLISM	36	0.3719	2.6785	0.0000	0.0006	0.0152
REACTOME_MITOTIC_PROMETAPHASE	79	0.2509	2.6198	0.0000	0.0008	0.0282
BIOCARTA_TNFR2_PATHWAY	18	0.5117	2.5967	0.0008	0.0010	0.036
SA_G1_AND_S_PHASES	12	0.6094	2.5752	0.0000	0.0010	0.0414
KEGG_HYPERTROPHIC_CARDIOMYOPATHY_HCM	75	0.2523	2.5725	0.0004	0.0010	0.0432
KEGG_EPITHELIAL_CELL_SIGNALING_IN_HELICOBACTER_PYLORI_INFECTION	62	0.2799	2.6016	0.0000	0.0010	0.0346
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	88	0.2354	2.5840	0.0000	0.0010	0.0386
REACTOME_MEMBRANE_TRAFFICKING	67	0.2743	2.6200	0.0000	0.0010	0.0278
KEGG_HEMATOPOIETIC_CELL_LINEAGE	63	0.2790	2.5993	0.0000	0.0010	0.033
REACTOME_CLATHRIN_DERIVED_VESICLE_BUDDING	52	0.3001	2.5287	0.0000	0.0015	0.0568
REACTOME_G_ALPHA_12_13_SIGNALLING_EVENTS	45	0.3179	2.5324	0.0000	0.0015	0.0572
BIOCARTA_TNFR1_PATHWAY	26	0.4119	2.5205	0.0004	0.0016	0.0628
REACTOME_PLATELET_ACTIVATION_TRIGGERS	54	0.2882	2.4998	0.0004	0.0017	0.0694
KEGG_DILATED_CARDIOMYOPATHY	81	0.2360	2.5037	0.0004	0.0017	0.0680

BIOCARTA_CELLCYCLE_PATHWAY	21	0.4560	2.5070	0.0000	0.0017	0.0686
KEGG_ARRHYTHMOGENIC_RIGHT_VENTRICULAR_CARDIOMYOPATHY_ARVC	68	0.2549	2.4950	0.0000	0.0018	0.0726
KEGG_OTHER_GLYCAN_DEGRADATION	16	0.5124	2.4900	0.0004	0.0018	0.0748
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	50	0.3007	2.4836	0.0004	0.0019	0.0788
REACTOME_G_PROTEIN_BETA_GAMMA_SIGNALLING	27	0.3949	2.4834	0.0004	0.0019	0.0782
BIOCARTA_NFKB_PATHWAY	22	0.4444	2.4891	0.0000	0.0019	0.0790
REACTOME_INTRINSIC_PATHWAY_FOR_APOPTOSIS	29	0.3877	2.4677	0.0000	0.0020	0.0874
SIG_PIP3_SIGNALING_IN_B_LYMPHOCYTES	29	0.3875	2.4658	0.0000	0.0021	0.0910
KEGG_PANCREATIC_CANCER	65	0.2568	2.4649	0.0000	0.0021	0.0892
REACTOME_SEMA4D_IN_SEMAPHORIN_SIGNALING	22	0.4374	2.4592	0.0004	0.0022	0.0976
KEGG_MAPK_SIGNALING_PATHWAY	231	0.1387	2.4289	0.0004	0.0025	0.1134
BIOCARTA_CASPASE_PATHWAY	22	0.4228	2.4105	0.0004	0.0029	0.1330
BIOCARTA_BLYMPHOCYTE_PATHWAY	7	0.7503	2.4193	0.0004	0.0029	0.1284
REACTOME_MAPK_TARGETS_NUCLEAR_EVENTS_MEDIATED_BY_MAP_KINASES	27	0.3830	2.3902	0.0004	0.0033	0.1596
REACTOME_TOLL_LIKE_RECEPTOR_9_CASCADE	19	0.4575	2.3809	0.0000	0.0034	0.1648
REACTOME_CHEMOKINE_RECEPTORS_BIND_CHEMOKINES	46	0.3002	2.3871	0.0008	0.0034	0.1634
SIG_CHEMOTAXIS	41	0.3101	2.3634	0.0000	0.0037	0.1848
BIOCARTA_MONOCYTE_PATHWAY	10	0.6087	2.3511	0.0000	0.0039	0.1998
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION	45	0.2983	2.3552	0.0004	0.0039	0.1952
REACTOME_NEF_MEDIATES_DOWN_MODULATION_OF_CELL_SURFACE_ RECEPTORS_BY_RECRUITING_THEM_TO_CLATHRIN_ADAPTERS	18	0.4599	2.3485	0.0004	0.0041	0.2080
REACTOME_SEMA4D_INDUCED_CELL_MIGRATION_AND_GROWTH_CONE_COLLAPSE	18	0.4575	2.3377	0.0004	0.0043	0.2188
BIOCARTA_DEATH_PATHWAY	29	0.3618	2.3376	0.0004	0.0043	0.2166
REACTOME_CASPASE_MEDIATED_CLEAVAGE_OF_CYTOSKELETAL_PROTEINS	11	0.5796	2.3329	0.0016	0.0044	0.2254
REACTOME_THE_ROLE_OF_NEF_IN_HIV1_REPLICATION_AND_DISEASE_PATHOGENESIS	23	0.4093	2.3277	0.0004	0.0045	0.2306
REACTOME_MITOTIC_M_M_G1_PHASES	143	0.1645	2.3314	0.0008	0.0045	0.2314

BIOCARTA_TID_PATHWAY	16	0.4904	2.3253	0.0004	0.0045	0.2346
REACTOME_FORMATION_OF_A_POOL_OF_FREE_40S_SUBUNITS	66	0.2444	2.3195	0.0012	0.0046	0.246
BIOCARTA_MPR_PATHWAY	29	0.3612	2.3093	0.0000	0.0048	0.2588
KEGG_GLIOMA	56	0.2589	2.3152	0.0008	0.0048	0.2562
ST_INTEGRIN_SIGNALING_PATHWAY	73	0.2278	2.2933	0.0016	0.0052	0.2836
BIOCARTA_MYOSIN_PATHWAY	24	0.3990	2.2999	0.0008	0.0052	0.275
BIOCARTA_SODD_PATHWAY	9	0.6297	2.3001	0.0012	0.0052	0.2786
REACTOME_NEF_MEDIATED_DOWNREGULATION_ OF_MHC_CLASS_I_COMPLEX_CELL_SURFACE_EXPRESSION	9	0.6264	2.2806	0.0004	0.0055	0.3038
REACTOME_COLLAGEN_MEDIATED_ACTIVATION_CASCADE	20	0.4201	2.2807	0.0008	0.0055	0.3044
BIOCARTA_PAR1_PATHWAY	29	0.3530	2.2764	0.0008	0.0059	0.3184
KEGG_CELL_CYCLE	116	0.1807	2.2718	0.0008	0.0060	0.3288
KEGG_P53_SIGNALING_PATHWAY	61	0.2421	2.2457	0.0016	0.0066	0.3686
ST_GAQ_PATHWAY	26	0.3709	2.2478	0.0012	0.0068	0.3754
BIOCARTA_MITOCHONDRIA_PATHWAY	20	0.4158	2.2419	0.0004	0.0069	0.3816
KEGG_PROSTATE_CANCER	82	0.2131	2.2382	0.0020	0.0069	0.3890
REACTOME_G_BETA_GAMMA_SIGNALLING_THROUGH_PI3KGAMMA	24	0.3810	2.2322	0.0016	0.0072	0.403
ST_B_CELL_ANTIGEN_RECEPTOR	33	0.3266	2.2272	0.0015	0.0073	0.4092
REACTOME_DOWNSTREAM_EVENTS_IN_GPCR_SIGNALING	390	0.0973	2.2280	0.0016	0.0074	0.409
REACTOME_DOWN_STREAM_SIGNAL_TRANSDUCTION	33	0.3263	2.2230	0.0028	0.0076	0.4302
REACTOME_VIRAL_MRNA_TRANSLATION	59	0.2432	2.2153	0.0016	0.0079	0.4392
BIOCARTA_CTCF_PATHWAY	20	0.4100	2.1992	0.0008	0.0083	0.4754
REACTOME_P75_NTR_RECEPTOR_MEDIATED_SIGNALLING	71	0.2212	2.1991	0.0012	0.0085	0.4772
BIOCARTA_IL22BP_PATHWAY	16	0.4568	2.1943	0.0020	0.0086	0.4862
REACTOME_PEPTIDE_CHAIN_ELONGATION	59	0.2432	2.1886	0.0020	0.0088	0.5018

