Mitogen- and Stress-Activated Protein Kinase 1 Regulates Status Epilepticus-Evoked Cell Death in the Hippocampus

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Abstract
Mitogen-activated protein kinase (MAPK) signaling has been implicated in a wide range of neuronal processes, including development, plasticity, and viability. One of the principal downstream targets of both the extracellular signal-regulated kinase/MAPK pathway and the p38 MAPK pathway is Mitogen- and Stress-activated protein Kinase 1 (MSK1). Here, we sought to understand the role that MSK1 plays in neuroprotection against excitotoxic stimulation in the hippocampus. To this end, we utilized immunohistochemical labeling, a MSK1 null mouse line, cell viability assays, and array-based profiling approaches. Initially, we show that MSK1 is broadly expressed within the major neuronal cell layers of the hippocampus and that status epilepticus drives acute induction of MSK1 activation. In response to the status epilepticus paradigm, MSK1 KO mice exhibited a striking increase in vulnerability to pilocarpine-evoked cell death within the CA1 and CA3 cell layers. Further, cultured MSK1 null neurons exhibited a heightened level of N-methyl-D-aspartate-evoked excitotoxicity relative to wild-type neurons, as assessed using the lactate dehydrogenase assay. Given these findings, we examined the hippocampal transcriptional profile of MSK1 null mice. Affymetrix array profiling revealed that MSK1 deletion led to the significant (>1.25-fold) downregulation of 130 genes and an upregulation of 145 genes. Notably, functional analysis indicated that a subset of these genes contribute to neuroprotective signaling networks. Together, these data provide important new insights into the mechanism by which the MAPK/MSK1 signaling cassette confers neuroprotection against excitotoxic insults. Approaches designed to upregulate or mimic the functional effects of MSK1 may prove beneficial against an array of degenerative processes resulting from excitotoxic insults.

Keywords
cell death, excitotoxicity, hippocampus, MAPK, MSK1, neuroprotection

Introduction
The molecular signaling events that regulate neuroprotection and excitotoxic cell death have been an area of intensive investigation for many years. Beyond the well-established roles of a subset of signaling pathways that underlie either neuroprotection (e.g., the Nrf2-Antioxidant Response Element signaling pathway) or cell death (e.g., the intrinsic apoptotic pathway), numerous cell signaling events and gene networks have the capacity to confer both protection and to enhance vulnerability to potentially excitotoxic insults (Mattson, 2003; Calabrese et al., 2005; Culmsee and Landshamer, 2006; Rueda et al., 2016). Consistent with this idea, the extracellular signal-regulated kinase (ERK)/MAPK pathway

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has been shown to function as both a regulator of neuroprotective and cell death signaling pathways (reviewed in Hetman and Xia, 2000; Zhuang and Schnellmann, 2006; Cagnol and Chambard, 2010; Martin and Pognonec, 2010; Subramaniam and Unsicker, 2010). Along these lines, a large number of in vitro and in vivo studies have shown that the abrogation of ERK/MAPK signaling suppresses neuronal death induced by multiple apoptotic- and necrotic-mediated mechanisms (Alessandrini et al., 1999; Kuroki et al., 2001; Lesuisse and Martin, 2002; Pedersen et al., 2002; Park et al., 2004). In contrast with these findings, studies have also shown that the ERK/MAPK pathway facilitates neuronal cell survival (reviewed in Ballif and Blenis, 2001; Portt et al., 2011). For example, ERK/MAPK signaling has been shown to stimulate preconditioning-mediated neuroprotection (Gonzalez-Zulueta et al., 2000; Bickler et al., 2005) and to drive the expression of neuroprotective genes, including BCL-2 and BDNF (Hetman et al., 1999; Cheng et al., 2013).

These profoundly discordant observations regarding ERK/MAPK signaling and cell viability may be explained by the route of injury, duration of activation, and the subcellular localization of ERK (Hetman and Xia, 2000; Zhuang and Schnellmann, 2006; Cagnol and Chambard, 2010; Martin and Pognonec, 2010). Here, we chose to further our understanding of the role of MAPK signaling in neuroprotection by focusing on one of its principal effector kinases: Mitogen- and Stress-activated protein Kinase 1 (MSK1). MSK1 (and its homolog MSK2) is a serine/threonine kinase that is formed by two distinct functional domains: an autoregulatory C-terminal kinase and an N-terminal substrate kinase (reviewed in Hauge and Frodin, 2001; Martin and Pognonec, 2010). In addition to its regulation by the ERK/MAPK cascade, MSK1 is downstream of the p38/MAPK pathway (Deak et al., 1998; McCoy et al., 2005).

MSK1 is localized to the cell nucleus and functions as a regulator of chromatin structure and transcription factor activation. For example, MSK1 phosphorylates histone H3 and the transcription factors ATF-1 and CREB (Wiggin et al., 2002; Soloaga et al., 2003; and reviewed in Arthur, 2008; Vermeulen et al., 2009; Reyskens and Arthur, 2016). Notably, via its phosphorylation of CREB at Ser133 (and the resulting increase in CRE-mediated gene expression), MSK1 appears to be a key route by which the ERK/MAPK pathway triggers long-term forms of neuronal plasticity. Consistent with this idea, MSKI/- deficient mice exhibit an array of synaptic and cognitive deficits (Chwang et al., 2007; Karešina et al., 2012; Correa et al., 2012). Further, MSK1 regulates progenitor cell proliferation in the subgranular zone of the dentate gyrus (Karešina et al., 2015), which could also contribute to the cognitive deficits observed in MSKI/ null mice.

As with signaling via the ERK/MAPK pathway (an upstream effector of MSKI), there are divergent findings regarding the role of MSK in cell death signaling, with reports showing that MSK is both protective and can enhance vulnerability to stress stimuli (Hughes et al., 2003; Kannan-Thulasiraman et al., 2006; Lang et al., 2015). Here, we furthered this line of inquiry and provide data showing that the MSK1 pathway plays an important role in conferring resistance against seizure-evoked cell death.

**Materials and Methods**

**Mice**

MSKI/ mice (also referred to here as MSKI null mice) and MSKI/ (also referred to here as MSKI WT mice) were provided by Dr. J. Simon C. Arthur (University of Dundee, Dundee, Scotland) and bred at the Ohio State University. MSKI/ and MSKI WT mice were genotyped via PCR profiling of DNA isolated from tail biopsies: The PCR cycling conditions and primers are described by Wiggin et al. (2002). The MSKI/ deletion line was bred into a C57Bl/6 line for >10 generations. For the experiments shown in Figures 2(d) and 3 to 7, which constitute the cell death profiling and array assays, experimental mice were derived from MSKI/ breeder cages; hence, MSKI/ (WT) and MSKI/ littermates with the same genetic background were used. Standard C57Bl/6 mice, originally acquired from Jackson Labs, were used for the MSKI, pMSK1, and pERK1/2 expression profiling assays (Figures 1 and 2(a), (b), (c), (e), and (f)). For all studies, adult, 6- to 14-week-old mice were used. Animals were entrained to a standard 12:12 light/dark cycle and were allowed ad libitum access to water and food. The studies reported here were conducted in compliance with the Ohio State University Institutional Animal Care and Use Committee guidelines.

**Pilocarpine-Induced SE**

The pilocarpine model was used to induce status epilepticus (SE) (Curia et al., 2008). Initially, mice received an intraperitoneal (IP) injection of atropine methyl nitrate (1.3 mg/kg in saline, Sigma, St Louis, MO). Thirty minutes later, mice were IP injected with pilocarpine (310 mg/kg, Sigma) diluted in physiological saline to evoke SE. The Racine grading scale (Racine, 1972) was used to assess seizure magnitude and SE onset. SE was defined as multiple Stage 5 motor seizures (tonic-clonic seizures observed in all four limbs, which resulted in a loss of balance) that persisted for ≥3 h. SE was not terminated with diazepam.

**Immunohistochemistry**

For all histological analysis, mice were sedated using ketamine/xylazine anesthetic (ketamine: 120 mg/kg of...
body weight and xylazine: 24 mg/kg body weight), and tissue was fixed using transcardial perfusion with paraformaldehyde (4%) diluted in phosphate-buffered saline (PBS). Isolated whole brains were then postfixed in paraformaldehyde (4% for 4 h at 4°C) followed by cryoprotection using 30% sucrose. Stereotaxic coordinates from anterior to posterior from bregma: −1.40 to −2.20 mm were used to cut 40-μm coronal sections through the dorsal hippocampus.

Immunolabeling commenced with a series of wash steps in PBS, followed by incubation in PBS with 0.3% hydrogen peroxide. Next, the tissue was blocked (2 h at room temperature) using 10% normal goat serum or 3% normal horse serum diluted in PBS with 1% Triton X-100 (PBST). Sections were then immunolabeled (overnight at 4°C) using rabbit polyclonal anti-pMSK1 (1:1,000 dilution, Cell Signaling, Danvers, MA; catalog number: 9594) or rabbit polyclonal anti-pERK1/2 (1:1,000 dilution, Cell Signaling, catalog number: 9101). Next, the tissue was processed using the ABC labeling method and then incubated with horseradish peroxidase (HRP) avidin (Vector Labs; San Carlos, CA). Visualization of the immunolabeling was achieved by incubating the tissue with nickel-intensified diaminobenzidine substrate (Vector Labs) for HRP. Tissue was then mounted on gelatin-subbed slides, cleared with xylenes and coverslipped using Permount (Fisher Scientific). Photomicrographs were acquired using a Leica DM IRB microscope (Nussloch, Germany).

Cresyl Violet Staining
Mice were transcardially perfused, as described earlier, and 40-μm-thick sections through the hippocampus were mounted on gelatin-coated slides, dehydrated in alcohol, and stained in cresyl violet solution (0.3%). Next, the sections were destained (0.1% glacial acetic acid in 95% ethanol), cleared with xylenes, and finally coverslipped with Permount. Photomicrographs were acquired as described earlier.

Fluoro-Jade B
Fluoro-Jade B (FJB) labeling was performed using the methods described in Choi et al. (2007). Image collection was performed using a Zeiss 510 confocal microscope.

Cell Quantitation
Photomicrographs of cresyl violet and FJB-labeled cells were acquired at 40× magnification, and digital images were captured and data quantified using MetaMorph software (Universal Imaging, West Chester, PA). Quantitation
was performed on the CA1, CA3, and hilar regions of the hippocampus. The hilus was defined as the region between the lower and upper granule cell layer (GCL) blades. The total number of FJB- and cresyl violet-positive cells in each of four dorsal hippocampal sections were counted. Each section was separated by a 200-μm interval (stereotaxic coordinate AP, approximately −1.40 to −2.20 mm). Cell counts were averaged for each animal and then used to generate group mean ± SEM values for each condition. For the 3-day post-SE data sets, six to eight mice were used for each group; for the 6-week time points, four to six animals were used for each group. Data are reported as the mean ± the SEM for each condition. Mean values were statistically analyzed between cell layers (e.g., control vs. experimental) using the Student’s t test, and a p < .05 was considered significant.

**Immunofluorescent Labeling**

Sections were washed with PBS and then blocked (2 h room temperature) with 10% normal goat serum in PBST. Next, sections were incubated overnight (at 4°C) with a rabbit polyclonal total MSK1 antibody (1:500 dilution, Cell Signaling, catalog number: 3489) and with a mouse monoclonal anti-NeuN antibody (1:1,000 dilution, Millipore, Billerica, MA; catalog code: MAB377). Tissue was then washed 5× in PBST and incubated for 2 h (at 22°C) with goat polyclonal Alexa 488- and donkey polyclonal Alexa 594- (1:1,000 dilution, Invitrogen, Carlsbad, CA) conjugated secondary antibodies. Next, sections were washed, and DNA was labeled with Hoechst (1 μg/ml: Cell Signaling). Finally, tissue was mounted with Cytoseal (Richard-Allan Scientific, Kalamazoo, MI), and images were acquired with a Leica SP8 confocal microscope.

**Western Blotting**

Animals were sacrificed as described earlier, and hippocampi were dissected from whole brains. Tissue was lysed in radioimmunoprecipitation assay buffer, and then protein extracts (5 μg/μL) were loaded onto 10% SDS-PAGE gels and electrophoresed and then transblotted onto polyvinylidene difluoride membranes (Immobilon-P; Millipore) using standard methodologies. Next, membranes were blocked with 10% milk in tris-buffered saline containing 0.1% Triton-X-100 (TBST: 1 hr) and then incubated overnight with the noted MSK1 (1:500 dilution) or pMSK (1:1,000, dilution) antibodies. After washing, membranes were treated (1 hr at room temperature) with an anti-rabbit IgG HRP-conjugated antibody (1:2,000 dilution, PerkinElmer Life Sciences), and the HRP signal was detected using the Renaissance bioluminescent detection system (New England Nuclear). Blots were then stripped and probed using a mouse monoclonal β-actin antibody (1:1,000, PhosphoSolutions Catalog code: 125-ACT), and the signal was detected using the noted HRP labeling and visualization steps.

**RNA isolation and microarray analyses**

Mice were sacrificed, and brains were isolated as described earlier. Bilateral hippocampal tissue was removed, and total RNA was purified using TRIzol (Invitrogen) following the manufacturer’s protocol. RNA quantity and quality was assayed using an Agilent 2100 Bioanalyzer (Agilent Technologies), and the RNA from three animals per genotype (WT and MSK1 null) was prepared for array profiling using the GeneChip one-cycle target labeling kit (Affymetrix). Biotinylated cRNA was profiled using the GeneChip 430 2.0 Mouse Genome Array, running one array per mouse: (e.g., three animals/arrays per genotype). cRNA preparation, microarray hybridization, and profiling were performed at the Ohio State University Microarray Core Facility. Raw data (.cel files) were processed using dChip software (http://www.hsph.harvard.edu/cli/complab/dchip/). The resulting data sets were filtered to identify genes that were significantly altered by the deletion of MSK1; a 1.25-fold change in expression with a p value of ≤ .05 was considered significant. Subsequently, Matlab R2016a (MathWorks) was used to generate the hierarchical clustering map based on the expression values of significantly altered genes. Finally, gene functional classification and clustering were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID), with significant enriched annotation terms set to p values of ≤ .05. Graphical representation of the analysis results was completed using the Cytoscape software Enrichment Map plug-in. Microarray data are available from the Gene Expression Omnibus website (http://www.ncbi.nlm.nih.gov/geo), under accession number: GSE98751.

