ONLINE DATA SUPPLEMENT

DIAPHRAGM UNLOADING VIA CONTROLLED MECHANICAL VENTILATION ALTERS THE GENE EXPRESSION PROFILE

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Methods

Experimental animals

These experiments were approved by the University of Florida animal use committee. Healthy, young adult (female, 4-months old) Sprague-Dawley rats were individually housed and fed rat chow and water *ad libitum* and were maintained on a 12 hour light/dark cycle for three weeks prior to initiation of these experiments. To minimize the possible influence of circadian rhythm on gene expression levels, animals were euthanized around the same time (± 2 hours) each day (E1).

Experimental design

To examine the time course of MV-induced alterations in diaphragmatic gene expression, animals were randomly assigned to one of three experimental groups: 1) control, 2) 6 hours MV (MV6), and 3) 18 hours MV (MV18).

Protocol for control animals

The animals in the control group were free of intervention prior to measurement of diaphragmatic gene expression. That is, these animals were not mechanically ventilated or exposed to long-term anesthesia prior to study. Animals in the control group received an IP injection of sodium pentobarbital (50 mg/kg body weight). After a surgical plane of anesthesia was achieved, the diaphragm was rapidly removed and segments from the ventral costal region were rapidly frozen in liquid nitrogen and stored at -80^{o}C for subsequent analysis of gene expression.

Protocol for mechanically ventilated animals

All surgical procedures were performed using aseptic techniques. Animals randomly selected for MV were anesthetized with an IP injection of sodium pentobarbital

(50 mg/kg body weight). After reaching a surgical plane of anesthesia the animals were tracheostomized and mechanically ventilated using a volume-driven small animal ventilator (Harvard Apparatus, Cambridge, MA). The ventilator delivered all breaths; hence, this mode of ventilation (i.e., controlled MV) results in complete diaphragmatic inactivity. The tidal volume was established at approximately 0.55 ml/100 grams of body weight with a respiratory rate of 80 breaths/min. This respiratory rate was selected to mimic the breathing frequency of adult rats at rest. Additionally, positive end-expiratory pressure (PEEP) of 1 cm H_2O was used throughout the protocol.

An arterial catheter was inserted into the carotid artery for constant measurement of systolic blood pressure, periodic blood sampling for analysis of arterial pH and blood gases, and infusion of isotonic saline. Arterial blood samples (~100 microliters per sample) were removed during the first and last hour of MV and analyzed for arterial PCO_2, PO_2, and pH using a blood gas analyzer (Instrumentation Laboratories, model 1610). Prior to analysis, the analyzer was calibrated using standardized gases and pH solutions.

A venous catheter was inserted into the jugular vein to permit the infusion of sodium pentobarbital when necessary. Anesthesia was maintained over the entire period of MV by continuous infusion of sodium pentobarbital (~10 mg/kg/hour). Throughout the experiments, technicians continuously monitored the level of anesthesia in MV animals by several methods (i.e., monitoring blood pressure and corneal/lid reflexes). Body temperature was maintained at ~37°C by use of a re-circulating heating blanket. Additionally, heart rate and electrical activity of the heart were monitored via a lead II ECG using needle electrodes placed subcutaneously.

Throughout MV, animals received enteral nutrition using the AIN-76 rodent diet. This planned feeding schedule was administered to the animals at a rate of 0.5 ml/2 hours. Further, body fluid homeostasis was maintained via the administration of 2.0 ml/kg/hour intravenous electrolyte solution.

Continuing care during MV included expressing the bladder, removing airway mucus, lubricating the eyes, rotating the animal and passive movement of the limbs. This care was maintained throughout the experimental period at hourly intervals. Finally, repeated intramuscular injections of glycopyrrolate (0.04 mg/kg/2 hours) were employed to reduce airway secretions. Upon completion of MV, segments of the costal diaphragm were removed, rapidly frozen in liquid nitrogen and stored at –80°C for subsequent analysis of gene expression.

Molecular Measurements

Isolation of total RNA

A portion of the costal diaphragm (~60 mg) was homogenized in 1.5 ml of Trizol reagent (Invitrogen, Carlsbad, CA) and processed according to the manufacturer's instructions. Samples were homogenized with a Polytron blade homogenizer at medium speed and centrifuged at 12,000 x g for 10 minutes (4°C) to remove insoluble material. The RNA portion was transferred to a new tube and extracted with 120 μL of bromochloropropane (Sigma, St. Louis, MO). Samples were vortexed briefly and centrifuged at 13,000 x g for 20 minutes (4°C). Following transfer of the aqueous phase to a new tube, RNA was precipitated with one volume of isopropanol and washed twice with two volumes of 75% ethanol. The RNA was pelleted via centrifugation and resuspended in RNAse free water (Sigma, St. Louis, MO). Concentration and purity of the extracted RNA was measured spectrophotometrically at 260 nm and at 280 nm in 1X TE buffer (Promega, Madison, WI). The integrity of the extracted total RNA was verified by gel electrophoresis of 1 μg RNA on a 1% agarose ethidium bromide-stained TBE gel. Total RNA was stored at –80°C.

Microarray Processing

Total RNA was subsequently prepared for hybridization according to the protocols outlined in the GeneChip® Expression Analysis Technical Manual (Affymetrix Santa Clara, Ca) as we previously reported (E2). Briefly, 8 μg of total RNA was used as template for cDNA synthesis (Superscript III, Life Technologies, Carlsbad, CA), which was primed with a T7-$(dT)_{24}$ primer containing a T7 RNA polymerase promoter sequence (Genset Oligos, La Jolla, CA). I*n vitro* transcription was performed on the purified cDNA product using biotinylated UTP and CTP (Bioarray High Yield RNA Transcript labeling Kit, Enzo Diagnostics, Farmingdale, NY). Biotinylated cRNA was incubated with a 40 mM Tris-acetate (pH 8.1), 100 mM KOAc, 30 mM MgOAc buffer for 35 min at 94°C in order to obtain fragments of 35-200 bases in length. Rat genomic (U34A) arrays (Affymetrix, Santa Clara, CA) were hybridized for 16 hr at 45°C with 15 μg of fragmented cRNA. Arrays were stained with a streptavidin-phycoerythrin conjugate (Molecular Probes, Eugene, OR) and scanned with a GeneArray^{TM} Scanner (Agilent, Palo Alto, CA). Scans were converted into image files (dat. and cel.) with the Affymetrix Microarray Suite Version 5.0 software. Hybridization and scanning of the arrays was performed in the University of Florida ICBR Microarray Core Facility (Gainesville, FL, USA).

Microarray Analysis

Microarray images were analyzed using statistically based Affymetrix Microarray Suite 5.0 software (Affymetrix, Santa Clara, CA). Comparison of the hybridization signals from the perfect match (PM) and mismatch (MM) probes was performed for the purpose of removing any nonspecific hybridization from the data analysis. Multiple criteria were applied to determine differential expression between treatments, as discussed in more detail by Bey et al. (E2). Results are reported only for transcripts that passed each of the analytical filters (described below) and therefore displayed the most robust responses to MV. We focus more on results for the fully annotated genes because of less confidence in the identity of expressed sequence tags (ESTs). Published statistical algorithms were used to determine whether a transcript was first detectable in a given sample based upon the 16–20 independent probe pairs for each transcript (E2). A one-sided Wilcoxon's signed rank test (WSR) was applied to the PM and MM intensities of each probe set to determine which genes were expressed above background. This nonparametric test was chosen because it has been shown to be robust, insensitive to outliers, and does not assume a normal data distribution (E2). Genes were reported as significantly expressed above background at $p \leq 0.04$. Using this criterion, we found 3,303 transcripts expressed on all arrays in at least one of the three groups, these transcripts were then considered for further statistical analysis. Statistical algorithms based on a WSR test were then used to determine significant differential expression ($p < 0.001$) in comparative analyses between treatment groups. A WSR test was applied to 32–40 probes of each possible comparison. Each MV group was compared with the control group, resulting in twenty-five comparisons for MV6 and twenty-five for MV18. Furthermore, we used the conservative criteria of at least ±1.5-fold differential expression for 25 out of 25 of the possible comparisons for any given gene to be included. It has been shown previously (E2) that no false calls for differential expression were made when the > 1.5 fold criteria of gene presence was applied to at least 3 arrays per group and in this study we used 5 per group.