BIOCARTA_TOLL_PATHWAY	34	0.3160	2.1874	0.0012	0.0089	0.5028
BIOCARTA_CDC42RAC_PATHWAY	15	0.4679	2.1879	0.0023	0.0089	0.5048
BIOCARTA_CBL_PATHWAY	13	0.5042	2.1890	0.0016	0.0090	0.5030
BIOCARTA_TEL_PATHWAY	17	0.4398	2.1797	0.0004	0.0093	0.5266
REACTOME_G_BETA_GAMMA_SIGNALLING_THROUGH_PLC_BETA	19	0.4182	2.1799	0.0008	0.0094	0.5264
BIOCARTA_CLASSIC_PATHWAY	11	0.5391	2.1769	0.0016	0.0095	0.5308
BIOCARTA_CD40_PATHWAY	15	0.4603	2.1654	0.0012	0.0098	0.5582
REACTOME_GOLGI_ASSOCIATED_VESICLE_BIOGENESIS	47	0.2651	2.1625	0.0028	0.0100	0.5664
BIOCARTA_THELPER_PATHWAY	11	0.5382	2.1623	0.0016	0.0101	0.5666
REACTOME_CELL_CYCLE_MITOTIC	277	0.1116	2.1581	0.0027	0.0102	0.5744
BIOCARTA_LYM_PATHWAY	9	0.5864	2.1582	0.0008	0.0102	0.5810
SA_MMP_CYTOKINE_CONNECTION	13	0.4951	2.1594	0.0028	0.0102	0.5714
BIOCARTA_GRANULOCYTES_PATHWAY	11	0.5300	2.1335	0.0020	0.0115	0.6290
ST_GRANULE_CELL_SURVIVAL_PATHWAY	24	0.3623	2.1303	0.0012	0.0116	0.6418
BIOCARTA_CELL2CELL_PATHWAY	14	0.4680	2.1245	0.0000	0.0118	0.6480
BIOCARTA_NO2IL12_PATHWAY	17	0.4260	2.1266	0.0040	0.0118	0.6508
REACTOME_GENERATION_OF_SECOND_MESSENGER_MOLECULES	19	0.4044	2.1189	0.0020	0.0121	0.6648
SA_B_CELL_RECEPTOR_COMPLEXES	18	0.4133	2.1130	0.0028	0.0123	0.6784
BIOCARTA_P53_PATHWAY	14	0.4657	2.1157	0.0028	0.0123	0.6748
SIG_BCR_SIGNALING_PATHWAY	41	0.2774	2.1163	0.0012	0.0124	0.6732
ST_GA13_PATHWAY	30	0.3254	2.1089	0.0032	0.0127	0.6854
BIOCARTA_CHEMICAL_PATHWAY	21	0.3859	2.1059	0.0020	0.0129	0.6962
BIOCARTA_ATM_PATHWAY	17	0.4258	2.1021	0.0024	0.0131	0.7016
KEGG_GAP_JUNCTION	74	0.2056	2.0914	0.0020	0.0137	0.7228
SIG_PIP3_SIGNALING_IN_CARDIAC_MYOCTES	57	0.2348	2.0873	0.0020	0.0138	0.7332

BIOCARTA_TCRA_PATHWAY	9	0.5665	2.0846	0.0039	0.0140	0.7362
REACTOME_INFLUENZA_LIFE_CYCLE	104	0.1752	2.0792	0.0025	0.0141	0.7456
REACTOME_CLASS_A1_RHODOPSIN_LIKE_RECEPTORS	249	0.1129	2.0824	0.0023	0.0141	0.7422
KEGG_PRIMARY_IMMUNODEFICIENCY	32	0.3097	2.0741	0.0008	0.0144	0.7592
REACTOME_ACTIVATION_OF_BH3_ONLY_PROTEINS	16	0.4315	2.0693	0.0028	0.0146	0.7674
BIOCARTA_PDGF_PATHWAY	30	0.3189	2.0738	0.0029	0.0148	0.7638
REACTOME_GPCR_LIGAND_BINDING	342	0.0970	2.0702	0.0031	0.0148	0.7664
BIOCARTA_CXCR4_PATHWAY	22	0.3686	2.0589	0.0040	0.0154	0.7896
BIOCARTA_INTEGRIN_PATHWAY	33	0.3027	2.0631	0.0028	0.0154	0.7784
REACTOME_TOLL_LIKE_RECEPTOR_3_CASCADE	56	0.2330	2.0507	0.0036	0.0161	0.8070
KEGG_GNRH_SIGNALING_PATHWAY	90	0.1840	2.0511	0.0028	0.0162	0.8062
KEGG_CHRONIC_MYELOID_LEUKEMIA	63	0.2174	2.0458	0.0055	0.0164	0.8166
REACTOME_INITIAL_TRIGGERING_OF_COMPLEMENT	12	0.4861	2.0519	0.0028	0.0164	0.8062
REACTOME_REGULATION_OF_GENE_EXPRESSION_IN_BETA_CELLS	74	0.2005	2.0418	0.0024	0.0166	0.8222
BIOCARTA_IL17_PATHWAY	12	0.4890	2.0453	0.0040	0.0166	0.8176
REACTOME_CELL_DEATH_SIGNALLING_VIA_NRAGE_NRIF_AND_NADE	52	0.2383	2.0431	0.0060	0.0166	0.8242
BIOCARTA_UCALPAIN_PATHWAY	15	0.4393	2.0434	0.0032	0.0168	0.819
REACTOME_ERK_MAPK_TARGETS	18	0.4014	2.0397	0.0057	0.0174	0.8254
REACTOME_PECAM1_INTERACTIONS	11	0.4958	2.0253	0.0054	0.0179	0.8578
REACTOME_SIGNALLING_BY_NGF	190	0.1259	2.0259	0.0072	0.0181	0.8492
KEGG_CELL_ADHESION_MOLECULES_CAMS	98	0.1715	2.0171	0.0059	0.0184	0.8718
REACTOME_FURTHER_PLATELET_RELEASATE	21	0.3648	2.0163	0.0036	0.0185	0.8696
BIOCARTA_DC_PATHWAY	20	0.3753	2.0137	0.0060	0.0188	0.8726
KEGG_AMYOTROPHIC_LATERAL_SCLEROSIS_ALS	46	0.2505	2.0093	0.0037	0.0191	0.8852
BIOCARTA_KERATINOCYTE_PATHWAY	42	0.2618	2.0116	0.0071	0.0191	0.8814