**Neuronal Toxicity Assays**

Neuronal cell death after an N-methyl-D-aspartate (NMDA) challenge in primary hippocampal neurons from MSK1 null and WT mice was assessed as described in Carrier et al. (2006). Briefly, neurons were isolated from the hippocampus of postnatal day 1 mice, dissociated with trypsin, and plated on polylysine-coated 12-mm glass coverslips in a 24-well plate. The cells were maintained in Neurobasal media supplemented with 2% B27, 1% penicillin/streptomycin, and 0.25 mM glutamine (all culture media were from Gibco) for 10 days. NMDA (50 μM) with 2 μM glycine (or control solution) was added to the cultures for 20 min, and the cell culture media was collected at 4 h and 8 h for the measurement of lactate dehydrogenase (LDH) release as a measure of loss of membrane integrity (measured as described in Carrier et al., 2006). Brightfield images of the cells were
also acquired as a record of cell health/death. Finally, at 8 h after NMDA/glycine treatment, cultures were fixed with 4% paraformaldehyde for 30 min at room temperature, permeabilized with 0.4% Triton X-100 for 10 min at 37°C, and blocked with 10% bovine serum albumin for 60 min at 37°C. The cultures were then incubated overnight (at 4°C) in monoclonal MAP2 antibody (1:500 dilution, HM-2 clone, Sigma, St. Louis, MO) in PBS containing 3% bovine serum albumin/0.4% Triton X-100. After washing (3×) with PBS, the cells were incubated 60 min (at 37°C) with an Alexa 488-conjugated antibody against mouse IgG (1:1000, Molecular Probes, Eugene, OR). Finally, the cells were stained with Hoechst (as described above), mounted on glass slides with PBS/glycerol (1:3), and sealed with nail polish. Fluorescence images were captured using a CoolSnap HQ digital camera (Roper Scientific, Tucson, AZ) connected to a Nikon TE2000S epifluorescence microscope (Nikon Instruments, Melville, NY). FITC excitation/emission filters were used to visualize MAP2 while DAPI filters were used for Hoechst 33258. Data were analyzed using MetaMorph software. Mean values were statistically analyzed between control and experimental conditions and between cell phenotypes using the Student’s t test, and a p < .05 was considered significant.

**Intracellular Calcium Measurement**

Hippocampal neurons cultured on 12 mm coverslips were loaded with 5 μM Fura-2 AM (Molecular Probes) for 45 min at room temperature in a HEPES-based buffer (HBSS) containing the following (in mM): 137 NaCl, 5.6 glucose, 20 HEPES, 5 KCl, 0.6 Na2HPO4, 0.6 KH2PO4, 10 NaHCO3, 0.9 MgSO4, and 1.4 CaCl2, pH 7.4. Coverslips were then placed in a laminar flow chamber and mounted on the stage of a Nikon TE2000S epifluorescence microscope. Single-cell ratiometric (alternating 340 nm/380 nm excitation wavelengths and 510 nm emission wavelength) fluorescence traces were acquired at 10-s intervals using MetaFluor software controlling a CoolSnap digital camera. Neurons were identified by morphology as assessed from bright-field images. Results are presented as background subtracted 340 nm/380 nm ratios. All NMDA-containing solutions were made in HBSS and contained 0.5 μM tetrodotoxin. NMDA solutions included 1 μM glycine and omitted MgSO4. Mean-evoked response values were statistically analyzed between cell phenotypes using the Student’s t test, and a p < .05 was considered significant.

**Electroencephalogram Recording**

Electroencephalogram (EEG) electrode placement, recordings, and analysis were performed as described in our previous study (Lee et al., 2009). Briefly, animals were surgically implanted with bipolar recording electrodes (Plastics One, Roanoke, VA); one within hippocampal area CA1 (anterior −1.8 mm from bregma; lateral 1.1 mm; and dorsoventral 1.2 mm) and the other within the cortex (anterior −2.8 mm from bregma; lateral 1.1 mm; and dorsoventral 1.2 mm). Animals were then allowed to recover from the electrode implantation procedure for 10 days prior to the initiation of the SE paradigm (described earlier). EEG recording was started 10 min prior to pilocarpine injection, and data were recorded for approximately 120 min post-SE onset. The MP150 data acquisition system (Biopac Systems, Santa Barbara, CA) was used to record polysomnographic signals, and data analysis was performed using Acknowledge 3.9.0 software (Biopac Systems). EEG data were analyzed at 10-min intervals, and the average peak-to-peak values were generated from 20-s EEG traces. Four WT and 4 MSK1 null mice were profiled for this study. Mean peak-to-peak response values were statistically analyzed between mouse lines using the Student’s t test, and a p < .05 was considered significant.

**Results**

**MSK1 Expression and Activation in the Hippocampus**

As a starting point for our analysis, we used immunohistochemical labeling to examine MSK1 expression in the hippocampus. Consistent with prior reports (Choi et al., 2012; Karelina et al., 2012), MSK1 was detected in all major neuronal cell layers, including the CA1, CA3, and the GCL (Figure 1(a)). MSK1 expression was low in the CA1 relative to expression in the CA3 and the GCL. Double immunofluorescent labeling for MSK1 and for the neuronal-specific marker NeuN (Figure 1(b)) confirmed the neuronal expression of MSK1, and double labeling with the DNA stain Hoechst showed that MSK1 was concentrated in cellular nuclei. Interestingly, although the vast majority of CA1 neurons exhibited a low level of MSK1, there was a subset of neurons that expressed high levels of the kinase (Figure 1(b): CA1 panel; arrows denote high-expressing cells). In the hilus, limited MSK1 expression was detected in NeuN-positive neurons, indicating low-level MSK1 expression in interneuron cell populations (Figure 1(b): GCL panel; boxed regions denote hilar neurons with limited MSK1 expression). MSK1 was also detected in nonneuronal cells, as noted in the CA3 panel of Figure 1(b) (arrowheads denote MSK1-positive, NeuN-negative, cells within the stratum radiatum). Finally, a MSK1 null mouse line (Figure 1(c)) was used to test the specificity of the MSK1 immunolabeling; importantly, MSK1-like immunoreactivity (using both immunohistochemistry and Western blotting) was not detected in tissue from the MSK1 null mouse line (Figure 1(d)).
Next, we examined MSK1 activation resulting from pilocarpine-evoked (310 mg/kg: IP injection) SE. Of note, the SE model system has been widely used to examine mechanisms of excitotoxic and neuroprotective response processes and mechanisms underlying epileptogenesis (White, 2002; Curia et al., 2008; Curia et al., 2014). Initially, mice were sacrificed 15 to 30 min after the induction of Stage 5 seizure activity, and hippocampal tissue was probed with an antibody against the Ser-360 phosphorylated form of MSK (pMSK), a marker of MSK activation (McCoy et al., 2005). Of note, this antibody does not distinguish between MSK1 and MSK2. In control, vehicle-injected mice, very limited pMSK was detected within the principal cell layers of the hippocampus, although high background staining was observed in the hippocampal subfields and fiber tracks (Figure 2(a)). In contrast, SE evoked marked MSK phosphorylation in the major hippocampal cell layers (CA1, GCL: Figure 2(b)) and in the CA3 (data not shown); this expression pattern is consistent with the nuclear expression pattern that was observed for total MSK1 expression (see Figure 1(b)). Immunohistochemistry was complemented with pMSK Western analysis of hippocampal lysates (probed with the same pMSK antibody used for

![Figure 2. Seizure activity stimulates MSK activation. WT mice were injected with vehicle (control) or with pilocarpine and sacrificed 15 to 30 min after the induction of Stage 5 seizure activity. (a) Immunohistochemical labeling revealed limited MSK phosphorylation in the CA1 and GCL of control mice. (b) Marked phosphorylation in the CA1 and GCL was detected following seizure activity. Boxed regions in the left panels in (a) and (b) are magnified and presented to the right. Bar: 50 μm. (c) Western analysis of hippocampal lysates (from WT mice) were also used to profile MSK phosphorylation (pMSK) following seizure activity: Note that the increased band intensity in lysates isolated from pilocarpine (seizure)-treated animals. As a loading control, the blot was also probed for β-actin expression. Each lane represents lysate from an individual animal. Data are representative of three separate trials. (d) EEG analysis of pilocarpine-evoked SE. Top: representative traces from a WT and MSK1 null mouse. Recordings are from the start of motor seizure activity and continue to SE. Arrows denote the approximate onset of SE. Bottom: Mean SE-evoked EEG activity amplitude (peak-to-peak: P–P) for WT and MSK1 null mice. Significant P–P differences were not detected between the genotypes at any of the time points. Data were averaged from four animals from each genotype. Immunohistochemical labeling for ERK1/2 activation in WT (e) and MSK1 null mice (f). Animals were sacrificed 30 min after vehicle injection (top panels) or ~15 min after pilocarpine-evoked Stage 5 seizure activity (bottom panels). Note the marked increase in seizure-evoked hippocampal ERK1/2 activation in both WT and MSK1 null mice. Data are representative of triplicate determinations.](image-url)
immunolabeling). Relative to control tissue, SE trigged an increase in the expression of an ~90 kDa band, consistent with the molecular weight of MSK1 (and MSK2). As a control, the blot was also probed for total β-actin expression. Together, these data reveal that MSK1 is expressed in hippocampal neurons, and that its activation is coupled to seizure activity.

**MSK1 Confers Neuroprotection Against Excitotoxic Cell Death**

Next, we examined the potential role of MSK1 signaling in the excitotoxic response induced by SE. This line of inquiry was predicated on a large body of work showing that the MSK1 effector pathways (ERK/MAPK and P38/MAPK) affect cell viability. To address this question, we used a MSK1 null mouse line (MSK1−/−; Figure 1(c) and (d)), in which the MSK1 allele was selectively deleted using homologous recombination (Arthur and Cohen, 2000). In our two prior studies (Choi et al., 2012; Karelina et al., 2012), we provided a detailed description of the line, noting that MSK1 null mice are fertile, and that no health issues were detected. Further, compared with the WT mice, gross morphological differences in the hippocampus were not detected in MSK1 null mice. Of note, degeneration has been described within the striatum of aged (9 months) MSK1 null mice (Martin et al., 2011). However, within the 6- to 14-week age range used in our study, hippocampal neurodegeneration was not detected (described later). Further, with respect to the SE paradigm, WT mice and MSK1 null mice showed similar seizure onset times following pilocarpine injection, and there were no marked differences in the motor manifestations, and the progression of seizure severity. Using the Racine scale (Racine, 1972) both lines exhibited the stepwise progression from Stage 1 to Stage 5 seizure activity. A subset of MSK1 null (35%) and WT (40%) mice transitioned to SE; SE-evoked mortality rates between the two lines were similar, with MSK1 nulls exhibiting a slightly higher rate than WT mice (45% vs. 40%, respectively, N = 20 per genotype). EEG recording revealed high-amplitude electrical discharges, and peak-to-peak analysis detected a similar level of SE-evoked electrical activity in WT and MSK1 null mice (Figure 2(d)). Finally, immunohistochemical labeling for the activated, dual phosphorylated, form of ERK1/2 was used to test whether seizure activity drives an expected increase in ERK/MAPK pathway activation. In both WT (Figure 2(e)) and MSK1 null (Figure 2(f)) lines, 15 min of Stage 5 seizure activity led to a robust, hippocampal wide, increase in ERK phosphorylation. Together, these data indicate that MSK1 null and WT mice exhibit similar sensitivities and response properties to pilocarpine. Further, when combined with the data described later, these results indicate that the MSK1 null cell death phenotype is likely not the result of an enhanced sensitivity to pilocarpine, but rather can be ascribed to an elevated cellular-level vulnerability to the excitatory insult.

To analyze the potential role of MSK1 in SE-evoked excitotoxic cell death, WT (referred to as MSK1+/+ mice in the figure) and MSK1 null mice were sacrificed 3 days after pilocarpine-evoked SE, and hippocampal tissue was examined for cell death via FJB labeling. Initially, under control conditions (no pilocarpine injection), FJB-positive cells were not detected in the WT or MSK1 null mice (Figure 3(a)–(d)). In WT mice, SE led to cell death within the CA1, CA3, and hilar region, whereas limited cell death was detected in the GCL (Figure 3(a)). Interestingly, compared to WT mice, MSK1 null mice exhibited a significant increase in SE-evoked cell death within the CA1 and CA3 cell layers (Figure 3(a)–(c)). However, within the hilus, similar high levels of cell death were detected in WT and MSK1 null mice (Figure 3(a) and (d)).

Nissl staining was used to complement the 3-day post-SE FJB labeling and extend the analysis of cell death out to 6-week post-SE (Figure 4)—a time point when animals exhibit spontaneous seizure activity. Nissl staining of tissue at the 3-day post-SE time point confirmed the findings using FJB: A significant increase in CA1 and CA3 cell death in MSK1 null mice relative to WT mice (Figure 4(a) and (c)). Interestingly, marked degeneration of the GCL was observed in 1 MSK1 null mice (Figure 4(a), bottom panel), which represents ~6% of the MSK1 null mice profiled (n = 18 in total); GCL degeneration was not detected in WT mice (n = 20 in total). Representative data and quantitative analysis for the 6-week time point revealed a significantly higher level of cell death in the MSK1 null line (Figure 4(b)–(d)). Together, these data indicate that MSK1 confers potent neuroprotection against SE-evoked excitotoxicity. Further, these data indicate that the abrogation of MSK1 signaling does not affect cell viability under normal, nonpathophysiological conditions. Here, it is worth noting that a prior study reported that MSK1 enhances neuronal cell death (Hughes et al., 2003). Clearly, this result is inconsistent with our work reported here. Possible explanations for these divergent results could be related to either the experimental methods used to stimulate an excitotoxic challenge or the different experimental methods used to disrupt MSK1 signaling (the work of Hughes et al. largely utilized small molecular inhibitor-based approaches). As noted in the Introduction section, signaling via the ERK/MAPK pathway can confer neuroprotection or facilitate neuronal cell death, depending on the stimulus conditions: Given that MSK1 is downstream of ERK/MAPK, it may also play a similar, context-specific, role.