In reporting differentially expressed genes we elected to employ a conservative approach to the data analysis in which three criteria for all genes needed to pass. To be included in the statistical analysis: 1) a gene transcript was required to have calls of present for all 5 chips within either the control or one of the two experimental groups. The purpose of this filter was to eliminate from the analysis "noisy" genes that displayed signal values close to background levels; 2) genes needed to be significant at the $p < 0.001$; and 3) only genes displaying at least ±1.5-fold change in either the MV6 or MV18 groups were considered. With this degree of stringency we expect the error rate to be < 1.0% (E2).

Gene classifications and descriptions were obtained from several public databases including gene ontology (Amigo; http://www.godatabase.org/cgi-bin/amigo/go.cgi), PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi), LocusLink (http://www.ncbi.nlm.nih.gov/projects/LocusLink/), Rat Genome Database (http://www.rgd.mcw.edu/), OMIM (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM), and NetAffx (https://www.affymetrix.com/analysis/netaffx/index.affx).

Reverse transcription and cDNA quantification for real-time PCR

Reverse transcription was performed using the Superscript III First-Strand Synthesis System for RT-PCR (Life technologies, Carlsbad, CA) according to the manufacturer's instructions. Reactions were carried out using 5 μg of total RNA and 2.5 μM oligo$(dT)_{20}$ primers. First strand cDNA was subsequently treated with 2 units of RNase H. Following the addition of 2 μL of GlycoBlue coprecipitant (Ambion, Austin, TX) to the RT product (21 μL), first strand cDNA was purified of RNA and unincorporated nucleotides by treatment with an RNase cocktail (Ambion, Austin, TX), brought to a volume of 100 μL with water, and applied to a NucAway spin column (Ambion, Austin, TX). Samples were then mixed with phenol:chloroform:isoamyl alcohol (pH=7.9) and the aqueous phase recovered using a 1.5 mL heavy phase lock gel (Eppendorf, Hamburg, Germany). The cDNA was precipitated by adding one volume of 5.0 M NH_2OAc and two volumes of 100% ethanol and stored at –20°C overnight. Following centrifugation at 13,000 x g for 20 minutes (4°C) the cDNA was washed with two volumes of 75% ethanol, centrifuged at 13,000 x g for 10 minutes (4°C) and resuspended in 50 μL of 1X Tris-EDTA buffer. The cDNA was subsequently quantified using the Oligreen ssDNA Quantitation Reagent Kit according to the manufacturer's instructions (Molecular Probes, Eugene, OR).

Quantitative real-time PCR

Quantitative real-time PCR was performed using the ABI Prism 7000 Sequence Detection System (ABI, Foster City, CA). The probes for all genes consisted of Taqman 5' labeled FAM reporters and 3' non-fluorescent quenchers. Primers and probes for Gadd45α (GenBank mRNA; L32591), and thioredoxin reductase-1 (TxnRd1) (GenBank mRNA; AF108213, U63923, AF220760) were obtained from ABI (Assays-on-Demand). The sequences used by the manufacturer in the design of primers and probes from this service are proprietary and are therefore not reported. The hypoxanthine guanine phosphoribosyl transferase (HPRT) primers and probe were obtained from ABI (Assays-by-Design). Primer and probe sequences for the HPRT gene are as follows: Forward, 5'-GTTGGATACAGGCCAGACTTTGT-3'; Reverse, 5'-AGTCAAGGGCATATCCAACAACAA-3'; Probe, 5'-ACTTGTCTGGAATTTCA-3'. Each 25 μL PCR reaction, performed in duplicate, contained 3.0 ng of cDNA template. Gene expression was calculated using the relative standard curve method as described in the ABI User Bulletin #2. HPRT was selected as the appropriate normalizer since the expression of this gene in the diaphragm is not significantly altered by mechanical ventilation ($p = 0.76$) (E3).

References

E1. Holzberg, D., and U. Albrecht. 2003. The circadian clock: a manager of biochemical processes within the organism. *J Neuroendocrinol* 15(4):339-43.

E2. Bey, L., N. Akunuri, P. Zhao, E. P. Hoffman, D. G. Hamilton, and M. T. Hamilton. 2003. Patterns of global gene expression in rat skeletal muscle during unloading and low-intensity ambulatory activity. *Physiol Genomics* 13(2):157-67.

E3. Shanely, R. A., D. Van Gammeren, K. C. DeRuisseau, A. M. Zergeroglu, M. J. McKenzie, K. E. Yarasheski, and S. K. Powers. 2004. Mechanical Ventilation Depresses Protein Synthesis in the Rat Diaphragm. *Am. J. Respir. Crit. Care Med* 170(4):994-9.

Table E1. Expression of transport genes in the diaphragm following MV6 and MV18.