SIG_INSULIN_RECEPTOR_PATHWAY_IN_CARDIAC_MYOCYTES	45	0.2520	2.0078	0.0024	0.0193	0.8906
BIOCARTA_GSK3_PATHWAY	25	0.3345	1.9976	0.0097	0.0202	0.8974
BIOCARTA_IL12_PATHWAY	21	0.3636	1.9945	0.0053	0.0202	0.9034
KEGG_VIBRIO_CHOLERAE_INFECTION	45	0.2513	1.9942	0.0067	0.0203	0.908
KEGG_NON_SMALL_CELL_LUNG_CANCER	47	0.2477	1.9887	0.0053	0.0208	0.9162
REACTOME_REGULATION_OF_BETA_CELL_DEVELOPMENT	86	0.1804	1.9864	0.0040	0.0211	0.917
KEGG_NEUROTROPHIN_SIGNALING_PATHWAY	109	0.1631	1.9825	0.0063	0.0214	0.9220
REACTOME_G1_PHASE	14	0.4376	1.9821	0.0051	0.0218	0.9212
KEGG_RIBOSOME	61	0.2154	1.9724	0.0056	0.0225	0.9312
BIOCARTA_SRCRPTP_PATHWAY	11	0.4803	1.9612	0.0078	0.0234	0.946
REACTOME_G_ALPHA_I_SIGNALLING_EVENTS	152	0.1345	1.9500	0.0053	0.0248	0.9542
REACTOME_INHIBITION_OF_INSULIN_SECRETION_BY_ADRENALINE_NORADRENALINE	26	0.3203	1.9460	0.0064	0.0252	0.9562
BIOCARTA_G1_PATHWAY	23	0.3353	1.9380	0.0065	0.0259	0.9628
BIOCARTA_FREE_PATHWAY	8	0.5569	1.9396	0.0096	0.0259	0.9604
SA_PROGRAMMED_CELL_DEATH	12	0.4639	1.9398	0.0073	0.0259	0.9606
SA_FAS_SIGNALING	6	0.6397	1.9321	0.0072	0.0266	0.9628
BIOCARTA_SALMONELLA_PATHWAY	12	0.4512	1.9265	0.0072	0.0271	0.9718
BIOCARTA_COMP_PATHWAY	16	0.4028	1.9277	0.0092	0.0271	0.9688
BIOCARTA_MAPK_PATHWAY	74	0.1915	1.9273	0.0074	0.0271	0.9714
KEGG_RIG_I_LIKE_RECEPTOR_SIGNALING_PATHWAY	61	0.2085	1.9277	0.0101	0.0271	0.9718
BIOCARTA_TCYTOTOXIC_PATHWAY	11	0.4717	1.9214	0.0093	0.0278	0.9744
KEGG_PRION_DISEASES	31	0.2891	1.9187	0.0071	0.0280	0.9758
REACTOME_GENES_INVOLVED_IN_APOPTOTIC_CLEAVAGE_OF_CELLULAR_PROTEINS	33	0.2826	1.9182	0.0049	0.0281	0.9748
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_12	19	0.3655	1.9167	0.0097	0.0284	0.9754
REACTOME_SYNTHESIS_OF_GPI_ANCHORED_PROTEINS	22	0.3399	1.9141	0.0081	0.0286	0.9736

REACTOME_TRANSMISSION_ACROSS_CHEMICAL_SYNAPSES	117	0.1507	1.9088	0.0095	0.0289	0.9792
REACTOME_NUCLEAR_EVENTS_KINASE_AND_TRANSCRIPTION_FACTOR_ACTIVATION	21	0.3458	1.9095	0.0056	0.0289	0.9802
REACTOME_SYNTHESIS_OF_GLYCOSYLPHOSPHATIDYLINOSITOL	16	0.3973	1.9088	0.0067	0.0292	0.9802
KEGG_ADHERENS_JUNCTION	65	0.1984	1.9015	0.0076	0.0299	0.9834
REACTOME_LYSOSOME_VESICLE_BIOGENESIS	21	0.3469	1.9008	0.0133	0.0301	0.9832
REACTOME_NRAGE_SIGNALS_DEATH_THROUGH_JNK	38	0.2631	1.8971	0.0077	0.0303	0.9852
REACTOME_ACTIVATION_OF_THE_AP1_FAMILY_OF_TRANSCRIPTION_FACTORS	10	0.4909	1.8974	0.0079	0.0303	0.9844
REACTOME_GTP_HYDROLYSIS_AND_JOINING_OF_THE_60S_RIBOSOMAL_SUBUNIT	76	0.1852	1.8982	0.0094	0.0303	0.9818
KEGG_JAK_STAT_SIGNALING_PATHWAY	135	0.1390	1.9005	0.0082	0.0304	0.9818
REACTOME_METAL_ION_SLC_TRANSPORTERS	20	0.3548	1.8956	0.0107	0.0305	0.9856
KEGG_RENIN_ANGIOTENSIN_SYSTEM	15	0.4087	1.8971	0.0097	0.0306	0.9844
ST_PHOSPHOINOSITIDE_3_KINASE_PATHWAY	31	0.2877	1.8891	0.0080	0.0313	0.9874
ST_TUMOR_NECROSIS_FACTOR_PATHWAY	27	0.3055	1.8848	0.0117	0.0317	0.989
BIOCARTA_NKCELLS_PATHWAY	15	0.4054	1.8833	0.0122	0.0320	0.9884
REACTOME_TRANSLOCATION_OF_ZAP70_TO_IMMUNOLOGICAL_SYNAPSE	7	0.5771	1.8830	0.0096	0.0321	0.989
BIOCARTA_CARDIACEGF_PATHWAY	16	0.3886	1.8684	0.0106	0.0341	0.9926
REACTOME_PROCESSING_OF_CAPPED_INTRON_CONTAINING_PRE_MRNA	113	0.1496	1.8617	0.0108	0.0351	0.9938
ST_GA12_PATHWAY	22	0.3310	1.8589	0.0136	0.0358	0.9936
REACTOME_ACTIVATION_OF_THE_PRE_REPLICATIVE_COMPLEX	29	0.2913	1.8553	0.0128	0.0362	0.9938
REACTOME_STRIATED_MUSCLE_CONTRACTION	28	0.2934	1.8472	0.0099	0.0379	0.9948
BIOCARTA_FAS_PATHWAY	26	0.3051	1.8434	0.0129	0.0382	0.9952
REACTOME_PHOSPHORYLATION_OF_CD3_AND_TCR_ZETA_CHAINS	9	0.4978	1.8389	0.0111	0.0389	0.9980
REACTOME_TCR_SIGNALING	45	0.2282	1.8393	0.0124	0.0390	0.9966
BIOCARTA_CCR5_PATHWAY	17	0.3657	1.8321	0.0145	0.0398	0.9972
REACTOME_TRAF6_MEDIATED_INDUCTION_OF_	50	0.2194	1.8282	0.0118	0.0407	0.9972

THE_ANTIVIRAL_CYTOKINE_IFN_ALPHA_BETA_CASCADE						
ST_INTERLEUKIN_4_PATHWAY	22	0.3246	1.8243	0.0163	0.0414	0.9976
REACTOME_REV_MEDIATED_NUCLEAR_EXPORT_OF_HIV1_RNA	29	0.2839	1.8237	0.0161	0.0416	0.998
KEGG_COLORECTAL_CANCER	57	0.2035	1.8186	0.0141	0.0425	0.9984
KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_BIOSYNTHESIS	21	0.3291	1.8135	0.0190	0.0435	0.9984
REACTOME_ERKS_ARE_INACTIVATED	12	0.4290	1.8013	0.0153	0.0459	0.9994
BIOCARTA_EGF_PATHWAY	29	0.2802	1.7897	0.0149	0.0482	0.9998
REACTOME_GLUCOSE_AND_OTHER_SUGAR_SLC_TRANSPORTERS	74	0.1772	1.7887	0.0167	0.0486	0.9998
REACTOME_TRANSPORT_OF_THE_SLBP_INDEPENDENT_MATURE_MRNA	30	0.2744	1.7810	0.0170	0.0502	0.9998
REACTOME_METABLISM_OF_NUCLEOTIDES	63	0.1903	1.7798	0.0166	0.0505	0.9998
REACTOME_ACTIVATION_OF_KAINATE_RECEPTORS_UPON_GLUTAMATE_BINDING	30	0.2726	1.7772	0.0228	0.0513	1.0000
REACTOME_G_ALPHA_Q_SIGNALLING_EVENTS	136	0.1303	1.7723	0.0208	0.0520	1.0000
KEGG_GLYCOSAMINOGLYCAN_DEGRADATION	21	0.3207	1.7654	0.0194	0.0538	0.9998
REACTOME_OTHER_SEMAPHORIN_INTERACTIONS	16	0.3669	1.7571	0.0180	0.0560	0.9998
BIOCARTA_ACH_PATHWAY	15	0.3740	1.7507	0.0248	0.0573	1
REACTOME_PYRIMIDINE_METABOLISM	19	0.3347	1.7487	0.0194	0.0578	1
REACTOME_REGULATION_OF_INSULIN_SECRETION_BY_GLUCAGON_LIKE_PEPTIDE_1	51	0.2080	1.7476	0.0206	0.0579	1
BIOCARTA_MCM_PATHWAY	17	0.3502	1.7451	0.0210	0.0585	1
KEGG_N_GLYCAN_BIOSYNTHESIS	43	0.2251	1.7437	0.0253	0.0589	1
REACTOME_ZINC_TRANSPORTATION	15	0.3704	1.7337	0.0246	0.0614	1
BIOCARTA_RANKL_PATHWAY	13	0.3987	1.7330	0.0221	0.0617	1
REACTOME_REGULATED_PROTEOLYSIS_OF_P75NTR	11	0.4267	1.7270	0.0246	0.0631	1
ST_DIFFERENTIATION_PATHWAY_IN_PC12_CELLS	37	0.2354	1.7232	0.0284	0.0641	1
REACTOME_VPR_MEDIATED_NUCLEAR_IMPORT_OF_PICS	29	0.2663	1.7181	0.0221	0.0651	1
REACTOME_INFLUENZA_VIRAL_RNA_TRANSCRIPTION_AND_REPLICATION	71	0.1719	1.7121	0.0258	0.0667	1