The increase in evoked cell death observed in MSK1 null mice could be due to a number of factors, including
an increase in SE-evoked excitatory drive and a decrease in cellular neuroprotection. To address these two possibilities, we prepared primary hippocampal neuronal cultures from postnatal day 1 MSK1 null and WT mice and tested their response profiles to NMDA stimulation. We initially tested NMDA-induced cell death in neurons cultured for 10 days using the LDH assay. For these studies, neurons were stimulated (20 min) with 50 μM NMDA (supplemented with 2 mM glycine), and LDH release was examined 4 h and 8 h later. Relative to WT neurons, NMDA-evoked cell death was markedly increased in MSK1 null cultures at both time points (Figure 5(a)). Photomicrographs of MSK1 null cultures at 8 h post-NMDA stimulation revealed a large number of shrunken cells with fragmented processes; in contrast, the cellular morphology of WT neurons was largely intact, with only a relatively small number of cells exhibiting signs of necrosis (Figure 5(b)). To confirm that cell death occurred in neurons, cultures were also labeled for the neuronal-specific cytoskeletal protein MAP2, which has been used to profile excitotoxic cell death in culture (Carrier et al., 2006). Consistent with the LDH data set, MSK1 null cultures treated with NMDA showed a reduction in MAP2 labeling relative to the control MSK1 null

Figure 3. SE-evoked cell death phenotype in MSK1 null mice. (a) MSK1 null mice (MSK1−/−) and WT (MSK1+/+) mice were challenged with pilocarpine-evoked SE (or saline vehicle), sacrificed 3 days later, and coronal sections through the hippocampus were labeled with FJB. In WT mice, SE evoked a stereotypical pattern of cell death in the hilus, CA3, and CA1 cell layers. Interestingly, in MSK1 null animals, there was a marked, relative, increase in cell death within the CA3 and CA1 cell layers. ((b)–(d)) Quantitative analysis of FJB-positive cells in the CA1 (b), CA3 (c), and hilus (d). *p < .01. Of note, in control mice (saline injection), cell death was not detected in either MSK1 null or WT mice.
cultures (mock stimulation) and compared to WT cultures treated with NMDA (Figure 5(c)). Together, these data indicate that MSK1 contributes to cell-autonomous neuroprotective response mechanisms.

To extend this line of work, we also examined NMDA-evoked calcium responses of MSK1 null neurons. For these studies, neurons were cultured for 10 days, loaded with the calcium-sensitive fluorophore Fura-2, and the response profiles of individual neurons were monitored following brief (∼30 s) treatments with NMDA (10–100 μM). Surprisingly, the peak-evoked responses to NMDA were significantly lower in the MSK1 null neurons than in WT neurons (Figure 6(a) and (b)). Near the end of the experiment (Figure 6(a)), neurons were exposed to 100 μM NMDA for 5 min; this long stimulus paradigm was used to assess whether the response profiles to chronically elevated Ca2+ levels were affected by MSK1 deletion. Compared to the WT cells, MSK1 null neurons exhibited a significantly reduced average response profile to the chronic Ca2+ load (Figure 6(c)). Of note, basal calcium levels were significantly higher in MSK1 null neurons compared to WT neurons (Figure 6(d)). Collectively, the cellular level analysis presented here indicates that the disruption of MSK1 signaling reduces excitatory drive, while increasing vulnerability to potentially excitotoxic stimuli.

**MSK1 Deletion Alters the Hippocampal Transcriptome**

Finally, the complex nature of the MSK1 cell death phenotype (reduced excitatory drive, elevated excitotoxic response to NMDA, and elevated SE-evoked cell death) led us to explore the contribution of MSK1 to the hippocampal transcriptional profile. To this end, hippocampal RNA was isolated from WT and MSK1 null mice and profiled via Affymetrix array (all array data are presented in a Supplemental Excel Spreadsheet). Using a 1.25-fold cutoff, and a p value of < .05, our data set revealed that the disruption of MSK1 reduced the expression of 130 genes and increased the expression of 145 genes (Figure 7(a) and Table 1). Gene ontology (GO) functional clustering analysis via the Database for Annotation, Visualization and Integrated Discovery (DAVID) revealed that
MSK1 deletion had significant effects on the expression of several classes of genes associated with membrane receptor signaling, cytoskeletal organization, and redox chemistry (Figure 7(b)). The GO term Neuronal Apoptosis exhibited clustering, although significance was just below the \( p < .05 \) cutoff (Figure 7(b)). Together, these data indicate that MSK1 regulates the expression of a large number of genes that underlie basic cellular biochemistry and neuronal-specific cellular signaling.

**Discussion**

Here, we provide evidence supporting a role for MSK1 as a critical component of a neuroprotective response pathway that limits cell death resulting from SE. Using a 3-day post-SE time point, we observed extensive cell death in the CA1, CA3, and hilar regions of the hippocampus and relatively modest cell death in the GCL. This cell death pattern is consistent with an extensive literature on pilocarpine-evoked cell death (Olney et al., 1983; Freund et al., 1992; Borges et al., 2003; Zhang et al., 2009; Tang and Loke, 2010). Further, this pattern of cell death was largely intact in MSK1 null mice; hence, MSK1 did not consistently confer vulnerability to any additional cell types; rather, the loss of MSK1 exacerbated cell death in inherently vulnerable cell populations (i.e., pyramidal neurons of CA1 and CA3 cell layers). Interestingly, cell death in the hilus was not affected in MSK1 null mice. One possible explanation for this is that SE has been shown to trigger very high levels of hilar interneuron cell death (Buckmaster and Dudek, 1997; Choi et al., 2007; Sun et al., 2007), and thus, this high degree of cell death could preclude any effects of MSK1 deletion. However, it is also worth noting that our immunofluorescent labeling revealed limited MSK1 expression in hilar neurons. Could this limited expression of MSK1 in hilar neurons contribute to their inherently high level of sensitivity to SE? Clearly, further studies that focus on hilar interneurons and MSK1 signaling will be needed to address this idea. As noted above, the GCL is relatively resistant to the excitotoxic effects of pilocarpine-evoked SE (Olney et al., 1983; Freund et al., 1992; Cavazos et al., 1994; Mori et al., 2004). Given the
high level of MSK1 expressed in the GCL, we speculated that MSK1 null mice could exhibit GCL vulnerability to SE. However, the data presented here showed that MSK1 deletion did not consistently enhance GCL neuronal sensitivity to SE (of note, we did observe that one out of 18 MSK1 null animals showed marked SE-evoked GCL degeneration, see Figure 4(a)). These data coupled with the data from the CA1 and CA3 cell layers indicate that factors working independently of the MSK1 signaling network regulate SE-evoked cell death in the GCL layer of the hippocampus.

Here, we detected robust inducible MSK1 phosphoactivation in response to seizure activity, and that under control conditions, MSK1 activation was relatively low throughout the hippocampus. This pattern of robust SE-evoked MSK1 activity is consistent with work showing that the ERK/MAPK and P38 pathways (the two upstream effectors of MSK1) are activated following multiple seizure induction paradigms in the hippocampus (Baraban et al., 1993; Gass et al., 1993; Kim et al., 1994; Garrido et al., 1998; Jiang et al., 2005; Choi et al., 2007; Lopes et al., 2012). This dynamic, inducible, activation of MSK1 raises a question: Is SE-evoked MSK1 activity required to confer neuroprotection or is the tonic, basal level of MSK1 activity sufficient to drive neuroprotection. As noted earlier, Martin et al. (2011) reported striatal deterioration in aged MSK1 null mice. This finding could be used to support the idea that the disruption of basal MSK1 activity is sufficient to drive vulnerability to stressful stimuli. However, it is also worth noting that a number of studies have shown that the disruption of basal ERK/MAPK activity does not affect cell health, but rather leads to the abrogation of an evoked neuroprotective response (Han and Holtzman, 2000; Kuroki et al., 2001; Pedersen et al., 2002; Park et al., 2004; Nguyen et al., 2005). Hence, it is likely that both basal and stress-evoked MSK1 signaling contribute to the neuroprotective response. Here, it is also worth noting that MSK1 deletion did not affect hippocampal neuronal cell viability under normal physiological conditions. Rather, the MSK1 null cell death phenotype was only revealed under stress conditions. In some respects, this is consistent with studies showing that the disruption of CREB (a downstream MSK1 target) does not, by itself, trigger cell death, but does increase neuronal vulnerability to excitatory insults (Lee et al., 2005; Lee et al., 2009). Notably, as with CREB, MSK1 has been implicated in a range of plasticity-dependent processes, including learning and memory, and activity dependent synapse formation (Chwang et al., 2007; Corrêa SA et al., 2012; Kareлина et al., 2012). Together, these data indicate that MSK1 plays at least two distinct roles in the central nervous system.
system: one that couples synaptic activity to changes in functional plasticity and a second role as an effector of neuroprotective signaling. Further work will be required to determine the relative contribution of CREB to the neuroprotective effects elicited by MSK1 signaling.

Given the enhanced cell death phenotype, it was surprising to find that MSK1 null neurons exhibited weaker NMDA-evoked excitatory drive compared to WT neurons, as assessed using Ca^{2+} imaging. Interestingly, reduced excitability may be consistent with studies showing that MSK1 null mice exhibit reduced functional plasticity, including activity-dependent spine formation, synaptic scaling, and cognition (Chwang et al., 2007; Corrêa SA et al., 2012; Karelina et al., 2012). Further, the weak-evoked Ca^{2+} response in MSK1 null neurons indicates that the enhanced cell death phenotype likely cannot be ascribed to aberrant excitatory drive. Rather, these data point to the compromised expression of neuroprotective genes and gene networks in MSK1 null neurons.

Could the enhanced SE-evoked cell death in MSK1 null mice result from dysregulated apoptotic and necrotic cell death mechanisms? With respect to apoptosis, extensive work in nonneuronal cells has shown that MSK regulates cell survival via the regulation of antiapoptotic cell death mechanisms (Mu et al., 2005; Kannan-Thulasiraman et al., 2006; Dumka et al., 2009; Joo and Jetten, 2010; Odgerel et al., 2010; Healy et al., 2012; Moens and Kostenko, 2013), including the regulation of NF-κB, BAD, and caspase activation (She et al., 2002; El Mchichi et al., 2007). Further, the CREB/CRE transcriptional pathway, a principal target of MSK1, has also been shown to regulate apoptotic cell death (reviewed in Sakamoto et al., 2011).

In contrast to the extensive work on MSK and apoptotic cell death, to our knowledge, limited work has
explored the potential contribution of MSK signaling to necrotic cell death. Necrotic cell death is typically associated with elevated intracellular Ca\textsuperscript{2+} levels, rapid ATP depletion, and mitochondrial swelling; these and other events lead to the collapse of the membrane potential and the rupturing of the plasma membrane. Although our data did not identify an effect of MSK1 deletion on Ca\textsuperscript{2+} homeostatic, or evoked responses, our array data indicate that MSK1 regulates the expression of several genes that could affect neuronal vulnerability. Many of these genes are associated with oxidation/reduction chemistry (alcohol dehydrogenase, phenylalanine hydroxylase, NOS2, sulfide quinone reductase) and membrane receptor signaling (epidermal growth factor receptor, GABA-A receptor subunit alpha 2) and cellular transport (e.g., alpha-synuclein, EHD2, coronin).

Interestingly, one of the strongest effects of MSK1 deletion was on the expression of galactosylceramidase (Galc): ~14-fold decrease in expression. Galc is highly expressed in both neurons and oligodendrocytes and serves as a key enzyme in the metabolism of galactolipids. Loss-of-function mutations in Galc underlie the development of Krabbe disease in humans (Wenger et al., 2000). Interestingly, the Twitcher mouse line (a model of Krabbe disease) bred onto a C57BL/6J and 129SvEv mixed background shows spontaneous neuronal cell death within the hippocampus (Tominaga et al., 2004). These observations raise the prospect that reduced Galc expression in MSK1 null mice may also contribute to the cell death phenotype reported here. However, it is worth noting that the developmental and motor phenotypes associated with the Twitcher line (i.e., stunted growth, twitching and limb weakness reported by Duchen et al. (1980)) were not observed in the MSK1 null line. Clearly, the list of genes that are regulated by MSK1 is extensive, and as such, the cell death phenotype observed here could have resulted from a complex interplay of affected genes and gene networks. It is also worth noting that the effects of MSK1 deletion on cell type-specific neuroprotective genes may have evaded detection, given that the whole hippocampus was used for our array profiling.

In conclusion, the data reported here reveal that MSK1 regulates neuroprotective signaling in the CA1 and CA3 sublayers of the hippocampus. This effect occurs on a cellular level and is not associated with increased cellular excitability. These findings justify further work examining the potential role of MSK1 in other mechanisms of cell stress and neuroprotection, including ischemia and preconditioning. Finally, the elevated levels of cell death observed in MSK1 null mice raise the prospect that approaches designed to enhance MSK1 activity could abrogate some of the pathophysiological effects associated with, and potentially underlying, the development of epilepsy.