	MV6/Control		MV18/Control		Gene	Description
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
				Transport		
AF047707_at	1.09E-02	2.2	3.94E-04	4.1	UDP-glucose:ceramide glycosyltransferase	carbohydrate transport
X66494_at	2.77E-02	-1.3	1.25E-04	-1.8	choline transporter	choline transporter
D50436_at	1.68E-01	-0.7	2.57E-05	-2.0	ferredoxin 1	electron transport
AF007107_s_at	6.94E-01	-0.5	9.35E-05	-2.6	cytochrome b5	electron transport
AF080468_g_at	1.03E-02	-1.7	2.24E-06	-2.8	glucose-6-phosphatase, transport protein 1	endoplasmic reticulum glucose-6-phosphate transport
AF080468_at	1.52E-02	-1.4	3.50E-07	-2.6	glucose-6-phosphatase, transport protein 1	endoplasmic reticulum glucose-6-phosphate transport
D14048_g_at	6.63E-01	0.3	9.96E-05	2.1	transporter protein; system N1 Na+ and H+-coupled glutamine transporter	glutamine transport
AB006450_at	2.05E-01	-0.8	1.53E-07	-2.5	translocator of inner mitochondrial membrane 17a (yeast)	intracellular protein transport
AF022774_g_at	1.74E-01	-0.6	3.65E-04	-1.7	rabphilin 3A-like (without C2 domains)	intracellular protein transport
L12383_at	6.99E-01	-0.3	1.52E-04	1.7	ADP-ribosylation factor 4	intracellular protein transport
rc_AI007857_at	6.04E-01	-0.3	9.32E-04	1.6	HGF-regulated tyrosine kinase substrate	intracellular protein transport
D17445_g_at	7.25E-01	-0.1	1.30E-05	1.5	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, eta polypeptide	intracellular protein transport
rc_AA944324_at	3.61E-02	1.2	5.15E-04	1.6	ADP-ribosylation factor 6	intracellular protein transport
rc_AA799645_g_at	3.48E-02	-1.5	1.33E-06	-2.1	FXYD domain-containing ion transport regulator 1	membrane ion transport
rc_AA799645_at	3.00E-02	-1.3	1.33E-06	-2.0	FXYD domain-containing ion transport regulator 1	membrane ion transport
U14007_at	6.18E-03	-2.1	7.41E-04	-3.6	aquaporin 4	membrane water channel
AB000507_at	1.24E-01	-0.5	6.33E-05	-2.1	aquaporin 7	membrane water channel
AF093139_s_at	2.00E-02	1.3	1.26E-04	1.8	nuclear RNA export factor 1 homolog (S. cerevisiae)	mRNA-nucleus export
AB015432_s_at	2.89E-02	1.4	1.38E-04	1.9	tumor-associated protein 1	neutral amino acid transporter
rc_AA875099_s_at	2.44E-01	1.1	4.08E-05	1.9	nuclear pore associated protein	nuclear protein export
AF015304_at	1.60E-03	-1.4	8.51E-05	-2.3	solute carrier family 29, member 1	nucleoside transport
D86039_at	4.88E-01	-0.6	8.12E-04	-2.2	potassium inwardly rectifying channel, subfamily J,	potassium ion transport
M94287_at	1.53E-01	1.2	8.89E-04	1.5	nucleolar phosphoprotein p130	protein-nucleolus import
rc_AI230614_s_at	2.35E-03	-2.0	7.07E-05	-2.9	ATPase Na+/K+ transporting beta 1 polypeptide	sodium/potassium-exchanging ATPase
rc_AI112173_at	1.66E-02	-1.7	2.04E-05	-2.7	ATPase Na+/K+ transporting beta 1 polypeptide	sodium/potassium-exchanging ATPase
D84450_at	1.75E-01	0.8	1.49E-04	1.7	ATPase, Na+/K+ transporting, beta 3 polypeptide	sodium/potassium-exchanging ATPase
rc_AA859652_at	7.94E-01	-0.7	5.47E-04	-2.1	solute carrier family 16 (monocarboxylic acid transporters), member 6	unknown
D17521_at	1.58E-01	-0.7	2.74E-06	-1.9	protein kinase C-regulated chloride channel	voltage-gated chloride channel
Y16774_at	9.42E-01	0.4	2.89E-04	1.8	Dri 27/ZnT4 protein	zinc ion transport
M22253_at	2.08E-02	-3.7	7.45E-04	-32.9	sodium channel, voltage-gated, type 1, alpha polypeptide	sodium ion transport

Table E2. Expression of regulation of transcription and structural protein genes in the diaphragm following MV6 and MV18.

	MV6/Control		MV18/Control		Gene	Description
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
			Regulation of Transcription			
X60769mRNA_at	3.40E-03	1.5	1.75E-04	2.2	CCAAT/enhancer binding protein (C/EBP), beta	acute phase response
L35271_at	1.58E-01	1.9	2.93E-06	4.1	runt related transcription factor 1	development
U04835_at	2.77E-01	1.0	7.93E-04	2.8	cAMP responsive element modulator (delta)	down-regulator of cAMP-induced transcription
D86745cds_s_at	8.07E-03	-1.0	7.52E-05	-2.0	nuclear receptor subfamily 0, group B, member 2	ligand-dependent nuclear receptor
M27151_at	1.45E-01	1.5	6.45E-04	2.7	myogenic factor 6	muscle development
M24393_at	1.46E-01	3.1	8.61E-05	6.4	myogenin	muscle development
Y00396mRNA_g_at	1.04E-03	3.4	1.06E-04	9.9	c-myc	proto-oncogene
Y00396mRNA_at	8.48E-03	17.5	2.82E-04	54.5	c-myc	proto-oncogene
rc_AI136891_at	1.24E-03	1.5	6.76E-04	1.7	zinc finger protein 36, C3H type-like 1	regulates response to growth factors
U53184_at	4.44E-03	2.7	8.73E-06	5.7	LPS-induced TNF-alpha factor	regulation of TNF-Alpha
AF016387_g_at	1.62E-02	-1.9	2.17E-05	-3.1	retinoid X receptor gamma	retinoic acid response pathway
M36074_at	1.16E-02	-1.3	1.75E-05	-2.8	nuclear receptor subfamily 3, group C, member 2	sodium ion homeostasis
rc_AA875121_at	4.05E-03	-1.2	3.58E-04	-1.5	nuclear transcription factor-Y gamma	transcriptional activation of various genes
L23148_g_at	1.04E-04	-2.5	2.15E-03	-2.6	Inhibitor of DNA binding 1, helix-loop-helix protein (splice variation)	transcriptional repressor
L25785_at	5.74E-02	1.9	1.23E-05	3.4	transforming growth factor beta 1 induced transcript 4	transcriptional repressor
rc_AA866257_at	2.48E-02	-0.8	2.82E-05	-1.9	cytosolic leucine-rich protein	tumor suppressor
U50736_s_at	6.65E-02	16.8	1.91E-06	56.5	ankyrin-like repeat protein	negative regulator of cardiac-specific gene expression
			Structural Proteins			
rc_AI169370_at	2.89E-01	0.9	9.12E-04	2.2	alpha-tubulin	microtubule component
X74800_at	4.02E-01	-0.8	2.33E-04	-1.6	unconventional myosin Myr2 I heavy chain	contractile apparatus
X80130cds_f_at	1.90E-01	0.0	5.40E-04	-1.5	alpha actin, cardiac	contractile apparatus
rc_AI227608_s_at	9.97E-03	-1.6	3.45E-05	-2.3	microtubule-associated protein tau	cytoskeleton
U05784_s_at	4.59E-06	1.7	9.04E-04	2.1	microtubule-associated proteins 1A/1B light chain 3	cytoskeleton
rc_AI104707_at	2.28E-03	-1.6	3.45E-05	-1.7	caveolin 3	endocytosis
Z46614cds_at	1.67E-03	-1.6	9.74E-04	-2.1	caveolin	endocytosis
rc_AI043968_g_at	5.89E-02	-1.2	5.07E-04	-2.1	caveolin 3	endocytosis
J04993_at	2.61E-01	-0.5	2.42E-04	-1.7	Troponin I, slow isoform	contractile apparatus
U48288_at	4.99E-01	-0.1	1.53E-04	1.6	A-kinase anchoring protein 11	mitochondria anchoring protein

Table E3. Expression of metal binding protein, nucleic acid/nucleotide metabolism, inflammatory response, and neuronal factor genes in the diaphragm following MV6 and MV18.