REACTOME_INSULIN_SYNTHESIS_AND_SECRETION	101	0.1466	1.7123	0.0229	0.0667	1
BIOCARTA_NTHI_PATHWAY	22	0.3028	1.7109	0.0272	0.0672	1
BIOCARTA_CDMAC_PATHWAY	15	0.3612	1.7082	0.0187	0.0677	1
ST_G_ALPHA_I_PATHWAY	31	0.2538	1.6934	0.0268	0.0716	1
BIOCARTA_EDG1_PATHWAY	26	0.2792	1.6915	0.0266	0.0719	1
KEGG_CYTOSOLIC_DNA_SENSING_PATHWAY	46	0.2106	1.6896	0.0327	0.0724	1
BIOCARTA_ARENRF2_PATHWAY	13	0.3868	1.6863	0.0300	0.0737	1
BIOCARTA_ERK_PATHWAY	26	0.2765	1.6857	0.0271	0.0738	1
KEGG_ASTHMA	15	0.3615	1.6844	0.0292	0.0740	1
BIOCARTA_BAD_PATHWAY	23	0.2905	1.6800	0.0305	0.0749	1
KEGG_BLADDER_CANCER	34	0.2425	1.6775	0.0289	0.0758	1
BIOCARTA_TH1TH2_PATHWAY	15	0.3613	1.6766	0.0335	0.0759	1
KEGG_ALDOSTERONE_REGULATED_SODIUM_REABSORPTION	37	0.2333	1.6772	0.0307	0.0760	1
REACTOME_NUCLEOTIDE_LIKE_PURINERGIC_RECEPTORS	14	0.3702	1.6721	0.0279	0.0766	1
KEGG_ACUTE_MYELOID_LEUKEMIA	51	0.1995	1.6687	0.0303	0.0779	1
REACTOME_P38MAPK_EVENTS	12	0.3977	1.6671	0.0349	0.0783	1
KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_METABOLISM	41	0.2185	1.6641	0.0362	0.0794	1
BIOCARTA_STEM_PATHWAY	13	0.3800	1.6557	0.0367	0.0810	1
BIOCARTA_IL2RB_PATHWAY	34	0.2407	1.6579	0.0312	0.0813	1
SIG_REGULATION_OF_THE_ACTIN_CYTOSKELETON_BY_RHO_GTPASES	32	0.2473	1.6541	0.0436	0.0818	1
BIOCARTA_RACCYCD_PATHWAY	24	0.2846	1.6557	0.0341	0.0819	1
REACTOME_SPHINGOLIPID_METABOLISM	30	0.2538	1.6524	0.0385	0.0825	1
KEGG_CARDIAC_MUSCLE_CONTRACTION	64	0.1755	1.6493	0.0398	0.0829	1
REACTOME_MAP_KINASES_ACTIVATION_IN_TLR_CASCADE	41	0.2158	1.6498	0.0332	0.0837	1
REACTOME_THROMBIN_SIGNALLING_THROUGH_PROTEINASE_ACTIVATED_RECEPTORS	26	0.2706	1.6482	0.0334	0.0838	1

BIOCARTA_G2_PATHWAY	22	0.2935	1.6449	0.0352	0.0843	1
REACTOME_TRANSLATION	89	0.1485	1.6453	0.0384	0.0848	1
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION	239	0.0919	1.6424	0.0296	0.0850	1
REACTOME_NEURORANSMITTER_RECEPTOR_BINDING_AND_ DOWNSTREAM_TRANSMISSION_IN_THE_POSTSYNAPTIC_CELL	75	0.1603	1.6415	0.0399	0.0851	1
SA_REG_CASCADE_OF_CYCLIN_EXPR	12	0.3891	1.6408	0.0326	0.0860	1
REACTOME_APOPTOSIS	120	0.1272	1.6367	0.0393	0.0865	1
REACTOME_NEP_NS2_INTERACTS_WITH_THE_CELLULAR_EXPORT_MACHINERY	27	0.2685	1.6351	0.0303	0.0867	1
REACTOME_G_PROTEIN_ACTIVATION	26	0.2678	1.6334	0.0340	0.0874	1
KEGG_HOMOLOGOUS_RECOMBINATION	26	0.2685	1.6307	0.0396	0.0878	1
BIOCARTA_P53HYPOXIA_PATHWAY	19	0.3115	1.6308	0.0294	0.0882	1
REACTOME_PD1_SIGNALING	12	0.3865	1.6247	0.0384	0.0898	1
BIOCARTA_STRESS_PATHWAY	24	0.2779	1.6219	0.0425	0.0911	1
BIOCARTA_RAC1_PATHWAY	21	0.2911	1.6189	0.0406	0.0918	1
REACTOME_MRNA_SPLICING	84	0.1507	1.6162	0.0476	0.0924	1
REACTOME_NUCLEAR_IMPORT_OF_REV_PROTEIN	28	0.2576	1.6163	0.0388	0.0925	1
KEGG_FC_EPSILON_RI_SIGNALING_PATHWAY	74	0.1600	1.6089	0.0413	0.0949	1
BIOCARTA_NKT_PATHWAY	26	0.2614	1.6076	0.0412	0.0954	1
BIOCARTA_BCELLSURVIVAL_PATHWAY	16	0.3348	1.6068	0.0452	0.0954	1
KEGG_TIGHT_JUNCTION	104	0.1348	1.6035	0.0408	0.0966	1
BIOCARTA_LAIR_PATHWAY	13	0.3697	1.6041	0.0441	0.0968	1
REACTOME_UNWINDING_OF_DNA	9	0.4363	1.5960	0.0447	0.0989	1
REACTOME_MUSCLE_CONTRACTION	46	0.1988	1.5939	0.0447	0.0999	1
REACTOME_HOST_INTERACTIONS_OF_HIV_FACTORS	109	0.1312	1.5916	0.0490	0.1007	1
REACTOME_POST_TRANSLATIONAL_PROTEIN_MODIFICATION	35	0.2243	1.5886	0.0473	0.1021	1