| Probe set    | Baseline mean's SE | Experiment mean's SE | Fold change | t statistic | p value |
|--------------|--------------------|----------------------|-------------|-------------|---------|
| 1431050_at   | 247.85             | 9.86                 | 2.76        | -3.185      | .000899 |
| 1440343_at   | 433.39             | 23.65                | 18.44       | 23.074      | .000006 |
| 1452907_at   | 438.54             | 9.59                 | 31.024      | 31.09       | .000006 |
| 1422360_at   | 6.76               | 1.12                 | 5.51        | 3.852       | .018297 |
| 1446525_at   | 10.59              | 2.05                 | 4.51        | 4.23        | .036928 |
| 1429511_at   | 13.49              | 1.83                 | 4.19        | 4.285       | .01315    |
| Probe set | Gene Name | Accession | Entrez Gene | Description | WT-1 Mean | WT-2 Mean | WT-7 Mean | Baseline Mean's SE | MSK-14 Mean | MSK-15 Mean | MSK-9 Mean | Experiment Mean | Experiment Mean's SE | Fold Change | t statistic | p value  |
|-----------|-----------|-----------|-------------|-------------|------------|------------|------------|-------------------|-------------|-------------|-------------|----------------|---------------------|--------------|------------|---------|
| 1430998_at | Sqrdl: sulfide quinone reductase-like (yeast) | BE626283 | 59010 | | 13.44 | 16.58 | 9.47 | 13.42 | 2.4 | 2.01 | 6.52 | 1.38 | 3.23 | 2.29 | -4.15 | -3.072 | 0.037307 |
| 1460269_at | Pnmt: Phenylethanolamine-N-methyltransferase | AV380429 | 18948 | | 4.34 | 5.7 | 6.69 | 5.47 | 1.01 | 1 | 1.79 | 1.84 | 1.38 | 1.04 | -3.97 | -2.828 | 0.047481 |
| 1432739_at | 2900060K15Rik: RIKEN cDNA 2900060K15 gene | AV154271 | 73041 | | 3.14 | 4.73 | 2.84 | 3.57 | 0.61 | 1 | 1.02 | 1.06 | 1.02 | 0.65 | -3.51 | -2.852 | 0.046521 |
| 1428038_at | Gm568: predicted gene 568 | BC028561 | 230143 | | 10.89 | 15.26 | 9.71 | 12.09 | 2.04 | 6.25 | 4.33 | 1 | 3.61 | 1.84 | -3.35 | -3.087 | 0.037174 |
| 1457878_at | C430042M11Rik: RIKEN cDNA C430042M11 gene | BB415623 | 320021 | | 28.03 | 22.19 | 16.26 | 22.22 | 3.46 | 14.06 | 5.8 | 1 | 6.91 | 3.83 | -3.22 | -2.969 | 0.041706 |
| 1420393_at | Nos2: nitric oxide synthase 2, inducible | AR065921 | 18126 | | 50.29 | 67.54 | 79.11 | 65.85 | 8.95 | 21.82 | 24.58 | 18.55 | 21.83 | 3.22 | -3.02 | -4.626 | 0.027847 |
| 1443153_at | Trip11: Thyroid hormone receptor interactor 11 | BB306866 | 109181 | | 7.62 | 8.41 | 7.68 | 8.01 | 1.28 | 3.1 | 3.78 | 1.01 | 2.65 | 1.32 | -3.02 | -2.917 | 0.043423 |
| 1444388_at | Mm.183515.1 | BB020727 | 13649 | | 16.68 | 14.27 | 11.08 | 14.12 | 1.9 | 4.3 | 4.47 | 8.39 | 5.75 | 1.78 | -2.45 | -3.217 | 0.032562 |
| 14515205_x_at | Gm6273 /// LOC381765 /// LOC665506 /// Tcrb-j: predicted gene 6273 /// similar to T cell antigen receptor C region /// T-cell receptor beta-2 chain region /// T-cell receptor beta, joining region | X67128 | 21580 /// 381765 /// 665506 | | 20.88 | 22.01 | 18.16 | 20.54 | 1.95 | 8.59 | 10.66 | 7.29 | 9.12 | 1.94 | -2.44 | -2.844 | 0.048518 |
| 1427717_at | Cd80: CD80 antigen | X60958 | 12519 | | 8.94747 | 13.12 | 12.49 | 10.69 | 12.18 | 1.37 | 5.72 | 4.5 | 5.23 | 5.01 | 1.44 | -2.43 | -3.606 | 0.022721 |
| 1447355_at | Acsl1: acyl-CoA synthetase long-chain family member 1 | BQ128552 | 14081 | | 50.29 | 67.54 | 79.11 | 65.85 | 8.95 | 21.82 | 24.58 | 18.55 | 21.83 | 3.22 | -3.02 | -4.626 | 0.027847 |
| 1432542_at | Gm10474C1Rik: RIKEN cDNA 2810474C18 gene | AK013405 | 72785 | | 15.84 | 14.47 | 14.31 | 14.89 | 1.45 | 4.75 | 5.65 | 8.84 | 6.43 | 1.67 | -2.32 | -3.829 | 0.01929 |
| 1418918_at | Igfbp1: insulin-like growth factor binding protein 1 | NM_008341 | 16006 | | 20.88 | 22.01 | 18.16 | 20.54 | 1.95 | 8.59 | 10.66 | 7.29 | 9.12 | 1.94 | -2.25 | -4.152 | 0.014233 |
| 1446391_at | Mm.209224.1 | BB450769 | 209224.1 | | 40.36 | 30.7 | 6.88 | 23.35 | 23.45 | 17.98 | 6.06 | -2.25 | -3.297 | 0.046554 |

(continued)
| Probe set | Gene Accession | Gene Description | WT -1 | WT -2 | WT -7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|----------------|------------------|-------|-------|-------|---------------|-----------------|--------|--------|--------|---------------|-----------------|-------------|------------|--------|
| 1453330_at | Ccdc88c: coiled-coil domain containing 88C | AK002458 | 68339 | Mm.45291.1 | 73.53 | 68 | 94.64 | 78.69 | 9.05 | 25.48 | 43.19 | 43.47 | 37.06 | 8.27 | -2.12 | -3.394 | 0.27765 |
| 1425447_at | Dkk4: dickkopf homolog 4 (Xenopus laevis) | BC018400 | 234130 | Mm.157322.1 | 11.78 | 13.87 | 14.1 | 13.31 | 1.32 | 5.66 | 9.64 | 3.76 | 6.33 | 1.91 | -2.1 | -3.003 | 0.46368 |
| 1427395_at | Aldh1a3: aldehyde dehydrogenase family 1, subfamily A3 | BC026667 | 56847 | Mm.140988.2 | 11.52 | 10.4 | 9.43 | 10.6 | 1.01 | 5.46 | 2.55 | 6.98 | 5.19 | 1.49 | -2.04 | -2.996 | 0.4721 |
| 1443483_at | Xlr5a /// Xlr5b /// Xlr5c: X-linked lymphocyte-regulated 5A /// X-linked lymphocyte-regulated 5B /// X-linked lymphocyte-regulated 5C | BM207672 | 27084 /// 574438 /// 627081 | Mm.139096.1 | 21.31 | 20.23 | 16.11 | 19.43 | 2.21 | 6.87 | 8.68 | 13.61 | 9.85 | 2.41 | -1.97 | -2.93 | 0.43207 |
| 1454248_at | Cib4: calcium and integrin binding family member 4 | AK006670 | 73259 | Mm.158977.1 | 16.11 | 19.23 | 21.9 | 19.13 | 2.24 | 7.79 | 9.06 | 12.46 | 9.9 | 1.81 | -1.93 | -3.199 | 0.34969 |
| 1457121_at | Obsl1: obscurin-like 1 | AV271877 | 98733 | Mm.213076.1 | 21.54 | 20.84 | 20.23 | 20.81 | 2.1 | 10.39 | 9.88 | 11.95 | 10.76 | 2.16 | -1.93 | -3.342 | 0.28807 |
| 1438877_at | Rbm31p: RNA binding motif 31, Y-linked | AK017055 | 74484 | Mm.159220.1 | 34.62 | 33.63 | 35.83 | 34.56 | 1.39 | 13.36 | 22.36 | 18.07 | 17.98 | 2.84 | -1.92 | -5.237 | 0.1467 |
| 1440766_at | Limch1: LIM and calponin homology domains 1 | BB709234 | 77569 | Mm.208624.1 | 11.7 | 10.5 | 7.94 | 10.21 | 1.34 | 5.93 | 5.8 | 4.01 | 5.3 | 0.97 | -1.92 | -2.964 | 0.46541 |
| 1439004_at | Rps6ka5: ribosomal protein S6 kinase, poly-peptide 5 | BE946999 | 73086 | Mm.101475.1 | 127.44 | 116.07 | 122.49 | 121.67 | 5.29 | 64.56 | 67.62 | 62.11 | 64.78 | 7.26 | -1.88 | -6.333 | 0.04286 |
| 1432163_at | 4930567K12Rik: RIKEN cDNA 4930567K12 gene | AK016242 | 75845 | Mm.159601.1 | 23.4 | 23.64 | 32.68 | 26.5 | 3.31 | 18.48 | 11.82 | 11.18 | 14.17 | 2.61 | -1.87 | -2.925 | 0.45943 |
| 1422343_at | Olfr155: olfactory receptor 155 | NM_019473 | 29845 | Mm.131262.1 | 21.86 | 18.92 | 23.07 | 21.12 | 1.41 | 13.65 | 11.72 | 10.32 | 11.58 | 1.33 | -1.82 | -4.91 | 0.00803 |
| 1439589_at | Cry1l: crystallin, lambda 1 | CB5932 | 68631 | Mm.200251.1 | 14.31 | 11.87 | 11.47 | 12.55 | 0.89 | 8.62 | 5.37 | 6.99 | 7 | 0.93 | -1.79 | -4.314 | 0.12568 |
| 1437721_at | Bbs43398 | Mm.200372.4 | 23790 | Mm.200025.1 | 14.31 | 11.87 | 11.47 | 12.55 | 0.89 | 8.62 | 5.37 | 6.99 | 7 | 0.93 | -1.79 | -2.927 | 0.43103 |

(continued)
| Probe set | Gene                                      | Accession | Entrez Gene | Description                                      | WT-1 mean | WT-2 mean | WT-7 mean | Baseline mean's SE | MSK-14 mean | MSK-15 mean | MSK-9 mean | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|-------------------------------------------|-----------|-------------|--------------------------------------------------|-----------|-----------|-----------|-------------------|-------------|-------------|-------------|-----------------|----------------------|-------------|------------|---------|
| 1430693_at | Pnpla5: patatin-like phospholipase domain containing 5 | AV250770  | 75772       |                                                   | 52.38     | 49.76     | 48.13     | 3.46              | 20          | 37.16       | 24.42       | 27.24          | 5.77                | -1.77       | -3.104     | .04719  |
| 1431193_at | Ta4b: TAF4B RNA polymerase II, TATA box binding protein (TBP)-associated factor | AK012135  | 72504       |                                                   | 29.82     | 37.11     | 29.6      | 32.26             | 2.73        | 19.26       | 18.07       | 16.99          | 1.84                | -1.77       | -4.278     | .017046 |
| 1449190_a_at | Entpd4 /// LOC100048085: ecto-nucleoside triphosphate diphosphohydrolase 4 /// similar to ectonucleoside triphosphate diphosphohydrolase 4 | NM_026174  | 100048085 /// |                                                   | 1825.03   | 1840.45   | 2179.94   | 118.91            | 1355.9     | 1020.69     | 982.16      | 1119.68        | 118.27              | -1.74       | -4.938     | .007827 |
| 1438553_x_at | Entpd4 /// LOC100048085: ecto-nucleoside triphosphate diphosphohydrolase 4 /// similar to ectonucleoside triphosphate diphosphohydrolase 4 | BB817087  | 67609       |                                                   | 175.4     | 183.43    | 195.83    | 6.98              | 105.24      | 108.98      | 106.53      | 106.84         | 4.37                | -1.73       | -9.51      | .001529 |
| 1438177_x_at | Entpd4 /// LOC100048085: ecto-nucleoside triphosphate diphosphohydrolase 4 /// similar to ectonucleoside triphosphate diphosphohydrolase 4 | AV255351  | 100048085 /// |                                                   | 1188.25   | 1260.97   | 1525.79   | 103.56            | 949.11      | 619.41      | 740.27      | 769.29         | 96.15               | -1.72       | -3.936     | .017192 |
| 1457944_at | Mm.215864.1 | BM218086  | 76992       |                                                   | 111.91    | 110.14    | 112.66    | 124.95            | 13.14       | 79.86       | 76.75       | 52.04          | 72.57               | -1.72       | -3.007     | .040656 |
| 1432514_at | Mm.170066624.1 | AKG06904  | 76992       |                                                   | 36.72     | 38.09     | 29.26     | 34.68             | 3.08        | 13.82       | 26.24       | 20.59          | 20.3               | 3.92        | -1.71      | -2.882   |
| 1457653_at | Mm.133457.1 | BB292252  | 76992       |                                                   | 111.91    | 110.14    | 112.66    | 124.95            | 13.14       | 79.86       | 76.75       | 52.04          | 72.57               | -1.72       | -3.007     | .040656 |
| 1424978_at | Mm.170066624.1 | AKG06904  | 76992       |                                                   | 36.72     | 38.09     | 29.26     | 34.68             | 3.08        | 13.82       | 26.24       | 20.59          | 20.3               | 3.92        | -1.71      | -2.882   |
| 1458228_at | Mm.208324.1 | BB244358  | 252868      |                                                   | 25.63     | 27.97     | 25.47     | 26.66             | 2.03        | 19.28       | 17.05       | 12.3           | 16.04               | 2.45        | -1.66      | -3.338   |
| 1435999_at | Mm.159647.1 | BB244358  | 207932      |                                                   | 111.91    | 110.14    | 112.66    | 124.95            | 13.14       | 79.86       | 76.75       | 52.04          | 72.57               | -1.72       | -3.007     | .040656 |
| 1456750_at | Mm.230303O12.1 | BB308463  | 319739      |                                                   | 35.44     | 35.94     | 37.61     | 3.27              | 18.67       | 26.66       | 24.81       | 23.21          | 2.78               | -1.62       | -3.938     | .017838 |