	MV6/Control		MV18/Control		Gene	Description
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
			Metal binding Proteins			
rc_AA900516_s_at	2.19E-02	-2.5	2.09E-04	-4.0	peptidyl arginine deiminase, type 2	calcium dependent deimination of protein arginine residues
rc_AI231807_at	3.33E-01	1.1	6.16E-05	3.2	ferritin light chain 1	trace metal metabolism
rc_AI231807_g_at	2.96E-01	1.5	2.27E-04	3.7	ferritin light chain 1	trace metal metabolism
AJ000347_g_at	1.58E-01	-0.7	2.75E-05	-2.5	3(2),5-bisphosphate nucleotidase	magnesium-dependent inositol/phosphatidylinositol phosphatase
rc_AI172247_at	4.82E-05	2.5	1.48E-02	2.2	xanthine dehydrogenase	purine metabolism
J03572_i_at	9.55E-02	-1.0	7.97E-04	-1.8	alkaline phosphatase	skeletal mineralization
			Nucleic Acid/Nucleotide Metabolism			
AJ011606_at	2.24E-01	-0.7	6.23E-04	-2.5	DNA polymerase alpha subunit II	DNA replication
AF044910_g_at	8.76E-01	0.3	6.54E-04	1.7	survival motor neuron	mRNA processing
rc_AI104524_s_at	1.50E-01	0.8	9.99E-06	1.9	heterogeneous nuclear ribonucleoprotein A/B	mRNA processing
AF044910_at	2.25E-02	1.3	6.61E-05	2.1	survival motor neuron	mRNA processing
M29293_at	2.71E-01	-0.6	8.48E-04	-1.6	small nuclear ribonucleoparticle-associated protein, clone Sm51	mRNA splicing
X73411mRNA_s_at	4.54E-01	0.1	4.29E-04	-2.3	snRNP-associated polypeptide N	mRNA splicing
AF036335_at	3.16E-01	0.5	3.58E-05	2.1	NonO/p54nrb homolog	mRNA splicing
AB022209_s_at	1.96E-01	1.1	2.97E-04	2.1	ribonucleoprotein F	mRNA splicing
AA848218_at	8.90E-02	1.2	5.19E-04	1.7	DNA topoisomerase I	DNA supercoiling
J03969_at	3.23E-01	0.6	1.02E-04	1.8	nucleophosmin 1	ribosome assembly
J04943_at	3.61E-01	1.1	6.00E-04	3.1	nucleophosmin 1	ribosome assembly
AF063447_at	2.08E-01	0.8	2.39E-06	2.3	nuclear RNA helicase, DECD	mRNA processing
rc_AA892562_g_at	4.48E-01	-0.2	9.29E-05	1.8	dyskeratosis congenita 1, dyskerin	rRNA processing
rc_AA892562_at	4.30E-01	0.8	3.25E-04	2.1	dyskeratosis congenita 1, dyskerin	rRNA processing
M55015cds_s_at	1.99E-01	1.0	1.30E-06	2.1	nucleolin	synthesis of ribosomes
U90888_at	2.89E-01	3.1	4.35E-04	5.3	adenosine monophosphate deaminase 3	purine ribonucleoside monophosphate biosynthesis
			Inflammatory Response			
U49062_g_at	4.08E-01	0.8	5.54E-04	3.7	CD24 antigen	cell adhesion
U49062_at	3.79E-01	1.0	6.05E-05	3.2	CD24 antigen	cell adhesion
rc_AI171462_s_at	1.13E-01	1.6	9.13E-04	3.8	CD24 antigen	cell adhesion
AF039583_s_at	3.12E-04	-5.7	1.07E-04	-18.8	decay-accelarating factor	complement activation
			Neuronal Factors			
S55427_s_at	4.94E-04	-1.6	3.00E-03	-1.3	peripheral myelin protein 22	cell cycle arrest
D88250_at	2.57E-01	1.2	1.52E-04	2.2	complement component 1	glial cell differentiation
AB007689_at	6.85E-01	-0.1	1.93E-04	-1.9	homer, neuronal immediate early gene, 2	metabotropic glutamate receptor signaling
AF016296_at	8.69E-04	-1.9	2.01E-03	-1.7	neuropilin	neurogenesis
X59267_at	3.62E-03	-0.9	8.47E-05	-1.6	drebrin 1	neurogenesis
AA801441_at	6.97E-02	1.3	1.14E-07	2.0	platelet-activating factor acetylhydrolase beta subunit	neurogenesis
S50879_g_at	6.77E-03	-1.4	7.97E-05	-2.4	acetylcholinesterase	neurotransmitter catabolism

Table E4. Expression of miscellaneous and unknown function genes in the diaphragm following MV6 and MV18.

	MV6/Control		**MV18/Control**		**Gene**	**Description**
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
					Miscellaneous	
rc_AA874874_at	4.30E-02	-1.0	3.16E-04	-1.6	alcohol dehydrogenase 4 (class II), pi polypeptide	ethanol oxidation
rc_AA892821_at	3.12E-03	-0.9	9.12E-04	-1.5	aldo-keto reductase family 7, member A2 (aflatoxin aldehyde reductase)	aldehyde metabolism
rc_AI171959_at	8.16E-03	-2.6	5.87E-06	-13.6	neuraminidase 2	hydrolyzes sialylated compounds
AF087431_g_at	2.92E-01	0.9	7.45E-05	1.8	glucosidase 1	N-linked glycoprotein processing
AF049344_at	4.85E-03	-1.0	5.56E-05	-1.6	UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase T5	O-linked glycosylation
D10587_g_at	1.54E-01	2.0	4.05E-04	3.2	85kDa sialoglycoprotein	lysosomal membrane
L12025_at	1.84E-01	5.2	3.92E-04	13.7	tumor-associated antigen 1	virus entry
D13623_g_at	1.03E-01	1.3	8.85E-05	2.3	P34 protein	membrane ribosome binding protein
D30649mRNA_s_at	1.65E-01	4.4	4.96E-05	11.1	alkaline phosphodiesterase	skeletal mineralization
D42137exon_s_at	2.83E-02	-1.1	9.35E-04	-1.7	annexin V	blood coagulation
AF090867_at	2.91E-01	-0.8	1.13E-04	-2.5	guanosine monophosphate reductase	response to cold
AF090867_g_at	4.50E-01	-0.8	4.23E-06	-2.4	guanosine monophosphate reductase	response to cold
					Unknown Function	
U95161_s_at	5.02E-02	-1.3	3.52E-05	-2.0	nuclear protein E3-3	
X62660mRNA_g_at	5.28E-02	-1.2	5.28E-05	-1.8	LRRGT00083 mRNA	
rc_AA893338_at	3.67E-02	-1.0	8.54E-04	-1.9	hypothetical RNA binding protein RDA288	
AF095741_g_at	1.07E-01	-1.0	6.41E-04	-1.8	Mg87 protein	
rc_AA891824_at	4.03E-02	-1.0	1.87E-04	-1.9	Rattus norvegicus clone ZG52 mRNA sequence	
S72637_s_at	6.80E-02	-1.0	3.71E-04	-2.0	tumor-suppressive gene	
X76453_at	3.57E-02	-0.8	3.63E-06	-1.7	HRAS like suppressor	
S45663_g_at	3.28E-01	-0.5	4.52E-05	-1.8	synaptic glycoprotein SC2	
S45663_at	3.28E-01	-0.5	3.04E-05	-2.1	synaptic glycoprotein SC2	
M13011cds_at	9.22E-01	-0.3	8.30E-04	-1.5	c-ras-H-1	
X89225cds_s_at	1.86E-01	1.1	1.73E-04	2.3	protein linked to system L-like neutral amino acid transport	
rc_AI071866_s_at	2.26E-01	1.2	9.89E-05	3.1	Rattus norvegicus Nclone10 mRNA	
U92081mRNA_s_at	2.47E-01	1.2	5.01E-05	3.5	epithelial cell transmembrane protein antigen precursor (RTI40)	

Table E5. Expression of growth and/or maintenance, and apoptosis genes in the diaphragm following MV6 and MV18.