REACTOME_ORC1_REMOVAL_FROM_CHROMATIN	62	0.1724	1.5862	0.0433	0.1026	1
BIOCARTA_EPONFKB_PATHWAY	11	0.3958	1.5836	0.0471	0.1039	1
REACTOME_COMPLEMENT_CASCADE	17	0.3145	1.5654	0.0573	0.1110	1
REACTOME_TRANSPORT_OF_MATURE_MRNA_ DERIVED_FROM_AN_INTRON_CONTAINING_TRANSCRIPT	40	0.2077	1.5650	0.0503	0.1116	1
BIOCARTA_RELA_PATHWAY	15	0.3360	1.5635	0.0564	0.1120	1
BIOCARTA_CSK_PATHWAY	19	0.2990	1.5576	0.0544	0.1136	1
ST_FAS_SIGNALING_PATHWAY	53	0.1813	1.5572	0.0563	0.1142	1
BIOCARTA_AGR_PATHWAY	33	0.2255	1.5530	0.0559	0.1157	1
REACTOME_SEROTONIN_NEUROTRANSMITTER_RELEASE_CYCLE	14	0.3452	1.5524	0.0532	0.1160	1
BIOCARTA_ACTINY_PATHWAY	18	0.3042	1.5512	0.0614	0.1164	1
REACTOME_METABOLISM_OF_PROTEINS	170	0.1011	1.5448	0.0582	0.1185	1
KEGG_ERBB_SIGNALING_PATHWAY	74	0.1543	1.5449	0.0560	0.1188	1
BIOCARTA_ATRBRCA_PATHWAY	16	0.3185	1.5437	0.0588	0.1191	1
REACTOME_DOPAMINE_NEUROTRANSMITTER_RELEASE_CYCLE	14	0.3452	1.5418	0.0635	0.1198	1
REACTOME_REGULATION_OF_GLUCOKINASE_BY_GLUCOKINASE_REGULATORY_PROTEIN	27	0.2484	1.5386	0.0670	0.1212	1
REACTOME_ACTIVATION_OF_RAC	11	0.3793	1.5347	0.0608	0.1228	1
REACTOME_TRANSPORT_OF_RIBONUCLEOPROTEINS_INTO_THE_HOST_NUCLEUS	27	0.2484	1.5349	0.0584	0.1228	1
BIOCARTA_STATHMIN_PATHWAY	18	0.3000	1.5350	0.0625	0.1228	1
BIOCARTA_TCR_PATHWAY	42	0.2016	1.5319	0.0637	0.1243	1
BIOCARTA_P27_PATHWAY	12	0.3685	1.5330	0.0623	0.1244	1
KEGG_VEGF_SIGNALING_PATHWAY	69	0.1562	1.5309	0.0550	0.1245	1
REACTOME_M_G1_TRANSITION	60	0.1665	1.5252	0.0661	0.1266	1
REACTOME_SIGNALING_BY_ROBO_RECEPTOR	23	0.2648	1.5242	0.0577	0.1269	1
BIOCARTA_CTL_PATHWAY	10	0.3889	1.5115	0.0658	0.1329	1

BIOCARTA_ARAP_PATHWAY	13	0.3451	1.5088	0.0660	0.1342	1
KEGG_STEROID_BIOSYNTHESIS	14	0.3352	1.5031	0.0728	0.1369	1
REACTOME_ADP_SIGNALLING_THROUGH_P2Y_PURINOCEPTOR_1	24	0.2558	1.5007	0.0714	0.1377	1
REACTOME_POST_CHAPERONIN_TUBULIN_FOLDING_PATHWAY	15	0.3185	1.4973	0.0671	0.1390	1
REACTOME_CTLA4_INHIBITORY_SIGNALING	20	0.2777	1.4976	0.0768	0.1392	1
KEGG_GALACTOSE_METABOLISM	23	0.2569	1.4889	0.0781	0.1435	1
BIOCARTA_FCER1_PATHWAY	33	0.2164	1.4852	0.0798	0.1456	1
KEGG_VASOPRESSIN_REGULATED_WATER_REABSORPTION	39	0.2019	1.4820	0.0730	0.1461	1
BIOCARTA_CCR3_PATHWAY	21	0.2699	1.4823	0.0808	0.1465	1
REACTOME_LOSS_OF_NLP_FROM_MITOTIC_CENTROSOMES	55	0.1691	1.4803	0.0794	0.1470	1
REACTOME_THROMBOXANE_SIGNALLING_THROUGH_TP_RECEPTOR	22	0.2615	1.4774	0.0803	0.1485	1
REACTOME_COSTIMULATION_BY_THE_CD28_FAMILY	50	0.1779	1.4752	0.0770	0.1502	1
KEGG_ENDOMETRIAL_CANCER	45	0.1851	1.4730	0.0787	0.1514	1
REACTOME_CELLEXTRACELLULAR_MATRIX_INTERACTIONS	14	0.3226	1.4661	0.0821	0.1540	1
REACTOME_CD28_DEPENDENT_VAV1_PATHWAY	10	0.3776	1.4653	0.0854	0.1546	1
REACTOME_G1_S_TRANSITION	98	0.1271	1.4628	0.0836	0.1562	1
BIOCARTA_SKP2E2F_PATHWAY	9	0.3999	1.4613	0.0846	0.1567	1
BIOCARTA_ECM_PATHWAY	22	0.2613	1.4581	0.0825	0.1582	1
KEGG_DRUG_METABOLISM_OTHER_ENZYMES	23	0.2561	1.4569	0.0843	0.1590	1
BIOCARTA_CERAMIDE_PATHWAY	21	0.2643	1.4561	0.0794	0.1594	1
BIOCARTA_D4GDI_PATHWAY	12	0.3419	1.4517	0.0943	0.1617	1
KEGG_THYROID_CANCER	24	0.2474	1.4501	0.0852	0.1622	1
REACTOME_FORMATION_OF_THE_TERNARY_ COMPLEX_AND_SUBSEQUENTLY_THE_43S_COMPLEX	36	0.2018	1.4447	0.0961	0.1658	1
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	82	0.1367	1.4438	0.0876	0.1660	1

REACTOME_E2F_MEDIATED_REGULATION_OF_DNA_REPLICATION	33	0.2112	1.4417	0.0832	0.1670	1
REACTOME_SYNTHESIS_AND_INTERCONVERSION_ OF_NUCLEOTIDE_DI_AND_TRIPHOSPHATES	16	0.2996	1.4413	0.0931	0.1672	1
REACTOME_SIGNALING_BY_EGFR	45	0.1792	1.4387	0.0963	0.1687	1
REACTOME_SIGNAL_AMPLIFICATION	29	0.2226	1.4312	0.0871	0.1733	1
REACTOME_E2F_TRANSCRIPTIONAL_TARGETS_AT_G1_S	22	0.2536	1.4272	0.1045	0.1752	1
SIG_CD40PATHWAYMAP	33	0.2098	1.4245	0.0989	0.1768	1
REACTOME_DEATH_RECEPTOR_SIGNALLING	10	0.3680	1.4198	0.0993	0.1792	1
BIOCARTA_ERK5_PATHWAY	14	0.3119	1.4079	0.1070	0.1870	1
KEGG_MELANOMA	66	0.1481	1.4067	0.1081	0.1878	1
REACTOME_P2Y_RECEPTORS	10	0.3674	1.4068	0.1085	0.1879	1
REACTOME_CENTROSOME_MATURATION	62	0.1501	1.4046	0.1000	0.1894	1
BIOCARTA_RAS_PATHWAY	21	0.2553	1.3998	0.1080	0.1929	1
BIOCARTA_P35ALZHEIMERS_PATHWAY	10	0.3582	1.3877	0.1065	0.2004	1
BIOCARTA_IL7_PATHWAY	17	0.2770	1.3780	0.1123	0.2064	1
REACTOME_TRAFFICKING_OF_AMPA_RECEPTORS	26	0.2250	1.3786	0.1277	0.2069	1
REACTOME_PEPTIDE_LIGAND_BINDING_RECEPTORS	148	0.0962	1.3702	0.1267	0.2122	1
BIOCARTA_TALL1_PATHWAY	14	0.2997	1.3703	0.1181	0.2124	1
REACTOME_BASIGIN_INTERACTIONS	23	0.2383	1.3704	0.1273	0.2125	1
BIOCARTA_AGPCR_PATHWAY	12	0.3234	1.3697	0.1214	0.2128	1
BIOCARTA_41BB_PATHWAY	15	0.2909	1.3657	0.1195	0.2152	1
REACTOME_SNRNP_ASSEMBLY	46	0.1708	1.3622	0.1219	0.2180	1
BIOCARTA_TPO_PATHWAY	22	0.2408	1.3604	0.1228	0.2186	1
KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_PRODUCTION	30	0.2091	1.3562	0.1265	0.2210	1
REACTOME_TRANSMEMBRANE_TRANSPORT_OF_SMALL_MOLECULES	198	0.0829	1.3562	0.1251	0.2218	1