(continued)
| Probe set | Gene | Accession | Entrez Gene | Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|------|-----------|-------------|-------------|-------|-------|-------|---------------|-----------------|--------|--------|--------|---------------|-------------------|-------------|------------|--------|
| 1456166_at | Ehd2: EH-domain containing 2 | BB358215 | 259300 | Mm.138215.1 | 36.02 | 33.97 | 38.04 | 36.04 | 3.09 | 25.05 | 20.96 | 21.41 | 22.45 | 1.79 | -1.61 | -3.808 | 0.28374 |
| 1418552_at | Opn1sw: opsin 1 (cone pigments), short-wave sensitive (color blindness, tritan) | AF190670 | 12057 | Mm.56987.1 | 26.24 | 25.45 | 22.73 | 24.79 | 1.75 | 12.16 | 19.88 | 15.16 | 15.7 | 2.56 | -1.58 | -2.933 | 0.049563 |
| 1459451_at | Mm.207852.1 | BB201499 | 12057 | Mm.56987.1 | 26.24 | 25.45 | 22.73 | 24.79 | 1.75 | 12.16 | 19.88 | 15.16 | 15.7 | 2.56 | -1.58 | -2.933 | 0.049563 |
| 1454218_at | 4930405D01Rik: RIKEN cDNA 4930405D01 gene | AK015093 | 73795 | Mm.159062.1 | 25.19 | 23.46 | 27.67 | 25.45 | 2.10 | 15.4 | 18.17 | 15.79 | 16.41 | 2.03 | -1.55 | -3.175 | 0.033712 |
| 1460064_at | BC028789: cDNA sequence BC028789 | BM237812 | 407802 | Mm.103545.1 | 178.74 | 142.83 | 147.97 | 156 | 11.67 | 93.72 | 107.19 | 100.39 | 100.78 | 5.59 | -1.55 | -4.266 | 0.025765 |
| 1453940_at | 2810404M03Rik: RIKEN cDNA 2810404M03 gene | AK012985 | 69966 | Mm.58693.1 | 25.18 | 25.89 | 24.93 | 25.36 | 1.09 | 17.83 | 14.46 | 17.64 | 16.43 | 1.75 | -1.54 | -4.339 | 0.018034 |
| 1457877_at | Mm.102971.1 | AW557111 | 43.05 | 35.17 | 43.94 | 40.46 | 3.38 | 31.21 | 23.01 | 23.17 | 26.28 | 3.05 | -1.54 | -3.117 | 0.036131 |
| 1440064_at | Etl4: enhancer trap locus 4 | BB502547 | 208618 | Mm.169632.1 | 28.41 | 34.56 | 31.93 | 31.79 | 2.37 | 18.69 | 23.67 | 19.62 | 20.77 | 2.41 | -1.53 | -3.266 | 0.031089 |
| 1445080_at | 4921511H03Rik: RIKEN cDNA 4921511H03 gene | AK014870 | 70920 | Mm.158494.1 | 77.6 | 78.23 | 77.72 | 77.89 | 2.68 | 52.52 | 52.55 | 50.77 | 51.78 | 2.71 | -1.5 | -6.861 | 0.002364 |
| 1430467_at | 9530010C24Rik: RIKEN cDNA 9530010C24 gene | BG069453 | 109279 | Mm.11474.1 | 24.48 | 26 | 23.56 | 24.7 | 2.21 | 16.47 | 16.64 | 17.1 | 16.65 | 1.48 | -1.48 | -3.032 | 0.04618 |
| 1420687_at | 4932438H23Rik: RIKEN cDNA 4932438H23 gene | NM_028905 | 73487 | Mm.35184.1 | 68.09 | 65.49 | 61.73 | 65.01 | 2.91 | 40.66 | 46.74 | 48.74 | 45.5 | 4.04 | -1.43 | -3.918 | 0.02077 |
| 1422273_at | Mmp1b: matrix metalloproteinase 1b (interstitial collagenase) | NM_032007 | 83996 | Mm.35184.1 | 68.09 | 65.49 | 61.73 | 65.01 | 2.91 | 40.66 | 46.74 | 48.74 | 45.5 | 4.04 | -1.43 | -3.918 | 0.02077 |
| 1426054_at | Npy1r: neuropeptide Y receptor Y1 | D63819 | 18166 | Mm.156951.1 | 33.34 | 28.09 | 28.03 | 29.51 | 2.25 | 19.57 | 19.07 | 23.36 | 20.63 | 1.96 | -1.43 | -2.975 | 0.041925 |
| 1452590_a_at | Gm9780 /// Plac9: predicted gene 9780 /// placenta specific 9 | BB609699 | 100039175 /// 211623 | Mm.29491.1 | 174.21 | 154.86 | 173.92 | 167.36 | 7.32 | 138.39 | 109.58 | 102.21 | 117.54 | 11.88 | -1.42 | -3.571 | 0.031712 |
| 1446429_at | P2rx4: purinergic receptor P2X, ligand-gated ion channel 4 | BB110945 | 18438 | Mm.207333.1 | 48.22 | 47.42 | 41.89 | 45.96 | 2.16 | 36.46 | 30.2 | 30.82 | 32.42 | 2.2 | -1.42 | -4.398 | 0.011718 |

(continued)
Table 1. Continued

| Probe set  | Gene                      | Accession | Entrez Gene | Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|------------|---------------------------|-----------|-------------|-------------|------|------|------|--------------|------------------|--------|--------|--------|-----------------|--------------------|-------------|------------|--------|
| 1418943_at | B230120H23Rik: RIKEN cDNA B230120H23 gene | NM_023057 | 65964       |             | 78.26 | 87.25 | 73.14 | 79.74 | 4.8           | 55.89 | 55.17 | 57.92 | 56.44           | 2.72               | -1.41       | -4.225     | .02179 |
| 1432791_at | 9030218A15Rik: RIKEN cDNA 9030218A15 gene | AK020251 | 77662       |             | 84.87 | 86.93 | 72.67 | 81.36 | 4.88          | 60.11 | 61.32 | 50.9  | 57.74           | 4.22               | -1.41       | -3.662     | .022334 |
| 1445611_at | Trappc9: trafficking protein particle complex 9 | BB349535 | 76510       |             | 42.64 | 53.04 | 50.98 | 48.62 | 3.75          | 31.68 | 37.76 | 33.25 | 34.39           | 2.69               | -1.41       | -3.081     | .042056 |
| 1443393_at | Mm.131148.1 | BB201890 |            |             | 84.87 | 86.93 | 72.67 | 81.36 | 4.88          | 60.11 | 61.32 | 50.9  | 57.74           | 4.22               | -1.41       | -3.341     | .039369 |
| 1446254_at | Mm.149067.1 | BB116559 |            |             | 42.64 | 53.04 | 50.98 | 48.62 | 3.75          | 31.68 | 37.76 | 33.25 | 34.39           | 2.69               | -1.41       | -4.168     | .014144 |
| 1429358_at | Fam135a: family with sequence similarity 135, member A | AK019549 | 68187       |             | 26.74 | 24.67 | 28.35 | 26.83 | 1.51          | 18.14 | 20.73 | 18.85 | 19.34           | 1.81               | -1.39       | -3.181     | .03506  |
| 1457308_at | Mm.4245.1 | BG070176 |            |             | 53.25 | 45.83 | 43.52 | 47.65 | 3.04          | 32.48 | 35.7  | 34.91 | 34.41           | 1.41               | -1.38       | -3.944     | .032405 |
| 1455000_at | Gpr68: G protein-coupled receptor 68 | BB538372 | 238377      |             | 39.43 | 34.83 | 33.98 | 36.18 | 17.77         | 264.67 | 271.46 | 249.4 | 262.82 | 8.79               | -1.38       | -4.998     | .016385 |
| 1417017_at | Cyp17a1: cytochrome P450, family 17, subfamily a, polypeptide 1 | NM_007809 | 13074       |             | 40.4  | 38.41 | 42.27 | 40.95 | 2            | 30.6  | 30.94 | 28.96 | 29.99           | 1.64               | -1.37       | -4.234     | .014427 |
| 1426305_at | Upk1a: uroplakin 1A | AF262335 | 109637      |             | 47.35 | 47.65 | 43.06 | 46.34 | 2.76          | 34.58 | 34.67 | 32.89 | 33.85           | 2.84               | -1.37       | -3.15      | .034557 |
| 1429957_at | Krtap26-1: keratin associated protein 26-1 | AK009086 | 69533       |             | 55.69 | 63.18 | 58.29 | 58.89 | 3.59          | 45.55 | 45.7  | 38.18 | 42.94           | 3.91               | -1.37       | -3.003     | .040213 |
| 1439674_at | Sloc4b: solute carrier family 4 (anion exchanger), member 8 | BB436482 | 59033       |             | 169.3 | 174.08 | 152.95 | 166.06 | 7.22          | 116.68 | 139.51 | 107.92 | 121.63 | 10.17              | -1.37      | -3.562     | .027951 |
| 1440191_s_at | Leng9: leukocyte receptor cluster (LRC) member 9 | AB47494 | 243813      |             | 300.04 | 285.18 | 259.66 | 281.54 | 12.12         | 195.64 | 215.32 | 206.5 | 205.28 | 6.72               | -1.37      | -5.501     | .010637 |
| 1420720_at | LOC100044234 /// Npo2: hypothetical protein LOC100044234 /// neuronal pentraxin 2 | NM_016789 | 100044234 /// 53324 |             | 704.44 | 660.98 | 662.72 | 676.22 | 14.5          | 471.43 | 504.6 | 510.57 | 495.71 | 13.63              | -1.36      | -9.072     | .000833 |
| 1421414_a_at | Sem6a: sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A | NM_018744 | 20358       |             | 63.6  | 64.02 | 54.08 | 60.28 | 3.7           | 39.32 | 50.08 | 44.06 | 44.31           | 4.06               | -1.36       | -2.91      | .044143 |
| 1459279_at | Mm.126689.1 | BB363958 | 126689.1    |             | 55.94 | 60.46 | 51.02 | 55.53 | 3.51          | 37.3  | 39.75 | 45.98 | 40.98           | 3.28               | -1.36       | -3.031     | .038982 |

(continued)
| Probe set  | Gene Accession | Description | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|------------|----------------|-------------|---------------|--------------------|---------|---------|-------|----------------|-------------------|-------------|------------|--------|
| 1419005_at | NM_021352      | Crybb3: crystallin, beta B3 | MM.40616.1 | 66.16 | 62.97 | 63.63 | 3.01 | 49.56 | 43.73 | 48.37 | 47.51 | 2.99 | -1.34 | -3.79 | .019144 |
| 1452243_at | BB282273       | Kcnj14: potassium inwardly rectifying channel, subfamily J, member 14 | MM.68170.1 | 108.27 | 104.81 | 94.68 | 103.54 | 5.47 | 85.87 | 71.34 | 75.43 | 77.48 | 5.03 | -1.34 | -3.56 | .025051 |
| 1440757_at | BB750206       | Def6: differentially expressed in FDCP 6 | MM.102276.1 | 42.91 | 43.21 | 46.81 | 44.11 | 2.3 | 30.81 | 32.14 | 36.29 | 32.81 | 2.9 | -1.34 | -3.05 | .040319 |
| 1459968_at | AW742677       | Ugt2b1: UDP glucuronosyltransferase 2 family, polypeptide B1 | MM.170575.1 | 89.81 | 89.86 | 80.55 | 86.07 | 3.76 | 60.96 | 66.98 | 64.23 | 64.24 | 3.83 | -1.34 | -4.06 | .015267 |
| 1457860_at | MM.25024.1     | Tnc: tenasin C | MM.25024.1 | 39.65 | 34.63 | 37.11 | 37.03 | 1.7 | 27.47 | 28.52 | 27.54 | 27.89 | 1.58 | -1.33 | -3.94 | .017096 |
| 1442934_at | BC027200       | C80068: expressed sequence C80068 | MM.26741.1 | 52.91 | 53.27 | 61.41 | 56.13 | 3.52 | 44.21 | 39.7 | 49.97 | 42.51 | 2.41 | -1.32 | -3.19 | .039515 |
| 1438755_at | BB327213       | Cdk6: cyclin-dependent kinase 6 | MM.980.1 | 94.53 | 81.95 | 87.47 | 88.46 | 3.01 | 60.56 | 63.73 | 65.71 | 67.17 | 5.03 | -1.34 | -3.04 | .040732 |
| 1448383_at | AW912463       | Mmp14: matrix metalloproteinase 14 (membrane-inserted) | MM.188194.1 | 53.29 | 53.27 | 61.41 | 56.13 | 3.52 | 44.21 | 39.7 | 49.97 | 42.51 | 2.41 | -1.32 | -3.19 | .039515 |
| 1442643_at | BF018617       | 4930452G13Rik: RIKEN cDNA 4930452G13 gene | MM.107775.1 | 47.47 | 47.2 | 47.85 | 47.57 | 2.27 | 37.65 | 33.2 | 35.96 | 35.96 | 2.5 | -1.32 | -3.42 | .027121 |
| 1445746_at | AW912463       | 4930452G13Rik: RIKEN cDNA 4930452G13 gene | MM.107775.1 | 47.47 | 47.2 | 47.85 | 47.57 | 2.27 | 37.65 | 33.2 | 35.96 | 35.96 | 2.5 | -1.32 | -3.42 | .027121 |
| 1441205_at | BB118894       | Cdsd1: CUB and Sushi multiple domains 1 | MM.54865.1 | 88.18 | 86.2 | 76.36 | 83.79 | 4.7 | 63.09 | 62.65 | 66.93 | 64.12 | 2.36 | -1.31 | -3.73 | .03466 |
| 1445746_at | BB385992       | Cdsd1: CUB and Sushi multiple domains 1 | MM.54865.1 | 88.18 | 86.2 | 76.36 | 83.79 | 4.7 | 63.09 | 62.65 | 66.93 | 64.12 | 2.36 | -1.31 | -3.73 | .03466 |
| 1460291_at | MM.009873      | Eif4h: Eukaryotic translation initiation factor 4H | MM.88747.1 | 73.38 | 80.95 | 68.53 | 74.06 | 4.55 | 60.63 | 56.01 | 54.45 | 57.12 | 2.66 | -1.3 | -3.21 | .044154 |
| 1446273_at | MM.65379.1     | Mm.65379.1 | MM.65379.1 | 7.4 | 7 | 8.21 | 7.54 | 0.36 | 6.6 | 5.79 | 4.98 | 5.79 | 0.47 | -1.3 | -2.95 | .045372 |
| 1421393_at | NM_008172      | Mm.65379.1 | NM_008172 | 7.4 | 7 | 8.21 | 7.54 | 0.36 | 6.6 | 5.79 | 4.98 | 5.79 | 0.47 | -1.3 | -2.95 | .045372 |
| Probe set     | Gene Description                                                                 | Accession | Entrez Gene | Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|---------------|----------------------------------------------------------------------------------|-----------|-------------|-------------|------|------|------|--------------|-------------------|--------|--------|--------|---------------|-------------------|-------------|-------------|---------|
| 1448786_at    | Grin2d: glutamate receptor, ionotropic, NMDA2D (epsilon 4)                      | LOC100045163 /// Plb1: similar to RIKEN cDNA 1100001H23 gene /// phospholipase B domain containing 1 |          |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
|               |                                                                                   | MM_025806 /// 66857                            |           |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1429862_at    | Pia2g4: phospholipase A2, group IVE                                            | AV235932  | 329502      |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1445205_at    | Mm.218112.1                                                                      | BM122392  |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1421865_at    | Dbil5: diazepam binding inhibitor-like 5                                         | AK006528  | 13168       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1427138_at    | Cdc88Bc: coiled-coil domain containing BBC                                       | AV556861  | 68339       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 143628_x_at   | Cntn3: contactin 3                                                               | BB559510  | 18488       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1441477_at    | Calu: calumenin                                                                  | BB120190  | 12321       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1441790_at    | Mm.101345.1                                                                      | AW489900  |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1447669_s_at  | Gng4: guanine nucleotide binding protein (G protein), gamma 4                    | AV347903  | 14706       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1458793_at    |                                      Mm.182870.1                         | BG076186  |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1421109_at    | Cml2: camello-like 2                                                             | NM_053096 93673 |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1431147_at    | Rint1: RAD50 interactor 1                                                        | BG807740  | 72772       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1445835_at    |                                      Mm.76734.1                         | AW123001  |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1426492_at    | Tdp1: tyrosyl-DNA phosphodiesterase 1                                            | AK014855  | 104884      |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1449537_at    | Msh5: mutS homolog 5 (E. coli)                                                   | NM_013600 17687 |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1452035_at    | CoHa1: collagen, type IV, alpha 1                                                | BF158638  | 12826       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1438203_at    | Scarf2: scavenger receptor class F, member 2                                    | BF467245  | 224024      |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1444108_at    | Dnaq125: Dnaq (Hsp40) homolog, subfamily C, member 21                            | AD141004  | 74249       |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1444810_at    |                                      Mm.182531.1                         | BG065305  |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |
| 1446975_at    |                                      BE949995 69743                      |           |             |             |     |      |      |              |                   |        |        |        |               |                   |             |             |         |