	MV6/Control		**MV18/Control**		**Gene**	**Description**
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
			Cell Growth and/or Maintenance			
M24067_at	3.20E-02	4.6	2.20E-04	20.6	serine (or cysteine) proteinase inhibitor, member 1	angiogenesis
L20913_s_at	1.04E-01	-0.9	3.34E-04	-1.6	vascular endothelial growth factor	angiogenesis
AF022952_at	5.59E-01	0.3	2.36E-05	-2.0	vascular endothelial growth factor B	angiogenesis
rc_AA892598_g_at	6.14E-02	2.0	2.25E-06	3.6	nucleostemin	cell proliferation
rc_AA892598_at	1.26E-01	2.2	5.26E-06	3.6	nucleostemin	cell proliferation
rc_AA859920_at	1.29E-01	0.9	2.84E-05	1.8	nucleosome assembly protein 1-like 1	DNA replication
AF062594_at	2.25E-01	0.9	7.77E-05	1.8	nucleosome assembly protein 1-like 1	DNA replication
AF062594_g_at	2.03E-01	1.1	4.83E-04	1.9	nucleosome assembly protein 1-like 1	DNA replication
M24604_g_at	1.76E-01	1.4	3.02E-06	2.6	proliferating cell nuclear antigen	DNA replication
M24604_at	1.77E-01	1.5	1.37E-08	2.8	proliferating cell nuclear antigen	DNA replication
U77829mRNA_i_at	2.24E-02	2.0	1.97E-05	3.9	Rattus norvegicus gas-5 growth arrest homolog non-translated mRNA sequence	growth arrest
U77829mRNA_s_at	3.51E-02	2.4	3.36E-05	5.3	Rattus norvegicus gas-5 growth arrest homolog non-translated mRNA sequence	growth arrest
rc_AI232078_at	8.71E-01	-0.4	6.11E-04	2.6	LanC (bacterial lantibiotic synthetase component C)-like 1	growth factor binding
rc_AI014163_at	1.84E-02	1.8	3.30E-05	3.1	interferon-related developmental regulator 1	muscle differentiation
L26268_g_at	4.78E-02	1.2	8.83E-04	2.7	B-cell translocation gene 1	cell proliferation
L26268_at	4.55E-03	1.7	3.34E-04	3.0	B-cell translocation gene 1	cell proliferation
rc_AA818593_g_at	2.71E-01	1.0	3.65E-04	2.4	phosphatidate phosphohydrolase type 2a	phospholipid degradation
Z83757mRNA_at	1.86E-01	-1.0	1.76E-05	-3.2	growth hormone receptor	cell diferentiation
Z83757mRNA_g_at	6.64E-01	-0.9	2.26E-04	-3.8	growth hormone receptor	cell diferentiation
rc_AI103957_g_at	2.92E-04	-1.4	1.95E-05	-1.9	CD 81 antigen	cell proliferation
AF037272_at	3.40E-02	-5.6	3.06E-06	-9.8	wap four-disulfide core domain 1	serine-type endopeptidase inhibitor activity
X61654_at	4.99E-02	1.3	9.95E-05	2.0	CD63 antigen	tumor progression
L13619_g_at	3.47E-02	1.5	5.39E-04	4.8	growth response protein (CL-6)	unknown
L13619_at	4.05E-02	2.3	1.11E-04	4.7	growth response protein (CL-6)	unknown
U31203_at	1.22E-02	1.7	8.43E-04	2.7	noggin	skeletal and CNS development
			Apoptosis			
rc_AA874794_g_at	1.77E-03	-1.5	1.63E-04	-2.0	nerve growth factor receptor associated protein 1	caspase activation
S76511_s_at	1.88E-01	1.2	5.55E-05	1.8	Bcl2-associated X protein (Bax)	caspase activation
M86564_at	9.04E-01	-0.4	3.99E-04	1.9	alpha-prothymosin	caspase inhibition
M64733Mrna_s_at	2.11E-02	1.8	2.49E-04	2.4	clusterin	unknown

Table E6. Expression of signal transduction genes following MV6 and MV18.

	MV6/Control		MV18/Control		Gene	Description
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
					Signal Transduction	
rc_AI176689_at	9.15E-01	-0.2	7.85E-04	-2.8	mitogen-activated pkk 6	activator of p38 MAPK
X58828_at	1.30E-01	1.2	9.87E-04	2.6	protein tyrosine phosphatase, non-receptor type 2	amino acid dephosphorylation
rc_AA893743_at	1.72E-02	-1.2	3.25E-04	-1.7	protein kinase inhibitor, alpha	cAMP-dependent protein kinase inhibitor
rc_AA893743_g_at	6.18E-02	-1.0	6.36E-06	-1.6	protein kinase inhibitor, alpha	cAMP-dependent protein kinase inhibitor
U78517_at	3.10E-01	-0.6	5.42E-04	-2.1	cAMP-regulated guanine nucleotide exchange factor II	cAMP-dependent protein kinase regulator
rc_AA892417_at	3.69E-02	1.5	3.49E-05	2.7	ephrin A1	cell-cell signaling
rc_AI232374_at	1.80E-02	-1.4	2.55E-05	-2.2	H1 histone family, member 0	chromosome organization
rc_AI232374_g_at	9.51E-02	-1.2	4.00E-04	-2.6	H1 histone family, member 0	chromosome organization
rc_AI177503_at	5.14E-01	0.6	1.39E-04	1.9	H3 histone, family 3B	chromosome organization
rc_AA875172_at	3.39E-01	-0.6	2.43E-06	-2.5	SH3-domain kinase binding protein 1	endocytosis
rc_AI104012_g_at	2.31E-01	0.8	5.19E-05	2.1	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A	endocytosis
U16655_at	8.53E-02	-2.2	2.31E-04	-3.1	phospholipase C, delta 4	generation of inositol 1,4,5-trisphosphate and diacylglycerol
U90312_at	5.50E-04	-1.4	7.80E-07	-3.1	synaptojanin 2	inositol/phosphatidyl-inositol phosphatase
D90401_g_at	8.08E-01	0.2	1.52E-04	-1.6	Afadin	intercellular junction
S74351_s_at	2.63E-05	-7.2	2.06E-03	-5.5	protein tyrosine phosphatase, non-receptor type 16	MAP kinase phosphatase
U02553cds_s_at	5.73E-04	-3.3	6.87E-03	-3.1	protein tyrosine phosphatase, non-receptor type 16	MAP kinase phosphatase
S81478_s_at	6.18E-04	-2.6	1.28E-02	-2.4	protein tyrosine phosphatase, non-receptor type 16	MAP kinase phosphatase
L27843_s_at	3.50E-02	1.5	1.13E-07	2.6	protein tyrosine phosphatase 4a1	nuclear protein tyrosine phosphatase
AF012714_at	3.94E-01	0.3	2.00E-04	1.7	multiple inositol polyphosphate histidine phosphatase 1	phosphoinositide-mediated signaling
AJ001320_at	1.87E-01	-1.2	5.75E-06	-2.0	multiple PDZ domain protein	protein binding
D78588_at	8.15E-04	-1.9	5.83E-05	-3.1	diacylglycerol kinase zeta	protein kinase C activation
rc_AI137862_s_at	7.73E-03	1.7	9.55E-04	2.4	P38 alpha	protein kinase cascade
rc_AI171630_s_at	2.37E-05	2.6	1.09E-06	4.3	P38 alpha	protein kinase cascade
U73142_g_at	7.30E-04	2.6	6.66E-07	4.6	P38 alpha	protein kinase cascade
U73142_at	4.82E-03	3.0	6.50E-04	4.2	P38 alpha	protein kinase cascade
L15619_at	1.66E-01	0.9	3.74E-04	1.7	casein kinase II beta subunit	protein kinase CK2 complex
rc_AI230632_at	7.49E-02	1.3	6.11E-06	1.7	P21 (CDKN1A)-activated kinase 2	protein serine-threonine kinase
rc_AI180424_at	9.87E-01	0.0	6.02E-05	2.1	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	protein targeting
D30740_g_at	1.66E-01	0.8	5.62E-05	1.8	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide	protein targeting
rc_AA799729_g_at	2.90E-01	0.7	8.38E-06	5.4	cAMP phosphodiesterase 4B	purine metabolism
J04563_at	4.57E-01	1.4	9.99E-04	11.3	cAMP phosphodiesterase 4B	purine metabolism
M25350_s_at	7.05E-02	2.1	3.80E-04	9.0	cAMP phosphodiesterase 4B	purine metabolism
U12187_at	1.02E-04	12.8	1.52E-05	17.1	ras-related protein	small GTPase mediated signal transduction
L29232_at	1.04E-01	3.6	1.22E-05	6.8	insulin-like growth factor 1 receptor	protein tyrosine kinase signaling pathway

Table E7. Expression of biosynthesis, receptor, and extracellular matrix genes in the diaphragm following MV6 and MV18.