REACTOME_DNA_REPLICATION_PRE_INITIATION	74	0.1336	1.3532	0.1398	0.2244	1
REACTOME_CD28_CO_STIMULATION	27	0.2171	1.3478	0.1386	0.2272	1
BIOCARTA_IL1R_PATHWAY	33	0.1967	1.3445	0.1221	0.2295	1
KEGG_OOCYTE_MEIOSIS	99	0.1157	1.3449	0.1330	0.2300	1
REACTOME_ACTIVATION_OF_CHAPERONES_BY_IRE1_ALPHA	9	0.3643	1.3401	0.1435	0.2329	1
REACTOME_TRAFFICKING_OF_GLUR2_CONTAINING_AMPA_RECEPTORS	14	0.2987	1.3408	0.1477	0.2330	1
BIOCARTA_IL3_PATHWAY	14	0.2966	1.3384	0.1453	0.2339	1
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	14	0.2966	1.3352	0.1347	0.2368	1
REACTOME_FANCONI_ANEMIA_PATHWAY	12	0.3166	1.3301	0.1391	0.2408	1
BIOCARTA_HCMV_PATHWAY	17	0.2670	1.3181	0.1441	0.2492	1

APPENDIX 3: Enrichment plots for selected gene expression sets

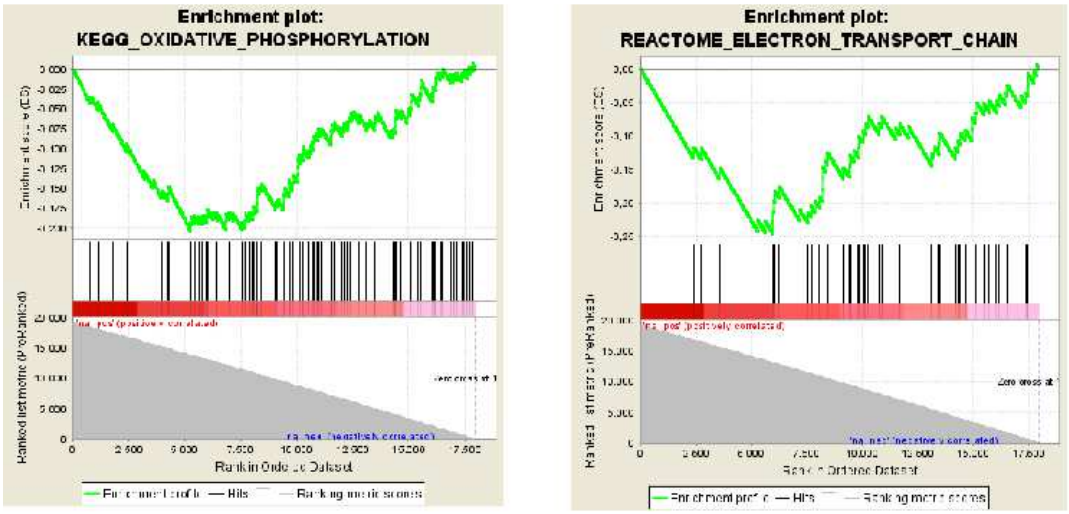

FIGURE 17. Enrichment plots for oxidative phoshporylation and electron transport chain gene expression sets in sActRIIB-Fc vs PBS. The color bar depicts the gene list used in the GSEA ordered by differential gene expression. Red indicates higher positive fold change (positively correlated) and white indicates higher negative fold change (negatively correlated) in treated with sActRIIB-Fc compared to untreated mdx mice.

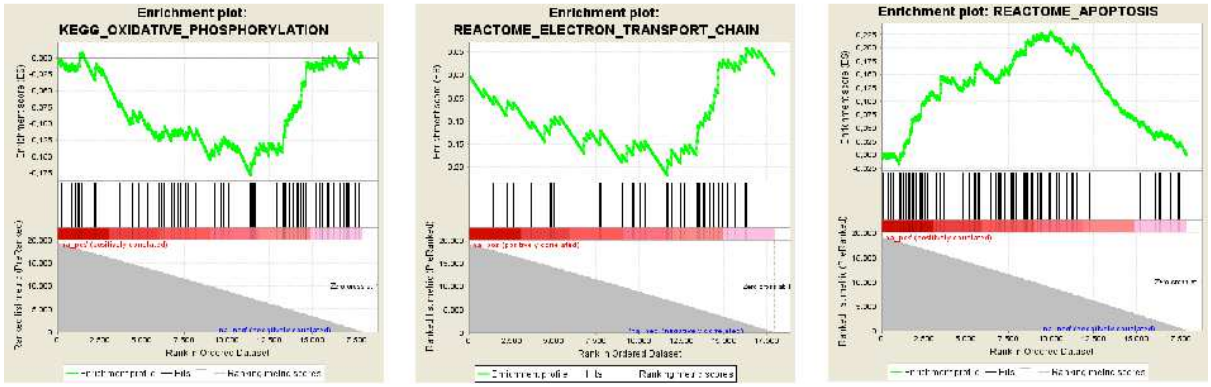

FIGURE 18. Enrichment plots for selected gene expression sets in sActRIIB-Fc + Running vs PBS + Running. The color bar depicts the gene list used in the GSEA ordered by differential gene expression. Red indicates higher positive fold change (positively correlated) and white indicates higher negative fold change (negatively correlated) in treated with sActRIIB-Fc compared to untreated mdx mice.

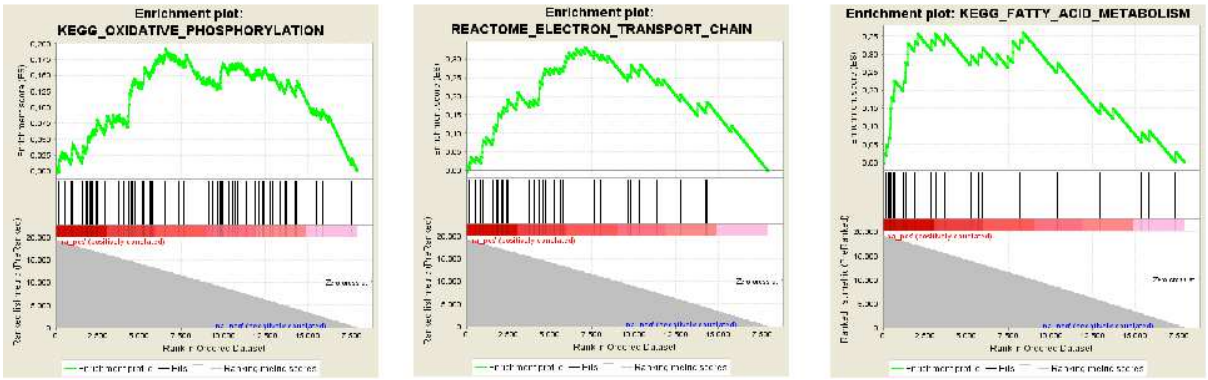

FIGURE 10. Enrichment plots for selected gene expression sets in PBS + Running vs PBS. The color bar depicts the gene list used in the GSEA ordered by differential gene expression. Red indicates higher positive fold change (positively correlated) and white indicates higher negative fold change (negatively correlated) in exercised (PBS+Running) compared to sedentary (PBS) PBS-receiving mdx mic

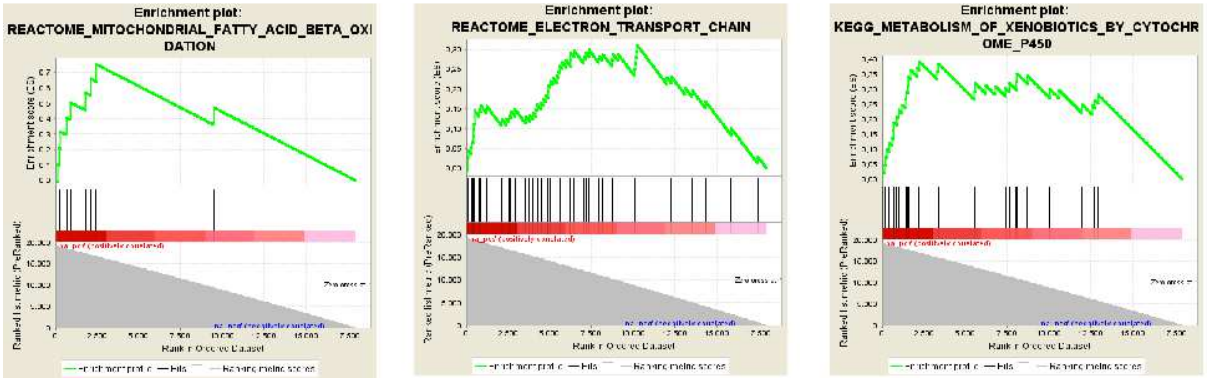

FIGURE 20. Enrichment plots for selected gene expression sets in sActRIIB-Fc + Running vs sActRIIB-Fc. The color bar depicts the gene list used in the GSEA ordered by differential gene expression. Red indicates higher positive fold change (positively correlated) and white indicates higher negative fold change (negatively correlated) in exercised (sActRIIB-Fc + Running) compared to sedentary (sActRIIB-Fc) sActRIIB-Fc-receiving mdx mice.