(continued)
| Probe set | Gene Description | Gene Accession | Entrez Gene | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|------------------|----------------|-------------|------|------|------|--------------|-------------------|--------|--------|-------|---------------|---------------------|-------------|------------|--------|
| 1447433_at | Casz1: Castor homolog 1, zinc finger (Drosophila) | BB743316 | 72145 | Mm.44007.1 | 321.95 | 375.48 | 343.8 | 347.02 | 16.18 | 248.72 | 279.7 | 295.72 | 274.77 | 14.46 | -1.26 | -3.329 | .029679 |
| 1456921_at | Wdly3: WD repeat and FYVE domain containing 3 | BE956991 | 5683 | Mm.151095.1 | 87.32 | 87.7 | 78.92 | 84.61 | 3.64 | 73.68 | 68.17 | 59.65 | 67.41 | 4.72 | -1.26 | -2.889 | .048063 |
| 1421821_at | Ldr: low density lipoprotein receptor | AF425607 | 16835 | Mm.3213.1 | 426.43 | 462.69 | 401.82 | 429.93 | 19.48 | 357.56 | 352.59 | 327.42 | 345 | 11.13 | -1.25 | -3.785 | .029189 |
| 1426591_at | Gfm2: G elongation factor, mitochondrial 2 | BB497484 | 320806 | Mm.219675.1 | 130.64 | 135.94 | 132.44 | 132.95 | 4.15 | 113.34 | 103.74 | 104.18 | 106.77 | 4.96 | -1.25 | -4.05 | .016453 |
| 1450971_at | Gadd45b: growth arrest and DNA-damage-inducible 45 beta | AK010420 | 17873 | Mm.1360.1 | 509.27 | 481.26 | 432.79 | 473.9 | 24.02 | 366.48 | 369.13 | 409.23 | 380.63 | 16.47 | -1.25 | -3.203 | .039018 |
| 1434973_at | Car7: carbonic anhydrase 7 | BE650380 | 12354 | Mm.63694.1 | 327.74 | 346.25 | 328.23 | 333.49 | 9.26 | 260.01 | 283.81 | 254.63 | 266.16 | 10.67 | -1.25 | -4.767 | .009299 |
| 1435116_at | 4933403G14Rik: RIKEN cDNA 4933403G14 gene | BB219003 | 7493 | Mm.41011.1 | 176.69 | 154.36 | 181 | 170.68 | 8.71 | 130.52 | 141.79 | 136.03 | 136.03 | 5.72 | -1.25 | -3.227 | .03638 |
| 1440834_at | Scl5a10: solute carrier family 5 (sodium/glucose cotransporter), member 10 | BB502441 | 109342 | Mm.133164.1 | 432.72 | 440.32 | 460.48 | 444.22 | 12.58 | 580.2 | 528 | 555.97 | 554.39 | 16.92 | 1.25 | 5.225 | .007956 |
| 1460478_at | 2200002J24Rik: RIKEN cDNA 2200002J24 gene | AK008620 | 69147 | Mm.45301.1 | 152.68 | 143.05 | 136.21 | 143.49 | 5.49 | 108.37 | 129.08 | 108.87 | 115.24 | 7.8 | -1.25 | -2.961 | .04754 |
| 1417170_at | Lztf1: leucine zipper transcription factor-like 1 | NM_033322 | 93730 | Mm.133164.1 | 432.72 | 440.32 | 460.48 | 444.22 | 12.58 | 580.2 | 528 | 555.97 | 554.39 | 16.92 | 1.25 | 5.225 | .007956 |
| 1417791_at | Znf7: zinc finger, matrin-like | BM238431 | 18139 | Mm.4503.1 | 603.83 | 584.34 | 597.44 | 594.66 | 13.66 | 749.91 | 678.98 | 797.88 | 742.26 | 36.46 | 1.25 | 3.791 | .042502 |
| 1423444_at | Rock1: Rho-associated coiled-coil containing protein kinase 1 | BI662863 | 19877 | Mm.6710.1 | 468.92 | 526.5 | 521.12 | 504.9 | 20.54 | 657.71 | 599.07 | 631.15 | 629.09 | 18.38 | 1.25 | 4.506 | .011078 |
| 1425095_at | BC002059: cDNA sequence BC002059 | BC002059 | 213811 | Mm.130624.1 | 138.71 | 131.84 | 140.27 | 136.08 | 4.63 | 174.89 | 169.53 | 167.14 | 170.4 | 4.4 | 1.25 | 5.375 | .005832 |
| 1425338_at | Pld4: phospholipase C, beta 4 | BB224034 | 18798 | Mm.132097.1 | 91.63 | 89.8 | 97.52 | 93.36 | 4.77 | 123.22 | 113.3 | 115.1 | 116.93 | 4.46 | 1.25 | 3.61 | .022714 |
| 1427089_at | Ccn2: cyclin T2 | BI872151 | 72949 | Mm.45584.1 | 268.1 | 284.73 | 311.81 | 289.1 | 15.34 | 390.92 | 349.47 | 351.96 | 361.9 | 15.93 | 1.25 | 3.292 | .030211 |

(continued)
| Probe set | Gene | Accession | Entrez Gene | Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|------|-----------|-------------|-------------|------|------|------|--------------|-----------------|--------|--------|-------|---------------|------------------|-------------|------------|--------|
| 1437461_s_at | Rnpc3: RNA-binding region (RNP1, RRM) containing 3 | BB557441 | 67225 | Mm.58104.2 | 131.32 | 150.69 | 145.85 | 142.79 | 7.28 | 173.91 | 164.41 | 196.42 | 178.35 | 9.79 | 1.25 | 2.914 | .047948 |
| 1452659_at | Dek: DEK oncogene (DNA binding) | AK007546 | 110052 | Mm.28343.1 | 1080.09 | 1042.17 | 1035.41 | 1015.28 | 19.33 | 1396.44 | 1259.97 | 1280.06 | 1310.85 | 44.07 | 1.25 | 5.393 | .015682 |
| 1443857_at | Hook3: hook homolog 3 (Drosophila) | BB825115 | 320191 | Mm.63527.1 | 195.2 | 202.63 | 236.78 | 211.28 | 13.35 | 254.63 | 257.81 | 281.53 | 264.66 | 9.71 | 1.25 | 3.233 | .036332 |
| 1441621_s_at | Ssb: Sjogren syndrome antigen B | BG796845 | 20823 | Mm.10508.1 | 378.2 | 349.83 | 335.58 | 354.22 | 13.47 | 472.79 | 403.84 | 464.68 | 446.72 | 22.64 | 1.26 | 3.511 | .010504 |
| 1424100_at | Ttc8: tetratricopeptide repeat domain 8 | BC017523 | 76260 | Mm.32347.1 | 199.2 | 202.63 | 236.78 | 211.28 | 13.35 | 254.63 | 257.81 | 281.53 | 264.66 | 9.71 | 1.25 | 3.233 | .036332 |
| 1445909_at | Utp11l: UTP11-like, U3 small nucleolar ribonucleoprotein, (yeast) | BB277054 | 268709 | Mm.156860.2 | 196.24 | 213.14 | 218.74 | 210.75 | 10.18 | 263.21 | 266.83 | 263.02 | 264.69 | 6.24 | 1.26 | 4.517 | .016314 |
| 1456381_at | Zfp772: zinc finger protein 772 | BC023179 | 232855 | Mm.217124.1 | 95.66 | 105.05 | 112.16 | 104.73 | 7.4 | 135.92 | 127.51 | 130.19 | 131.61 | 3.59 | 1.26 | 3.265 | .049469 |
| 1453348_at | Hist2h2be: histone cluster 2, H2be | BB667233 | 319190 | Mm.5220.1 | 209.9 | 227.54 | 206.27 | 214.25 | 8.88 | 249.8 | 285.1 | 275.44 | 269.87 | 12.24 | 1.26 | 3.678 | .032037 |
| 1455095_at | Kdr: kit ligand | BB815530 | 17311 | Mm.4235.1 | 386.79 | 459.82 | 395.51 | 414.16 | 25.01 | 523.41 | 530.84 | 516.9 | 524.2 | 8.05 | 1.27 | 4.189 | .037463 |

(continued)
| Probe set | Gene | Accession | Baseline mean | Baseline mean’s SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean’s SE | Fold change | t statistic | p value |
|-----------|------|-----------|---------------|--------------------|--------|--------|------|---------------|---------------------|-------------|------------|---------|
| 1424043_at | Ppil4: peptidylprolyl isomerase (cyclophilin)-like 4 | BC004652 | 67418 | 604.42 | 580.85 | 585.05 | 600.33 | 20.62 | 1.27 | 4.91 | .009727 |
| 1456319_at | Mm.196322.1 | BG065719 | 7676 | 558.04 | 539.62 | 535.05 | 550.33 | 12.62 | 1.27 | 3.04 | .047636 |
| 1436446_at | C310007011Rik: RIKEN cDNA 2310007011 gene | BQ174649 | 74177 | 562.38 | 519.75 | 546.66 | 530.29 | 10.31 | 1.27 | 3.61 | .049595 |
| 1440902_at | Ermm: ermm, ERM-like protein | A1154960 | 77767 | 1063.38 | 1252.69 | 1138.13 | 1150.5 | 57.68 | 1.27 | 2.85 | .046827 |
| 1442982_at | Cdc66: coiled-coil domain containing 66 | BG075305 | 320234 | 327.06 | 291.38 | 332.61 | 316.44 | 14.24 | 1.27 | 4.08 | .023368 |
| 1455738_at | Cdc55: coiled-coil domain containing 55 | BB066444 | 237859 | 173.48 | 193.62 | 170.54 | 179.24 | 9.33 | 1.27 | 3.51 | .036148 |
| 1423445_at | Rock1: Rho-associated coiled-coil containing protein kinase 1 | B1662863 | 19877 | 441.93 | 397.23 | 419.64 | 420.36 | 14.01 | 1.28 | 5.01 | .00806 |
| 1422575_at | Eph3: Eph receptor A3 | M68513 | 13837 | 166.22 | 184.06 | 172.18 | 174.08 | 6.61 | 1.28 | 3.19 | .040815 |
| 1452110_at | Mtrr: 5-methyltetrahydrofolate-homocysteine methyltransferase reductase | BB757908 | 210009 | 303.1 | 286.52 | 257.77 | 282.59 | 14.11 | 1.28 | 3.04 | .038106 |
| 1456510_at | Higd1c /// Metl7a2: HIG1 domain family, member 1 /// methyltransferase like 7A2 | BB703414 | 380975 /// 393082 | 360.13 | 345.26 | 329.26 | 344.53 | 11.3 | 1.28 | 4.46 | .011376 |
| 1436139_at | Mm.115096.1 | AV328974 | 143.44 | 187.47 | 186.62 | 206.57 | 193.79 | 7.3 | 1.28 | 4.33 | .012597 |
| 1443986_at | Cdc73: cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae) | BB211070 | 214498 | 226.38 | 211.75 | 235.19 | 224.69 | 7.83 | 1.28 | 3.45 | .032441 |
| 1428052_at | Zym1: zinc finger, MYM domain containing 1 | BC027750 | 68310 | 333.63 | 284.92 | 348.43 | 323.04 | 19.29 | 1.29 | 3.47 | .048966 |
| 1439103_at | Cdc73: cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae) | BB183750 | 214498 | 204.74 | 194.33 | 216.84 | 205.52 | 7.05 | 1.29 | 5.63 | .00934 |
| 1449972_at | NM_011765 /// 449000 | Mm.4596.1 | 223.44 | 234.21 | 213.43 | 207.91 | 214.67 | 6.04 | 1.27 | 4.31 | .001877 | (continued) |
| Probe set | Gene Accession | Baseline mean | WT-1 | WT-2 | WT-7 | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|----------------|---------------|------|------|------|--------------------|--------|--------|--------|----------------|------------------------|------------|------------|--------|
| BC018101 /// Zfp97: cDNA sequence | BB826168 | 27377 | Mm.23335.1 | 435.19 | 451.57 | 452.69 | 446.91 | 11.12 | 585.37 | 567.45 | 582.19 | 578.33 | 10.67 | 1.29 | 8.528 | .001045 |
| 1450954_at | Yme1l: YME1-like 1 (S. cerevisiae) | AA611589 | 73091 | Mm.158940.1 | 70.77 | 70.64 | 65.47 | 65.74 | 4.37 | 86.16 | 81.65 | 87.44 | 84.78 | 3.61 | 1.29 | 3.36 | .029859 |
| 1431381_at | 310005L24Rik: RIKEN cDNA 310005L24 gene | AV538049 | 67500 | Mm.196371.2 | 926.59 | 930.69 | 999.8 | 952.06 | 25.61 | 1267.85 | 1302.87 | 1118.49 | 1228.9 | 58.62 | 1.29 | 4.328 | .027373 |
| 1436157_at | Ccar1: cell division cycle and apoptosis regulator 1 | BB109183 | 100986 | Mm.131768.1 | 146.82 | 157.46 | 168.26 | 157.88 | 7.16 | 189.82 | 197.27 | 223.68 | 203.53 | 10.64 | 1.29 | 3.559 | .029388 |
| 1447913_x_at | Akap9: A kinase (PRKA) anchor protein (yotiao) | BB109183 | 100986 | Mm.144816.1 | 205.41 | 198.8 | 205.2 | 203.72 | 5.39 | 284.91 | 264.22 | 236.06 | 261.87 | 15.28 | 1.29 | 3.59 | .049852 |
| 1452750_at | 5530601H04Rik: RIKEN cDNA 5530601H04 gene | AV315180 | 71445 | Mm.86328.1 | 127.25 | 115.89 | 115.45 | 119.5 | 5.25 | 163.33 | 154.15 | 144.21 | 153.93 | 6.31 | 1.29 | 4.194 | .014735 |
| 1456027_at | Rbm41: RNA binding motif protein 41 | AL92455 | 234358 | Mm.10509.1 | 103.25 | 92 | 92.85 | 95.42 | 4.51 | 125.7 | 116.81 | 129.15 | 123.72 | 4.84 | 1.3 | 4.281 | .012974 |
| 1427518_at | D10627: cDNA sequence D10627 | BB183240 | 209707 | Mm.32012.3 | 188.36 | 191.61 | 221.02 | 200.51 | 11.72 | 243.12 | 247.32 | 291.93 | 260.54 | 16.55 | 1.3 | 2.96 | .047371 |
| 1439272_at | Lcorl: ligand dependent nuclear receptor corepressor-like | AV245518 | 74239 | Mm.23778.1 | 49.8 | 51.48 | 46.73 | 48.95 | 2.64 | 67.09 | 59.48 | 62.95 | 63.4 | 2.67 | 1.3 | 3.847 | .013835 |
| 1457897_at | Iqce: IQ motif containing E | NM_011584 | 353187 | Mm.26587.1 | 1633.2 | 1745.68 | 1893.36 | 1757.7 | 79.82 | 2412.63 | 2241.99 | 2271.59 | 2306.32 | 56.26 | 1.31 | 5.618 | .006753 |
| 1416958_at | Nrd1: nuclear receptor subfamily 1, group D, member 2 | AV171622 | 380975 /// 393082 /// 70152 | Mm.220975.2 | 408.26 | 453.92 | 404.75 | 422.32 | 18.25 | 573.73 | 550.07 | 527.89 | 552.11 | 14.53 | 1.31 | 5.564 | .005899 |
| 1434150_a_at | Higd1c /// Mettl7a1 /// Mettl7a2: HIG1 domain family, member 1C /// methyltransferase like 7A1 /// methyltransferase like 7A2 | BB173752 | 83946 | Mm.54737.1 | 106.83 | 111.25 | 103.61 | 106.99 | 5.12 | 145.62 | 136.2 | 138.98 | 139.78 | 5.31 | 1.31 | 4.445 | .01132 |