	MV6/Control		**MV18/Control**		**Gene**	**Description**
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**		
					Biosynthesis	
U89905_at	1.72E-01	-0.5	7.86E-04	-2.1	alpha-methylacyl-CoA racemase	bile acid synthesis
X52625_at	9.93E-02	-0.5	9.41E-06	-1.7	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	cholesterol synthesis
J03190_g_at	5.18E-02	-1.4	1.68E-04	-2.6	5-aminolevulinate synthase	heme synthesis
Y00350_at	3.29E-02	-1.1	3.59E-05	-1.6	uroporphyrinogen decarboxylase	heme synthesis
rc_AI232783_s_at	7.32E-03	1.5	9.13E-04	1.6	glutamine synthetase 1	nitrogen metabolism
D10354_s_at	6.94E-01	-0.1	2.29E-04	-1.7	glutamic-pyruvate transaminase (alanine aminotransferase)	nitrogen metabolism
AB000717exons#1-8_s_at	3.20E-03	1.5	3.17E-05	2.9	S-adenosylmethionine synthetase (non-hepatic-type)	S-adenosylmethionine synthesis
D26073_g_at	9.42E-03	1.3	3.22E-04	1.5	phosphoribosylpyrophosphate synthetase-associated protein (39 kDa)	nucleotide synthesis
U90888_at	2.89E-01	3.1	4.35E-04	5.3	adenosine monophosphate deaminase 3	purine ribonucleoside monophosphate synthesis
					Receptor	
S61973_g_at	1.85E-02	1.4	1.30E-04	1.6	NMDA receptor glutamate-binding chain	cation channel activity
rc_AI639001_at	7.47E-02	-1.4	1.18E-05	-2.2	protein tyrosine phosphatase, receptor-type, M	protein tyrosine kinase signaling pathway
rc_AI232379_at	2.53E-01	1.0	3.84E-05	2.0	platelet derived growth factor receptor, alpha polypeptide	protein tyrosine kinase signaling pathway
					Extracellular Matrix	
rc_AA900848_at	2.92E-02	-1.4	5.90E-04	-1.6	laminin, beta 2	cell adhesion
AF020046_s_at	4.96E-03	-2.2	3.03E-04	-3.9	integrin alpha E1, epithelial-associated	cell-matrix adhesion
rc_AI177366_at	3.34E-01	0.8	2.11E-04	1.7	integrin beta 1	cell-matrix adhesion
X02918_g_at	1.23E-01	0.4	6.61E-04	1.5	prolyl 4-hydroxylase, beta polypeptide	collagen synthesis
S77494_s_at	1.53E-01	3.3	4.81E-04	6.7	lysyl oxidase	collagen synthesis
S66184_s_at	1.62E-01	7.3	8.54E-04	22.8	lysyl oxidase	collagen synthesis
rc_AI639233_s_at	5.54E-01	0.6	6.98E-05	2.0	decorin	extracellular matrix organization and biogenesis
M64780_at	9.83E-03	-1.3	1.42E-04	-1.9	agrin	neuromuscular junction formation
M64780_g_at	4.61E-03	-1.3	8.36E-05	-1.9	agrin	neuromuscular junction formation
L02896_at	3.24E-01	-0.4	7.90E-05	-2.0	glypican 1	proteoglycan integral to plasma membrane
S61868_at	2.05E-01	1.2	2.47E-04	4.0	syndecan 4	skeletal muscle development and regeneration
U75405UTR#1_f_at	2.17E-02	-1.6	4.18E-05	-3.6	collagen, type 1, alpha 1	structural protein

Table E8. Expression of ESTs in the diaphragm following MV6 and MV18.

	MV6/Control		MV18/Control		Gene
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**	
rc_AI639060_at	2.94E-02	-4.9	4.09E-04	-48.1	weak similarity to protein ref:NP_060351.1 (H.sapiens) hypothetical protein FLJ20559 [Homo sapiens]
rc_AA799812_g_at	3.72E-03	-4.8	4.23E-10	-8.0	moderate similarity to protein pir:A41109 (H.sapiens) A41109 protein-tyrosine-phosphatase (EC 3.1.3.48), nonreceptor type 3 - human
rc_AA800202_at	3.67E-03	-2.9	3.74E-04	-2.6	
rc_AA800298_at	4.56E-03	-2.6	9.59E-04	-2.6	similar to type XV collagen (LOC298069), mRNA
rc_AA799812_at	5.86E-02	-2.3	1.44E-05	-5.1	moderate similarity to protein pir:A41109 (H.sapiens) A41109 protein-tyrosine-phosphatase (EC 3.1.3.48), nonreceptor type 3 - human
rc_AA874873_at	6.77E-03	-1.9	1.19E-04	-3.5	
rc_AA874873_g_at	8.80E-04	-1.9	9.19E-06	-2.5	
rc_AA891288_g_at	4.66E-03	-1.7	8.63E-05	-1.7	
rc_H31144_g_at	2.23E-03	-1.7	2.46E-04	-2.3	similar to non-receptor protein tyrosine kinase Ack (LOC303882), mRNA
rc_AI639267_at	1.52E-01	-1.5	9.29E-05	-3.2	
rc_AI639495_at	9.31E-04	-1.5	6.43E-07	-4.2	
rc_AA800272_at	3.17E-04	-1.5	1.98E-03	-1.9	similar to mitochondrial ribosomal protein L3 (L3mt) (LOC300974), mRNA
rc_H31802_at	2.11E-02	-1.4	4.67E-05	-2.4	moderate similarity to protein pir:S12207 (M.musculus) S12207 hypothetical protein (B2 element) - mouse
rc_AA874802_s_at	6.59E-03	-1.4	4.67E-05	-2.5	
rc_AA800686_at	6.34E-03	-1.3	2.00E-05	-2.2	moderate similarity to protein pir:I39175 (H.sapiens) I39175 SH2-domain protein Grb-IR - human
rc_AA799518_at	3.56E-02	-1.3	5.13E-04	-1.8	similar to mmDj4 (LOC300721), mRNA
rc_AA875633_at	3.35E-02	-1.3	8.66E-06	-2.0	similar to polymerase I-transcript release factor; PTRF (LOC287710), mRNA
rc_AA800184_at	1.22E-01	-1.3	1.04E-05	-2.2	
rc_AI639268_at	4.96E-02	-1.3	1.06E-04	-2.0	similar to DKFZP566O084 protein (LOC287411), mRNA
rc_AA892777_at	4.43E-02	-1.3	1.13E-04	-2.3	similar to Ribonuclease HI large subunit (RNase HI large subunit) (LOC364974), mRNA
rc_AA860015_at	5.17E-03	-1.2	5.16E-04	-1.8	similar to KIAA1592 protein (LOC363216), mRNA
rc_AA892570_at	6.17E-03	-1.2	8.46E-07	-2.7	
rc_AA866299_g_at	3.97E-02	-1.2	1.81E-04	-2.0	
rc_AA799442_at	9.43E-02	-1.2	1.41E-05	-1.8	hypothetical LOC293114 (LOC293114), mRNA
rc_AA892303_g_at	1.26E-02	-1.2	1.03E-04	-1.9	similar to dynein, axonemal, light chain 4 (LOC294376), mRNA
AA848268_at	1.16E-01	-1.2	3.02E-04	-2.4	similar to mmDj4 (LOC300721), mRNA
rc_AA799570_at	3.93E-02	-1.2	8.23E-05	-1.7	similar to mmDj4 (LOC300721), mRNA
rc_AA875495_at	1.43E-03	-1.1	4.71E-04	-2.1	
rc_AA891037_at	4.61E-02	-1.1	2.37E-04	-1.8	similar to 60S ribosomal protein L3-like (LOC287122), mRNA
rc_AA866299_at	2.05E-02	-1.1	3.12E-04	-1.9	
rc_AA799771_at	1.87E-02	-1.1	4.46E-05	-1.9	
rc_AA799442_g_at	6.50E-02	-1.1	5.32E-04	-2.1	hypothetical LOC293114 (LOC293114), mRNA
rc_AA799607_at	1.07E-02	-1.0	6.22E-05	-1.7	
rc_AA799829_at	1.68E-02	-1.0	4.96E-04	-1.7	similar to mitochondrial ATP synthase regulatory component factor B (LOC362749), mRNA
rc_AA892799_s_at	2.60E-01	-1.0	5.60E-04	-2.8	similar to glyoxylate reductase/hydroxypyruvate reductase (LOC298085), mRNA
rc_AA891802_at	6.92E-02	-0.9	1.82E-05	-2.6	strong similarity to protein ref:NP_062269.1 (M.musculus) cysteine and histidine rich 1; cysteine and histidine-rich cytoplasmic protein [Mus musculus]
rc_AA892422_at	2.76E-01	-0.9	6.49E-04	-1.8	similar to mitochondrial ribosomal protein L11 (LOC293666), mRNA
rc_H31711_at	4.05E-02	-0.9	1.51E-05	-1.8	similar to mitochondrial ribosomal protein L44 (LOC301552), mRNA
rc_AA891220_at	2.20E-01	-0.9	1.68E-04	-1.6	