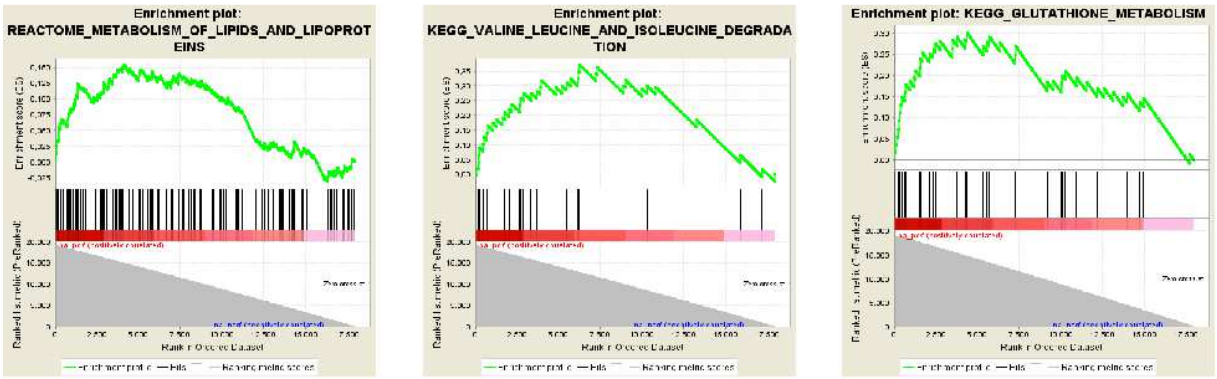

FIGURE 211. Enrichment plots for selected gene expression sets in sActRIIB-Fc + Running vs PBS. The color bar depicts the gene list used in the GSEA ordered by differential gene expression. Red indicates higher positive fold change (positively correlated) and white indicates higher negative fold change (negatively correlated) in treated with sActRIIB-Fc, exercised (sActRIIB-Fc + Running) compared to sedentary (sActRIIB-Fc) mdx mice.

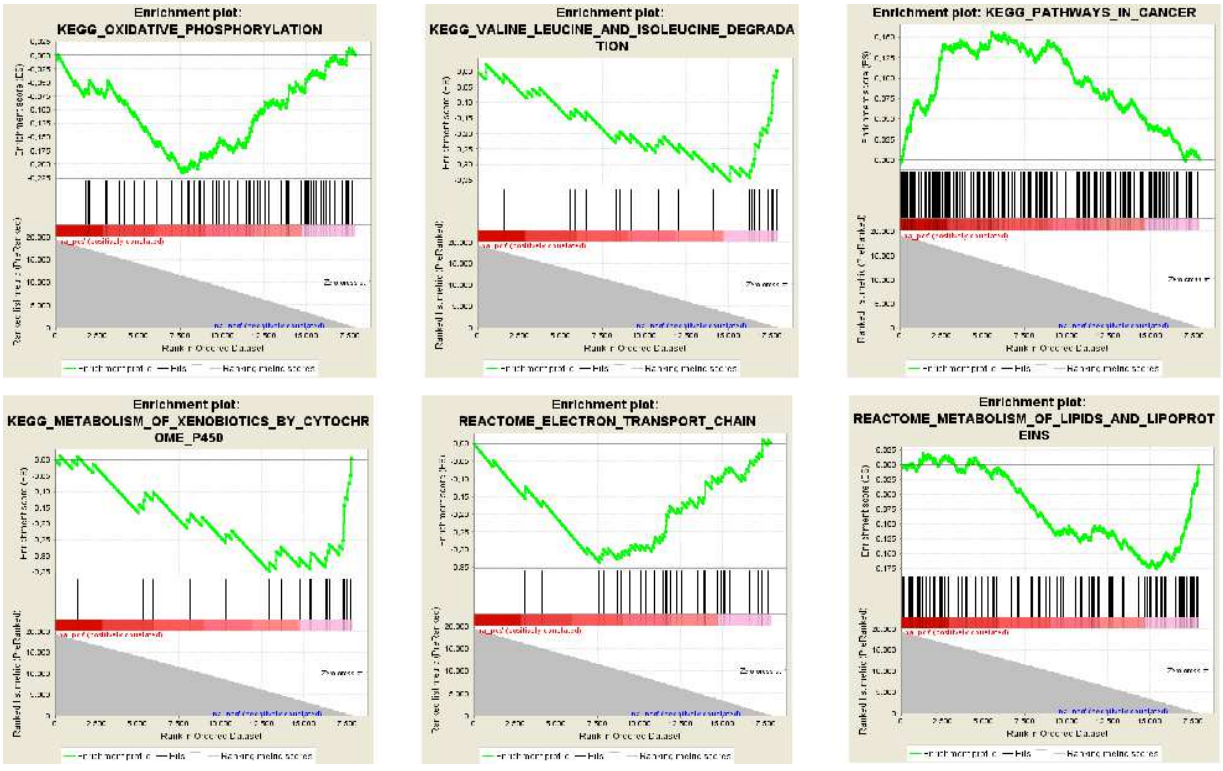

FIGURE 22. Enrichment plots for selected gene expression sets in PBS vc Control (both rows). The color bar depicts the gene list used in the GSEA ordered by differential gene expression. Red indicates higher positive fold change (positively correlated) and white indicates higher negative fold change (negatively correlated) in mdx (P) compared to normal healthy (C) mice.

APPENDIX 4: Leading-edge genes in selected pathways and comparisons

TABLE 24. Leading-edge genes in PBS running vs. PBS sedentary genes

Centroid	Leading-edge genes
Electron transport chain	NDUFB6, NDUFS4, COX6C, ETFA, ETFDH, SDHB, COX7B, UQCRC2,ETFB, NDUFB3,NDUFA8, NDUFA1, SDHC, NDUFA3, NDUFA9, NDUFV3, COX6B1, NDUFV2, NDUFB7, NDUFS8, NDUFB2, NDUFS2, NDUFB4, NDUFA4, CYC1, NDUFA10, UQCRH, NDUFA12, NDUFA5 ,UQCRC1, NDUFC1, NDUFS3, NDUFV1, NDUFA6, NDUFA2, NDUFS7, NDUFA7, UQCR11, COX5A, NDUFS6, SDHD, UQCR10
Oxidative phosphorylation	NDUFB6, NDUFS4, COX8C, COX6C, ATP6V0E2, COX7A1, ATP5O, SDHB, COX7B, UQCRC2, NDUFB3, NDUFA8, NDUFA1, SDHC, NDUFA3, NDUFA9, ATP6V0D2, NDUFV3, COX6B1, COX7B2, NDUFV2, NDUFB7, TCIRG1, NDUFS8, NDUFB2, NDUFS2, PPA2, ATP5C1, ATP6V1C2, NDUFB4, NDUFA4, CYC1, ATP5J, NDUFA10, UQCRH, ATP6V1G1, COX4I2, COX11, NDUFA5, UQCRC1, NDUFC1, NDUFS3, COX17, NDUFV1, ATP6V1G3, ATP6V1D, NDUFA6, ATP5A1, NDUFA2, NDUFS7, NDUFA7, UQCR11, ATP6V1E2, COX5A, PPA1, NDUFS6
TCA (Krebs) cycle	ACLY, OGDH, PDHA1, IDH2, SUCLG2, SDHB, CS, MDH1, DLD, ACO2, SDHC,