(continued)
| Probe set | Gene Accession | Gene Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean’s SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean’s SE | Fold change | t statistic | p value |
|-----------|----------------|------------------|------|------|------|--------------|------------------|--------|--------|--------|----------------|------------------|--------------|------------|--------|
| 1429690_at | 1300003B13Rik: RIKEN cDNA 1300003B13 gene | AK004870 | 74149 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1436045_at | Tsga10: testis specific 10 AV377349 | 211484 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1447854_s_at | Hist2h2be: histone cluster 2, H2be AV127319 | 319190 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1457584_at | A1B48100: expressed sequence A1B48100 AV377565 | 226551 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1420340_at | Cspp1: centrosome and spindle pole associated protein 1 NM_026493 | 211660 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1424672_at | Dmxi1: Dmx-like 1 BC020141 | 240283 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1429907_at | 1700094D03Rik: RIKEN cDNA 1700094D03 gene AK007060 | 73545 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1438736_at | Tho2: THO complex 2 BB703762 | 331401 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1436540_at | Mirlet7d: microRNA let7d BQ031149 | 387247 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1437556_at | Zfhx4: zinc finger homeodomain 4 AI385586 | 80892 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1438937_x_at | Ang: angiogenin, ribonuclease, RNase A family, 5 AI385586 | 17277 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1445723_at | Plic1: phospholipase C-like 1 BB451636 | 227120 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1436213_a_at | 1110028C15Rik: RIKEN cDNA 1110028C15 gene AV023018 | 68691 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1434097_at | D10627: cDNA sequence D10627 BM218328 | 234358 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1424854_at | Hist1h4a // Hist1h4b // Hist1h4c // Hist1h4d // Hist1h4m: histone cluster 1, H4a // histone cluster 1, H4b // histone cluster 1, H4c // histone cluster 1, H4d // histone cluster 1, H4m BC019757 | 319157 // 319158 // 319159 // 319160 // 319161 // 319162 // 319163 // 319164 // 319165 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |
| 1451640_a_at | Rsrc2: arginine/serine-rich coiled-coil 2 BC008229 | 208606 | Mm.30767.1 | 228.26 | 240.06 | 231.88 | 233.59 | 7.01 | 314.78 | 284.94 | 324.72 | 307.12 | 13.19 | 1.31 | 4.923 | .015498 |

Table 1. Continued
| Probe set | Gene | Accession | Entrez Gene | Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|------|-----------|-------------|-------------|------|------|------|--------------|-----------------|--------|--------|------|---------------|-----------------|-------------|------------|--------|
| 1433743_at | Dach1: dachshund 1 (Drosophila) | BG075820 | 13134 | Mm.10877.1 | 66.84 | 58.07 | 68.82 | 64.93 | 4.03 | 92.87 | 78.23 | 90.29 | 87.11 | 4.72 | 1.34 | 3.576 | .024218 |
| 1435230_at | Ankrd12: ankyrin repeat domain 12 | BB277613 | 106585 | Mm.34706.1 | 478.84 | 465.52 | 467.21 | 470.68 | 9.21 | 674.29 | 603.84 | 613.33 | 628.85 | 25.6 | 1.34 | 5.814 | .016311 |
| 1437433_at | B3gal2: UDP-GalbetaGlCNA beta 1,3-galactosyltransferase, polypeptide 2 | BB254922 | 26878 | Mm.110912.1 | 179.28 | 150.89 | 159.42 | 159.42 | 10.59 | 229.18 | 201.40 | 218.88 | 215.94 | 8.77 | 1.35 | 4.109 | .015801 |
| 1418526_at | Srs13a: splicing factor; arginine/serine-rich 13A | NM_010178 | 14105 | Mm.10229.1 | 259.28 | 283.51 | 264.54 | 264.54 | 10.63 | 388.10 | 363.75 | 328.44 | 359.99 | 17.79 | 1.36 | 4.606 | .015941 |
| 1418527_a_at | Srs13a: splicing factor; arginine/serine-rich 13A | NM_010178 | 14105 | Mm.10229.1 | 364.64 | 392.31 | 377.18 | 377.18 | 11.55 | 568.88 | 479.73 | 488.76 | 512.76 | 28.77 | 1.36 | 4.373 | .028901 |
| 1449571_at | Trhr: thyrotropin releasing hormone receptor | MS9811 | 22045 | Mm.3946.1 | 238.58 | 216.25 | 221.24 | 221.24 | 9.75 | 322.89 | 261.02 | 319.94 | 300.89 | 20.54 | 1.36 | 3.503 | .042507 |
| 1436156_at | Ccrl1: cell division cycle and apoptosis regulator 1 | AWS38049 | 67500 | Mm.196371.2 | 523.53 | 537.22 | 548.21 | 537.30 | 12.02 | 768.50 | 751.71 | 672.18 | 730.16 | 30.99 | 1.36 | 5.801 | .015139 |
| 1439340_at | D630036G22Rik: RIKEN cDNA D630036G22 gene | BB501833 | 442807 | Mm.170453.1 | 38.19 | 42.33 | 42.79 | 42.79 | 3.57 | 59.63 | 62.37 | 53.04 | 58.22 | 28.77 | 1.36 | 4.295 | .042411 |
| 1423084_at | B3gal2: UDP-GalbetaGlCNA beta 1,3-galactosyltransferase, polypeptide 2 | BB223909 | 26878 | Mm.123510.1 | 331.43 | 341.30 | 333.24 | 333.24 | 7.32 | 463.78 | 452.19 | 478.35 | 455.37 | 16.85 | 1.37 | 6.649 | .009263 |
| 1448738_at | Calb1: calbindin 1 | BB246032 | 12307 | Mm.354.1 | 170.68 | 184.23 | 184.23 | 184.23 | 11.55 | 215.59 | 234.09 | 234.64 | 228.2 | 8.23 | 1.37 | 4.305 | .015653 |
| 1446261_at | D1Ertd507e: DNA segment, Chr 1, ERATODi 507, expressed | BG068111 | 52356 | Mm.155161.1 | 38.46 | 29.53 | 34.84 | 34.84 | 3.43 | 49.18 | 48.35 | 46.58 | 47.9 | 15.9 | 1.37 | 3.454 | .04499 |
| 1455686_at | Lcorl: ligand dependent nuclear receptor corepressor-like | BB077342 | 209707 | Mm.131615.1 | 266.79 | 270.23 | 247.61 | 247.61 | 20.94 | 332.55 | 337.08 | 343.37 | 338.76 | 7.44 | 1.37 | 4.101 | .036974 |
| 1458112_at | Adarb2: adenosine deaminase, RNA-specific, B2 | BB527550 | 94191 | Mm.190112.1 | 305.74 | 279.66 | 290.6 | 290.6 | 10.51 | 419.59 | 381.19 | 394.81 | 398.53 | 13.78 | 1.37 | 6.229 | .004223 |
| 1458571_at | D430047D06Rik: RIKEN cDNA D430047D06 gene | BB488016 | 320716 | Mm.135160.1 | 28.97 | 30.16 | 27.76 | 27.76 | 2.62 | 37.76 | 37.64 | 38.44 | 37.93 | 2.01 | 1.37 | 3.076 | .040416 |
| 1423982_at | AF060490 | 14105 | Mm.10229.2 | 581.56 | 610.62 | 26.81 | 869.42 | 853.14 | 880.5 | 852.39 | 13.88 | 1.4 | 8.009 | .004063 | (continued) |
| Probe set | Gene Accession | Entrez Gene | Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean’s SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean’s SE | Fold change | t statistic | p value |
|------------|----------------|-------------|-------------|------|------|------|---------------|-------------------|--------|--------|--------|---------------|-------------------|-------------|------------|--------|
| 143322_at  | Sfrs13a: splicing factor, arginine/serine-rich 13A | AK015932 | 75226 | Mm.159470.1 | 36.51 | 32.37 | 29.73 | 32.88 | 2.62 | 50.8 | 44.24 | 41.63 | 46.03 | 3.39 | 1.4 | 3.069 | .040568 |
| 1447815_x_at | 6430527G18Rik: RIKEN cDNA 6430527G18 gene | BB057169 | 238330 | Mm.161505.1 | 50.64 | 41.91 | 39 | 44.6 | 4.81 | 60.44 | 63.71 | 64.84 | 63.05 | 4 | 1.41 | 2.95 | .043675 |
| 1419014_at | Rhag: Rhesus blood group-associated A glycoprotein | NM_011269 | 19743 | Mm.159470.1 | 21.82 | 19.73 | 18.16 | 19.94 | 1.71 | 31.77 | 24.51 | 28.68 | 28.25 | 2.31 | 1.42 | 2.886 | .049339 |
| 1456934_at | Calb1: calbindin 1 | BB177770 | 12307 | Mm.121403.1 | 238.21 | 187.65 | 224.7 | 216.7 | 15.62 | 337.85 | 296.9 | 300.88 | 308.67 | 15.52 | 1.42 | 4.176 | .01396 |
| 1430781_at | Ak7: adenylate kinase 7 | AV256298 | 78801 | Mm.59172.1 | 150.21 | 147.82 | 138.59 | 144.92 | 6.53 | 228.65 | 207.74 | 185.15 | 207.07 | 13.38 | 1.43 | 4.173 | .026715 |
| 1437980_at | 1437980_at | BB814947 | 1E+08 | Mm.190421.1 | 25.6 | 21.14 | 26.71 | 24.54 | 2.4 | 35.37 | 33.64 | 35.19 | 35.03 | 1.96 | 1.43 | 3.386 | .029338 |
| 1439820_at | 1439820_at | BB364548 | 12037 | Mm.167368.1 | 87.31 | 76.95 | 69.27 | 77.64 | 6.01 | 123.88 | 111.09 | 97.3 | 111.05 | 8.06 | 1.43 | 3.323 | .032963 |
| 1457373_at | 1457373_at | BB495006 | 155.53 | Mm.135415.1 | 152.24 | 155.53 | 181.84 | 163.77 | 10.45 | 251.24 | 251.9 | 200.53 | 234.96 | 17.83 | 1.43 | 3.444 | .036695 |
| 1443050_at | Fn3krp: fructosamine 3 kinase related protein | BB072270 | 238024 | Mm.117394.1 | 501.97 | 591.9 | 679.4 | 590.99 | 52.8 | 847.83 | 830.92 | 870.55 | 849.75 | 15.1 | 1.44 | 4.712 | .031289 |
| 1458040_at | D7Wsu130e: DNA segment, Chr 7, Wayne State University 130, expressed | BM213832 | 28017 | Mm.33177.1 | 47.59 | 46.86 | 51.41 | 49.05 | 3.07 | 72.64 | 74.88 | 65.4 | 71.03 | 3.68 | 1.45 | 4.587 | .010898 |
| 1455087_at | D7Etd715e: DNA segment, Chr 7, ERATO Doi 715, expressed | AV328498 | 52480 | Mm.21243.1 | 180.24 | 158.92 | 168.84 | 169.31 | 6.5 | 257.55 | 245.19 | 236.27 | 246.39 | 6.63 | 1.46 | 8.302 | .001152 |
| 1441938_x_at | Cables1: CDSK and Abl enzyme substrate 1 | BB071777 | 63955 | Mm.63141.1 | 103.77 | 103.88 | 145.15 | 118.01 | 14.28 | 166.82 | 182.08 | 170.07 | 173.23 | 6.02 | 1.47 | 3.563 | .049539 |
| 1450208_a_at | Elmo1: engulfment and cell motility 1, ced-12 homolog (C. elegans) | NM_080288 | 140580 | Mm.214934.1 | 157.5 | 179.25 | 187.87 | 174.63 | 10.48 | 264.19 | 303.84 | 222.94 | 263.69 | 24.15 | 1.51 | 3.383 | .049539 |
| 1419347_x_at | Svs5: seminal vesicle secretory protein 5 | NM_009301 | 20944 | Mm.140154.1 | 15.98 | 16.31 | 12.5 | 14.93 | 1.7 | 25.24 | 20.45 | 22.69 | 22.86 | 1.69 | 1.53 | 3.304 | .029812 |
| 1448421_s_at | Asprn: asporin | NM_025711 | 66695 | Mm.25755.1 | 15.78 | 17.96 | 14.48 | 15.96 | 2.14 | 22.98 | 26.37 | 24.44 | 24.73 | 1.88 | 1.55 | 3.076 | .03789 |
| 1417602_at | Per2: period homolog 2 (Drosophila) | AF035830 | 18627 | Mm.8471.1 | 165.31 | 180.23 | 249.94 | 198.58 | 26.58 | 347.55 | 318.29 | 266.59 | 310.75 | 24.1 | 1.56 | 3.126 | .035779 |
| 1422163_at | NM_008018 | 14218 | Mm.20446.1 | 9.84 | 9.73 | 12.25 | 11.01 | 1.45 | 15.77 | 18.59 | 16.62 | 17.16 | 1.59 | 1.56 | 2.859 | .046421 |