Table E8. Expression of ESTs in the diaphragm following MV6 and MV18, continued.

	MV6/Control		MV18/Control		Gene
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**	
rc_AA859719_at	1.50E-01	-0.8	1.87E-05	-2.0	similar to mitochondrial ribosomal protein S14 (LOC289143), mRNA
rc_AA891814_at	6.37E-02	-0.8	3.50E-05	-2.8	similar to RIKEN cDNA 2010001O09 (LOC298282), mRNA
rc_H31990_at	6.06E-02	-0.8	8.83E-07	-2.1	
rc_AA859688_at	2.72E-01	-0.8	2.17E-04	-1.6	similar to Auh protein (LOC361215), mRNA
rc_AA859919_at	2.63E-01	-0.8	7.68E-05	-1.7	similar to chromosome 14 open reading frame 52 (LOC299154), mRNA
rc_AA799654_g_at	1.70E-01	-0.8	1.90E-04	-1.6	similar to WD repeat-containing F-box protein FBW5 (LOC362082), mRNA
rc_AA799721_g_at	2.25E-02	-0.8	1.96E-05	-1.9	similar to cysteine and histidine-rich protein (LOC315097), mRNA
rc_AA874995_at	8.19E-02	-0.7	4.80E-04	-2.2	similar to BC002216 protein (LOC313771), mRNA
rc_H33149_at	1.96E-02	-0.7	3.27E-05	-1.5	similar to RIKEN cDNA 1810047C23 (LOC361151), mRNA
rc_AA892863_at	6.75E-02	-0.7	2.45E-05	-1.7	similar to mitochondrial carrier homolog 2 (LOC295922), mRNA
rc_AA800243_at	2.24E-01	-0.7	8.97E-04	-2.3	similar to cell death activator CIDE-A (LOC291541), mRNA
rc_AA891733_at	1.76E-01	-0.7	2.36E-06	-2.5	
rc_H32977_at	4.19E-01	-0.6	2.67E-04	-1.7	similar to RIKEN cDNA 2010015J01 (LOC316365), mRNA
rc_AI639499_s_at	1.90E-01	-0.6	1.61E-05	-2.4	
rc_AA859788_at	1.57E-01	-0.6	2.05E-06	-2.1	similar to mitochondrial ribosomal protein S11 (LOC365291), mRNA
rc_AA891171_s_at	6.72E-01	-0.6	4.00E-05	-1.8	similar to RIKEN cDNA 1810004I06 (LOC293130), mRNA
rc_AA892234_at	3.47E-01	-0.5	8.52E-04	-1.8	similar to microsomal glutathione S-transferase 3 (LOC289197), mRNA
rc_AA800216_at	8.70E-01	-0.5	3.02E-07	-2.8	weak similarity to protein ref:NP_005442.2 (H.sapiens) enigma protein; LIM domain protein [Homo sapiens]
AA684537_at	9.63E-01	-0.5	6.25E-05	-2.1	
M59814_at	2.09E-01	-0.5	3.30E-04	-2.1	
rc_AA866383_at	6.17E-01	-0.5	7.65E-05	-2.8	
rc_AA799472_g_at	6.55E-01	-0.4	1.72E-04	-1.7	similar to RIKEN cDNA 2900091E11 (LOC299707), mRNA
rc_AA891800_g_at	1.48E-01	-0.4	1.09E-05	-1.7	similar to RIKEN cDNA 1110013G13 (LOC310856), mRNA
rc_AA891742_at	2.06E-01	-0.4	1.55E-06	-2.1	similar to cDNA sequence BC019806 (LOC306270), mRNA
rc_AA892572_at	3.98E-01	-0.4	5.61E-05	-2.0	similar to RIKEN cDNA D130059P03 gene (LOC312248), mRNA
rc_AA892572_g_at	4.16E-01	-0.4	4.66E-04	-1.7	similar to RIKEN cDNA D130059P03 gene (LOC312248), mRNA
rc_AI232012_at	7.88E-01	-0.4	6.96E-05	-2.0	similar to Aa2-258 (LOC296658), mRNA
rc_AA799788_s_at	2.21E-01	-0.4	6.96E-04	-1.6	similar to cell division cycle 34; ubiquitin-conjugating enzyme E2-32 KDA complementing; ubiquitin carrier protein; ubiquitin-protein ligase (LOC299602), mRNA
rc_AI639093_at	6.21E-01	-0.4	8.49E-04	-1.7	moderate similarity to protein pdb:1LBG (E. coli) B Chain B, Lactose Operon Repressor Bound To 21-Base Pair Symmetric Operator Dna, Alpha Carbons Only
rc_AA891785_at	2.00E-01	-0.4	1.47E-04	-1.5	similar to NADP+-specific isocitrate dehydrogenase (LOC293043), mRNA
rc_AA891800_at	2.42E-01	-0.3	1.61E-05	-1.9	similar to RIKEN cDNA 1110013G13 (LOC310856), mRNA
rc_AI639030_f_at	9.18E-02	-0.3	1.50E-05	-1.7	
rc_AA799888_at	4.03E-01	-0.3	3.29E-05	-1.9	similar to mitochondrial ribosomal protein L40 (LOC287962), mRNA
rc_AA891580_at	2.46E-01	-0.3	3.32E-04	-1.8	similar to RIKEN cDNA 2010013M14 (LOC291910), mRNA
AA685112_at	4.21E-01	-0.2	1.83E-04	-2.2	similar to NADH dehydrogenase:ubiquinone Fe-S protein 8 (LOC293652), mRNA
rc_AI639381_at	2.49E-01	-0.2	4.40E-04	-1.7	similar to checkpoint suppressor 1 (LOC314367), mRNA
rc_AA799479_g_at	8.19E-01	-0.2	2.55E-04	-1.5	similar to NADH dehydrogenase:ubiquinone Fe-S protein 8 (LOC293652), mRNA
rc_AI104679_s_at	8.48E-01	-0.2	1.13E-04	-1.6	moderate similarity to protein ref:NP_002485.1 (H.sapiens) NADH dehydrogenase (ubiquinone) 1, subcomplex unknown, 1 (6kD, KFYI) [Homo sapiens]
rc_AA892378_at	6.68E-01	-0.2	3.07E-04	-1.7	similar to tetratricopeptide repeat domain 11 (LOC288584), mRNA