	IDH1, IDH3G, DLAT, SUCLG1, MDH2, PDHA2, PCK2, IDH3A, PDHB, SDHD, ACO1, DLST
Valine, Leucine and Isoleucine degradation	HMGCS2, ACAT1, ACAD8, OXCT1, ACAA2, BCAT2, IVD, ALDH9A1, BCKDHA, ECHS1, PCCA, MCEE, BCKDHB, ACADM, DLD, ALDH6A1, HMGCL, ALDH2, ABAT, DBT, HADHB, PCCB, MCCC1, ACAT2, AOX1, ACADSB, HADH, HIBADH, ACADS, MUT
Fatty acid metabolism	ACAT1, ACADVL, ACAA2, DCI, ALDH9A1, ACADL, GCDH, ECHS1, PECI, ADH4, ACADM, ACSL6, ADH7, CPT2, ALDH2, ACSL4, CPT1B, ACOX3, HADHB, ACAT2, ACADSB, HADH, ACADS, ACSL1, ADH5, EHHADH, ACSL3, ACOX1
Peroxisome	SLC27A2, SCP2, EPHX2, ABCD2, PRDX5, PXMP2, PAOX, DDO, IDH2, PECI, GSTK1, PHYH, AGXT, CRAT, DHRS4, PEX5, XDH, ACSL6, HMGCL, ECH1, PEX11A, PMVK, IDH1, ACSL4, ABCD1, CAT, ACOX2, MPV17, PEX19, ACOX3, GNPAT, ABCD3
Metabolism of lipids and lipoproteins	APOA2, APOA1, APOC3, CYP8B1, SLC27A1, HMGCS2, SLC27A5, ACAT1, SLC27A2, SLC27A2, BDH1, SPTLC3, CYP7A1, ACADVL, ACER2, SLC25A20, OXCT1, LDLRAP1, ACACA, DCI, DHCR24, ACADL, CYP7B1, HSD3B2, ECHS1, SCARB1, SPHK1, PHYH, GPD1, CRAT, SDC1, CYP39A1, NR1H3, LASS4, LASS6, PRKD1, ACADM, FABP4, CYP11B2, CPT2, HSD17B3, HMGCL, PEX11A, PMVK, DECR1, LCAT, IDH1, CLPS, ABCG5, CYP19A1, ABCD1, ABHD5, SLC2A2, ACOX2, AGPAT2, MED1, CPT1B, VAPB, NCOA2, SLC10A2, GPD2, ACOX3,

	SMARCD3, GNPAT, PPP1CB, TM7SF2, HSD11B1, HADHB, HSD3B7, CAV1, TBL1XR1, CYP27A1, DGAT1, TGS1, EBP, SLC10A1, STAR, APOC2, NCOA6, CYP11A1, SQLE, SC4MOL, NCOA3, LPL, ABCA1, COL4A3BP, CYP46A1, FABP6, APOA4, IDI2, PNLIP, CREBBP, HADH, PPM1L, HSD17B7, PPAP2B, ALB, DHCR7, MGLL, HACL1, PRKACA, FAR1, ACADS, VAPA, GGPS1, TBL1X, STARD5, ABCG8, UCP1, CSNK1G2, ACSL1, DEGS2, RXRA, NCOR1, SIN3A, AGPAT1
Glucose regulation of insulin secretion	NDUFB6, ALDOB, NDUFS4, COX6C, OGDH, PDHA1, ETFA, IDH2, ETFDH, ATP5O, SUCLG2, SDHB, COX7B, UQCRC2, CS, ETFB, DLD, NNT, NDUFB3, NDUFA8, NDUFA1, ACO2, SDHC, NDUFA3, NDUFA9, PFKP, PFKFB2, NDUFV3, COX6B1, CACNA1D, NDUFV2, NDUFB7, SLC2A2, NDUFS8, IDH3G, PKLR, NDUFB2, DLAT, NDUFS2, ENO2, ATP5C1, SUCLG1, NDUFB4, MDH2, NDUFA4, PGK1, CYC1, ATP5J, NDUFA10, UQCRH, PFKL, NDUFA12, NDUFA5, UQCRC1, NDUFC1, RAPGEF4, NDUFS3, NDUFV1, NDUFA6, ATP5A1, GAPDHS, NDUFA2, ABCC8, NDUFS7, NDUFA7, PFKFB4, IDH3A, UQCR11, CACNA1C, COX5A, NDUFS6, PRKACA, RAPGEF3, PDHB, SDHD, STXBP1, UQCR10, PFKM

TABLE 25. Leading-edge genes in sActRIIB-Fc running vs. PBS sedentary genes

Pathway	**Leading-edge genes**
Metabolism of xenobiotics by cytochrome P450	GSTA3, CYP2E1, MGST1, GSTT2, GSTT1, EPHX1, ADH7, UGT1A10, GSTM5, ALDH3A1, GSTZ1, GSTK1, GSTA1, ALDH3B1, MGST2, GSTP1, GSTO2, MGST3, GSTM3, ADH4, CYP1B1, GSTM4, GSTA4, CYP2S1
Drug metabolism of cytochrome P450	GSTA3, CYP2E1, MGST1, GSTT2, GSTT1, ADH7, UGT1A10, GSTM5, ALDH3A1, GSTZ1, GSTK1, MAOB, GSTA1, ALDH3B1, FMO1, MGST2, GSTP1, FMO3, GSTO2, MGST3, GSTM3, ADH4, GSTM4, GSTA4, AOX1, ALDH1A3, FMO4, GSTO1, CYP1A2, UGT2A3, FMO5, GSTM2
Glutathione metabolism	GSTA3, MGST1, GSTT2, GPX7, IDH1, GSTT1, RRM2, IDH2, GSTM5, GPX3, GSTZ1, GSTK1, GSTA1, MGST2, GSTP1, GGT5, GSTO2, MGST3, GSTM3, GPX5, GSTM4, RRM2B, GSTA4, TXNDC12, GSS
Peroxisome	PRDX5, SLC27A2, PAOX, IDH1, IDH2, EPHX2, SCP2, ACOX1, HAO1, GSTK1, HACL1, AGXT, PEX5, DDO, XDH, PXMP2, ABCD3, MPV17, HMGCL, ACSL3, PECI, PEX3, ACOX2, PEX11A, ACSL4, DHRS4, BAAT, FAR1, NUDT19, SLC25A17, SOD1, PEX19, FAR2, ACSL6, PEX7, ABCD2, PEX16, CAT, PHYH, MPV17L, ECH1, ABCD4, AMACR, PEX10, PEX11B, PXMP4, EHHADH, ACSL5, AGPS, HSD17B4, MLYCD, CROT, PIPOX, HAO2, NOS2, PMVK, CRAT, PRDX1
Mitochondrial fatty acid beta oxidation	ACADL, ECHS1, ACADS, ACADVL, DCI, DECR1, HADHB, ACADM, HADH

TABLE 26. Leading-edge genes in Apoptosis pathway in sActRIIB-Fc running vs. PBS running

Pathway	**Leading-edge genes**
Apoptosis	APAF1, BID, PSMB9, PSMD6, LMNB1, PSMA7, CASP6, PSMD10, PSMA5, PSMB1, PSMA2, BMX, VIM, PSMD14, PSMB6, CASP3, PSME2, CASP7, PSMD8, TFDP1, PSMD11, GAS2, BAX, CYCS, PSMD2, BCAP31, BAD, DSP, AKT1, PSMA4, LMNA, CASP8, CLSPN, PSMD9, PSMD13, PSMC1, TNFRSF10B, E2F1, BCL2, DYNLL2, PSMB3, FAS, PSMB8, PSMA1, PSMD7, DIABLO, PSMD4, PSMB4, PSMD12, TJP1, PMAIP1, PKP1, BBC3, TNF, BCL2L1, H1F0, NMT1, TNFSF10, PSMB2, PSMF1, PRKCD, PSMC5, DSG2, DBNL, MAPT, PSMD5, CDH1, PSMD1, PPP3CC, PSMB10, RIPK1, HIST1H1C, PSMC6, STK24, PPP3R1, CTNNB1, FNTA, DFFB, PSMB7, HIST1H1A, PSMC3, PSMB5, ARHGAP10, GZMB, CASP9, BCL2L11, PLEC, PSMC4, ADD1, FADD, BIRC2, DYNLL1, PSMC2, CFLAR