(continued)
| Probe set | Gene Description | Accession | Gene Accession | Entrez Gene | Description | WT -1 | WT -2 | WT -7 | Baseline mean | Baseline mean’s SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean’s SE | Fold change | t statistic | p value |
|-----------|------------------|-----------|----------------|-------------|-------------|--------|--------|--------|---------------|-------------------|--------|--------|--------|----------------|-------------------|------------|------------|--------|
| 1457534_at | Sh3pxd2a: SH3 and PX domains 2A | BB481074 | Mm.210151.1 | 30.93 | 38.4 | 27.12 | 32.4 | 4.79 | 47.71 | 55.5 | 51.01 | 50.79 | 3.89 | 1.57 | 2.981 | .042874 |
| 1459281_at | Mm.208534.1 | BB182935 | Mm.208534.1 | 4.97 | 6.95 | 5.75 | 5.73 | 0.89 | 8.91 | 9.47 | 9.21 | 9.13 | 0.81 | 1.59 | 2.817 | .048443 |
| 1436330_x_at | Gm7072: predicted gene 7072 | BG244780 | 631624 | 6.67 | 68.19 | 71.69 | 69.42 | 3.45 | 102.57 | 105.84 | 126.76 | 111.61 | 8.06 | 1.61 | 4.813 | .02151 |
| 1439717_at | Gabrg3: gamma-aminobutyric acid (GABA) A receptor, subunit gamma 3 | BB316100 | 14407 | 18.62 | 20.14 | 29.18 | 22.49 | 3.94 | 36.54 | 37.03 | 38.24 | 37.31 | 2.89 | 1.66 | 3.033 | .043228 |
| 1437303_at | Il6st: interleukin 6 signal transducer | BI102913 | 16195 | 203.93 | 239.51 | 305.91 | 249.74 | 30.94 | 374.55 | 491.68 | 387.59 | 418.57 | 38.63 | 1.68 | 3.412 | .029031 |
| 1430444_at | S010060.L08Rik: RIKEN cDNA 0610060.L08 gene | AK002255 | 76253 | 1 | 1 | 1 | 1 | 0.16 | 1.84 | 1.53 | 1.84 | 1.71 | 0.14 | 1.71 | 3.259 | .031831 |
| 1430376_at | Lrrc9: leucine rich repeat containing 9 | AK019545 | 78257 | 19.07 | 20.79 | 19.38 | 19.77 | 1.85 | 33.09 | 38 | 29.83 | 34.22 | 3.68 | 1.73 | 3.511 | .040127 |
| 1425618_at | Dhx9: DEAH-(Asp-Glu-Ala-His) box polypeptide 9 | U91922 | 13211 | 5.67 | 5.61 | 8.63 | 6.65 | 1.15 | 11.46 | 11.61 | 12.71 | 11.79 | 0.86 | 1.77 | 3.591 | .026201 |
| 1442809_at | Scn9a: sodium channel, voltage-gated, type IX, alpha | BB452274 | 20274 | 15.3332 | 1 | 16.86 | 19.48 | 15.07 | 17.4 | 2.12 | 34.02 | 34.22 | 25.01 | 31.01 | 3.3 | 1.78 | 3.473 | .032983 |
| 1419962_at | Mm.195371.1 | C80871 | 195371.1 | 8.34 | 8.47 | 5.99 | 7.4 | 1.45 | 11.81 | 14.21 | 14.27 | 13.39 | 1.19 | 1.81 | 3.19 | .035035 |
| 1446552_at | Slc12a3: solute carrier family 12, member 3 | BB503574 | 20497 | 4.23 | 4.32 | 5.85 | 4.85 | 1.05 | 6.49 | 7.18 | 6.82 | 6.88 | 0.97 | 2.11 | 2.888 | .039988 |
| 1420547_at | Galc: galactosylceramidase | BF168119 | 14420 | 68.87 | 69.19 | 79.15 | 72.08 | 7 | 153.99 | 147.99 | 103.53 | 135.17 | 16.53 | 1.88 | 3.514 | .046204 |
| 1437824_at | Grid2: glutamate receptor, ionotropic, delta 2 | BB334542 | 14804 | 131503.1 | 6.78 | 4.29 | 7.46 | 6.16 | 1.39 | 12.72 | 10.53 | 11.66 | 11.71 | 1.19 | 1.9 | 3.028 | .039988 |
| 1421317_x_at | Myb: myeloblastosis oncogene | NM_033597 | 17863 | 32.94 | 26.31 | 21.77 | 27.11 | 4.36 | 57.83 | 55.9 | 44.56 | 52.87 | 4.67 | 1.95 | 4.033 | .015838 |
| 1449807_x_at | Gabra2: gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2 | AV379247 | 14395 | 45112.2 | 960.49 | 1094.93 | 1173.79 | 1074.31 | 77.38 | 1969.95 | 2148.45 | 2248.45 | 2114.56 | 90.21 | 1.97 | 8.753 | .001041 |
| 1454561_at | 9430087B13Ric: RIKEN cDNA 9430087B13 gene | AK020508 | 77437 | 159920.1 | 7.7 | 2.5 | 6.09 | 5.58 | 1.64 | 9.82 | 12.79 | 11.87 | 11.51 | 1.29 | 2.06 | 2.84 | .049778 |
| 1430218_at | AK016899 | 67548 | Mm.148731.1 | 7.54 | 7.78 | 4.31 | 6.61 | 1.8 | 12 | 12.79 | 17.22 | 14.02 | 1.89 | 2.12 | 2.838 | .047103 |
| Probe set | Gene Accession | Gene Description | WT-1 | WT-2 | WT-7 | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|-----------|----------------|------------------|------|------|------|---------------|---------------------|--------|--------|--------|---------------|---------------------|------------|------------|--------|
| 4933424M12Rik: RIKEN cDNA 4933424M12 | NM_010172 14068 | F7: coagulation factor VII | 7.75 | 9.98 | 12.2 | 9.72 | 2.06 | 25.68 | 18.56 | 18.03 | 20.69 | 2.67 | 2.13 | 3.255 | .034136 |
| 1453435_at | AK009753 55990 | Fmo2: flavin containing monoxygenase 2 | 18.14 | 18.33 | 17.86 | 18.11 | 2.09 | 39.95 | 43.63 | 33.32 | 38.96 | 3.52 | 2.15 | 5.095 | .011921 |
| 1443577_at | AV261494 | Mm.72499.1 | 4.98 | 5.78 | 5.82 | 5.49 | 0.51 | 10.74 | 10.72 | 14.71 | 12.04 | 1.38 | 2.2 | 4.468 | .029512 |
| 1445438_at | AV106920 18478 | Pah: phenylalanine hydroxylase | 3.36 | 2.37 | 2.27 | 0.75 | 4.52 | 5.68 | 5.26 | 5.11 | 0.58 | 2.25 | 2.985 | .043983 |
| 1420300_at | AV379247 | Mm.45112.2 | 3.58 | 34.13 | 30.06 | 33.09 | 2.96 | 69.5 | 73.24 | 81.05 | 74.48 | 3.97 | 2.25 | 8.355 | .001566 |
| 1420774_at | NM_026358 67749 | 4930583H14Rik: RIKEN cDNA 4930583H14 gene | 8.56 | 4.51 | 5.51 | 6.46 | 2.07 | 15.78 | 12.7 | 17.34 | 15.41 | 2.2 | 2.39 | 2.963 | .04165 |
| 1440510_at | BB407702 319707 | F430002N11Rik: RIKEN cDNA F430002N11 gene | 1.24 | 2.96 | 2.23 | 0.26 | 2.44 | 4.09 | .01519 |
| 1442860_at | BB429621 217480 | Dgkb: diacylglycerol kinase, beta | 5.71 | 9.12 | 5.74 | 2.02 | 15.91 | 12.73 | 14.13 | 14.18 | 1.37 | 2.47 | 3.467 | .031537 |
| 1440754_at | BG797192 | Mm.193602.1 | 3.58 | 3.32 | 4.9 | 1.73 | 12.89 | 11.3 | 12.33 | 12.3 | 1.07 | 2.51 | 3.633 | .030159 |
| 1429481_at | AK014772 17974 | Nck2: non-catalytic region of tyrosine kinase adaptor protein 2 | 3.3 | 6.21 | 2.6 | 4.1 | 1.51 | 10.66 | 11.01 | 9.63 | 10.45 | 1.23 | 2.55 | 3.258 | .032988 |
| 1423340_at | AV334599 | Tcfap2b: transcription factor AP-2 beta | 1.87 | 3.53 | 2.1 | 0.9 | 6.49 | 4.95 | 5.2 | 0.59 | 2.63 | 3.18 | .014073 |
| 1425434_at | L04274 20288 | Msr1: macrophage scavenger receptor 1 | 3.95 | 5.87 | 3.45 | 1.56 | 8.98 | 7.75 | 10.51 | 9.07 | 1.17 | 2.63 | 2.889 | .048776 |
| 1418783_at | AF228681 56843 | Trpm5: transient receptor potential cation channel, subfamily M, member 5 | 9.47 | 10.65 | 3.88 | 7.72 | 2.65 | 21.44 | 20.09 | 19.89 | 20.42 | 1.38 | 2.65 | 4.254 | .023746 |
| 1453812_at | AK018295 76217 | Jakmip2: janus kinase and microtubule interacting protein 2 | 3.53 | 6.64 | 6.07 | 5.21 | 2.42 | 17.24 | 11.99 | 13.61 | 14.22 | 1.97 | 2.73 | 2.895 | .046562 |
| 1455444_at | BB339336 14395 | Gabra2: gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2 | 691.96 | 660.47 | 646.13 | 666.54 | 19.81 | 1812.1 | 1792.15 | 1931.73 | 1843.54 | 48.15 | 2.77 | 22.607 | .000409 |
| 1451510_s_at | BC025001 99035 | T rpm5: transient receptor potential cation channel, subfamily M, member 5 | 1.4 | 3.4 | 1.49 | 0.76 | 6.42 | 6.8 | 4.87 | 6.04 | 0.91 | 2.82 | 3.291 | .031751 |

(continued)
| Probe set  | Gene Description                  | Accession | Entrez Gene | Description | WT-1          | WT-2          | WT-7          | Baseline mean | Baseline mean's SE | MSK-14 | MSK-15 | MSK-9 | Experiment mean | Experiment mean's SE | Fold change | t statistic | p value |
|------------|-----------------------------------|-----------|-------------|-------------|---------------|---------------|---------------|---------------|------------------|--------|--------|-------|-----------------|----------------------|-------------|-------------|--------|
| 1421044_at | Oleoyl-ACP hydrolase              | BB528408  | 17534       |             | 1.00          | 0.99          | 1.00          | 1.00          | 0.99             | 1.00   | 0.99   | 1.00   | 2.85            | 2.85                  | 2.85        | 0.27566     |        |
| 1432837_at | Mannose receptor, C type 2        | AK012542  | 67969       |             | 2.82          | 2.1           | 5.55          | 3.43          | 1.43             | 9.26   | 8.57   | 11.03 | 2.85            | 3.57                  | 2.85        | 0.02643     |        |
| 1421738_at | Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2 | NM_008066 | 14395       |             | 5.86          | 565.56        | 595.82        | 582.45        | 12.02            | 1703.77 | 1644.43 | 1765.13 | 1705.63         | 39.55             | 2.93        | 27.174      | 0.000522 |
| 1459553_at | Fructose-1,6-bisphosphate-1phosphate 1, UDP-glycosyltransferase, 2 | BG068521  | 172145.1    |             | 3.54          | 1.35          | 2.01          | 0.93          | 5.98             | 5.42   | 7.25   | 6.31  | 3.14            | 3.445                 | 2.31        | 0.026706    |        |
| 1451349_at | EF-hand calcium binding domain 7 | BC020077  | 230500      |             | 56.46         | 59.29         | 53.06         | 12.18         | 177.8            | 140.15 | 182.95 | 166.99 | 3.15            | 6.152                 | 2.85        | 0.00376     |        |
| 1430751_at | Serine (or cysteine) peptidase inhibitor, clade A, member 3 | AK019935  | 628900      |             | 2.06          | 3.42          | 2.44          | 0.87          | 7.19             | 7.63   | 8.76   | 7.8    | 0.73            | 3.2                   | 4.739       | 0.009707    |        |
| 1424233_at | Mesenchyme homeobox 2             | BC02076   | 17286       |             | 4.38          | 4.83          | 3.33          | 1.82          | 13.33            | 8.69   | 9.68   | 10.7  | 1.62            | 3.031                 | 3.21        | 0.039425    |        |
| 1443865_at | Gamma-aminobutyric acid (GABA) A receptor, subunit alpha 2 | BC0174589 | 14395       |             | 268.13        | 278.64        | 283.81        | 11.89         | 975.16           | 892.33 | 949.03 | 939.04 | 24.63           | 3.31                   | 23.956      | 0.00207     |        |
| 1457044_at | Metastasis associated in colon cancer 1 | BB007136  | 238455      |             | 3.07          | 3.52          | 3.29          | 3.31          | 1.45             | 11.72  | 9.59   | 14.05 | 1.88            | 3.52                   | 3.507       | 0.027354    |        |
| 1450573_at | Anti-Mullerian hormone             | NM_007445 | 11705       |             | 3.06          | 2.53          | 4.46          | 3.67          | 1.6              | 12.2   | 10.17  | 16.74 | 1.96            | 3.54                   | 3.693       | 0.022496    |        |
| 1449393_at | LOC100046930 // Sh2d1a: similar to T cell signal transduction molecule SAP // SH2 domain protein 1A | NM_011364 | 100046930 // 20400 |             | 5.67          | 1.62          | 3.59          | 1.7           | 18.47            | 12.96  | 10.76  | 14.07 | 2.31            | 3.92                   | 3.663       | 0.024942    |        |
| 1419100_at | Serine (or cysteine) peptidase inhibitor, clade A, member 3N | NM_009252 | 20716       |             | 51.19         | 422.34        | 563.08        | 502.61        | 49.2             | 2450.79 | 2136.39 | 1426.57 | 2004.13         | 303.07             | 3.99        | 4.89        | 0.03549 |

(continued)
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