Table E8. Expression of ESTs in the diaphragm following MV6 and MV18, continued.

	MV6/Control		MV18/Control		Gene
Affymetrix Probe	**p-value**	**Fold**	**p-value**	**Fold**	
rc_AA799412_g_at	8.57E-01	-0.1	7.60E-04	-1.7	similar to Steroid hormone receptor ERR1 (Estrogen-related receptor, alpha) (ERR-alpha) (Estrogen receptor-like 1) (LOC293701), mRNA
rc_AA891396_at	4.16E-01	0.0	6.98E-04	-1.5	
rc_AA892789_at	8.71E-01	0.1	3.88E-06	-2.3	
rc_AI639422_at	6.47E-01	0.1	1.30E-05	-1.7	strong similarity to protein pir:A60424 (H.sapiens) A60424 calsequestrin precursor, fast skeletal muscle - human
rc_AI639058_s_at	3.42E-01	0.1	4.39E-05	2.7	similar to Nedd4 WW binding protein 4 (LOC311676), mRNA
rc_AA964320_at	7.08E-01	0.2	6.37E-04	-1.8	similar to NADH dehydrogenase (ubiquinone) 1 beta subcomplex 8 (LOC293991), mRNA
rc_AI639108_at	2.72E-01	0.2	7.23E-05	-2.0	
rc_AA799724_g_at	5.29E-01	0.2	6.74E-05	1.5	similar to hypothetical protein MGC9850 (LOC360762), mRNA
rc_AA799511_g_at	7.31E-01	0.4	9.27E-04	1.6	similar to heterogeneous nuclear ribonucleoprotein A2/B1 (LOC362361), mRNA
rc_AA892831_s_at	4.96E-01	0.4	7.62E-08	2.0	similar to 26S proteasome non-ATPase regulatory subunit 11 (26S proteasome regulatory subunit S9) (26S proteasome regulatory subunit p44.5) (LOC303353), mRNA
rc_AI228674_s_at	4.04E-01	0.4	6.49E-06	1.9	moderate similarity to protein pdb:2RMA (H.sapiens) S Chain S, Cyclophilin A (E.C.5.2.1.8) Complexed With Cyclosporin A
rc_AA891535_at	3.93E-01	0.7	3.33E-04	1.7	similar to hippocampus abundant gene transcript 1; tetracycline transporter-like protein (LOC295398), mRNA
rc_AA818152_f_at	3.10E-01	0.7	3.26E-06	1.8	
rc_AA799744_at	4.45E-01	0.7	4.69E-04	2.8	moderate similarity to protein pdb:1JZ5 (E. coli) B Chain B, E. Coli (Lacz) Beta-Galactosidase In Complex With D-Galctopyranosyl-1-On
rc_AA800576_at	5.25E-01	0.7	9.87E-05	1.7	
rc_AA875205_g_at	1.84E-01	0.8	4.17E-06	1.7	similar to D5Wsu45e protein (LOC288516), mRNA
rc_AA866482_at	2.49E-01	0.8	1.68E-04	1.8	similar to faciogenital dysplasia homolog (LOC363460), mRNA
rc_AA893082_at	2.29E-01	1.1	2.81E-04	3.0	
rc_AA799330_at	2.82E-01	1.1	3.82E-07	3.4	similar to Pelo protein (LOC294754), mRNA
rc_AA891108_at	1.85E-01	1.1	6.31E-04	1.6	
rc_AA860049_at	7.64E-02	1.2	4.35E-04	1.9	strong similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
rc_AA892042_at	8.57E-03	1.2	3.15E-05	1.9	similar to RNA helicase (LOC317335), mRNA
rc_AA800199_at	4.53E-02	1.2	5.44E-04	2.0	similar to RIKEN cDNA 1110020C13 (LOC363004), mRNA
rc_AA875438_at	1.64E-02	1.2	6.77E-04	1.5	
rc_AA859585_at	5.86E-02	1.2	5.45E-06	2.1	hypothetical LOC302941 (LOC302941), mRNA
rc_AA799803_at	2.27E-01	1.3	1.17E-04	2.6	similar to complement component 1, r subcomponent (LOC312705), mRNA
rc_AA859954_at	2.51E-01	1.3	3.33E-04	3.3	
rc_AI180108_at	1.74E-02	1.4	6.84E-04	1.5	similar to Wbscr1 (LOC288599), mRNA
rc_AA891821_at	4.11E-02	1.4	1.19E-04	1.9	Unknown (protein for MGC:72616)
rc_AA893860_at	3.40E-02	1.4	4.12E-05	2.1	similar to hypothetical protein D15Wsu59e (LOC294810), mRNA
rc_AI639277_at	7.63E-03	1.5	8.22E-05	2.1	
rc_AA800245_at	8.92E-03	1.5	4.66E-04	1.7	similar to RING-finger protein MURF (LOC362708), mRNA
rc_AA894188_at	1.31E-01	1.6	1.19E-05	3.0	moderate similarity to protein sp:P00722 (E. coli) BGAL_ECOLI Beta-galactosidase (Lactase)
U21719mRNA_s_at	6.82E-02	1.6	2.63E-06	3.1	similar to Nucleolar RNA helicase II (Nucleolar RNA helicase Gu) (RH II/Gu) (DEAD-box protein 21) (LOC317399), mRNA
rc_AA859633_g_at	2.66E-03	1.7	1.21E-04	2.1	similar to PD2 protein (LOC361531), mRNA
AF069782_at	3.14E-02	1.9	4.27E-06	3.9	
rc_AI237535_s_at	5.88E-02	1.9	2.57E-04	3.2	
rc_AA875126_at	5.15E-02	2.6	5.23E-06	7.2	similar to Myosin Id (Myosin heavy chain myr 4) (LOC289785), mRNA
rc_AA892333_at	5.65E-02	2.7	2.04E-04	6.0	similar to tubulin, alpha 6; tubulin alpha 6 (LOC300218), mRNA
rc_AI176456_at	8.83E-05	35.1	5.01E-05	41.0	moderate similarity to protein sp:P04732 (H.sapiens) MT1E_HUMAN METALLOTHIONEIN-IE (MT-1E)