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Genome wide transcriptional profiling in breast cancer cells reveals distinct changes in hormone receptor target genes and chromatin modifying enzymes after proteasome inhibition

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Abstract

Steroid hormone receptors, like glucocorticoid (GR) and estrogen receptors (ER), are master regulators of genes that control many biological processes implicated in health and disease. Gene expression is dependent on receptor levels which are tightly regulated by the ubiquitin-proteasome system. Previous studies have shown that proteasome inhibition increases GR, but decreases ER-mediated gene expression. At the gene expression level this divergent role of the proteasome in receptor-dependent transcriptional regulation is not well understood. We have used a genomic approach to examine the impact of proteasome activity on GR and ER-mediated gene expression in MCF-7 breast cancer cells treated with dexamethasone (DEX) or 17β-estradiol (E2), the proteasome inhibitor MG132 (MG) or MG132 and either hormone (MD or ME2) for 24h. Transcript profiling reveals that inhibiting proteasome activity modulates gene expression by GR and ER in a similar manner in that several GR and ER target genes are up-regulated and down-regulated after proteasome inhibition. In addition, proteasome inhibition modulates receptor-dependent genes involved in the etiology of a number of human pathological states, including multiple myeloma, leukemia, breast/prostate cancer, HIV/AIDS and neurodegenerative disorders. Importantly, our analysis reveals that a number of transcripts encoding histone and DNA modifying enzymes, prominently histone/DNA methyltransferases and demethylases, are altered after proteasome inhibition. As proteasome inhibitors are currently in clinical trials as therapy for multiple myeloma, HIV/AIDs and leukemia, the possibility that some of the target molecules are hormone regulated and by chromatin modifying enzymes is intriguing in this era of epigenetic therapy.

Keywords

Proteasome inhibitor; receptors: glucocorticoid; estrogen; gene expression profiling; microarray analysis

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Introduction

Glucorticoids and estrogens play a crucial role in regulating transcription of many genes that are important regulators of diverse physiological processes, including development, reproduction, bone formation/resorption, energy metabolism, cholesterol mobilization and immunity. The physiological actions of glucocorticoids and estrogens are mediated primarily through the glucocorticoid receptor (GR) and estrogen receptor (ER). Glucocorticoid and estrogen receptors are ligand-dependent transcription factors and members of the nuclear hormone receptor super family [1]. Upon hormone binding these receptors localize in the nucleus where they associate with specific hormone response elements within promoter sequences embedded in chromatin [2]. To activate or repress target genes, steroid hormone receptors recruit various co-regulator complexes, including chromatin remodeling complexes to modify local chromatin structure [3,4]. Receptor and coregulator levels play key roles in controlling appropriate physiological outcomes in specific target tissues. Similar to other steroid hormone receptors, GR and ER are tightly regulated by the ubiquitin proteasome system (UPS) [reviewed in [5,6]. Additionally, levels of nuclear hormone receptor co-regulators are also regulated by the UPS [7-9]. Briefly, the UPS plays an important role in a variety of cellular functions primarily via its proteolytic activity, although recent studies implicate the components of the pathway in direct regulation of specific transcriptional processes [reviewed in [10,11]. The 26S proteasome is the principal biochemical machinary that degrades short lived cellular proteins and rids the cell of damaged and misfolded polypeptides, in addition to providing basic housekeeping functions [12]. The 26S proteasome is a multi-enzyme complex made of a 20S catalytic ‘core’, capped by the 19S regulatory complex [13,14]. The 19S complex is composed of two sub-complexes: the lid and the base composed of six AAA-type ATPases and two non-ATPase subunits. Proteolysis of a target protein by the 26S proteasome, involves two intricate steps [13,14]. First, the protein is tagged with ubiquitin (Ub), a conserved 76 amino acid polypeptide, or, more precisely, with a poly-Ub chain of defined length and topology to generate the polyubiquitin degradation signal [14]. Secondly, the tagged protein is degraded by the 26S proteasome complex. Conjugation of ubiquitin to the protein substrate is mediated by a multi-enzyme cascade consisting of an Ub-activating enzyme (E1), an Ub-conjugating enzyme (E2), and an Ub ligase (E3) [15].

Control of cellular protein levels by the ubiquitin–proteasome system is essential for various cellular functions and ultimately dysregulation of the system is associated with many pathological conditions [16,17]. Although the role of the ubiquitin-proteasome system in regulating many transcription factors, such as p53, is well established, the system has only recently been linked to steroid hormone receptor function. There is a general agreement that the ubiquitin-proteasome system and particularly the proteolytic activity of the proteasome is critical for promoting the exchange of transcriptional factors on chromatin and possibly facilitating multiple rounds of transcription initiation, hence controlling receptor mediated gene expression [6,10,11,18,19. In addition, a number of ubiquitin proteasome pathway enzymes, such as E6 associated protein (E6-AP) and the marine double minute-2 (Mdm2), have been identified as steroid receptor co-activator [reviewed by {Kinyamu, 2005 #388]. Furthermore, specific components of the proteasome, such as the 19S subunit, thyroid interacting protein 1 (TRIP1/Sug1) and the 20S beta subunit low molecular mass polypeptide 2 (LMP2) are implicated in receptor-mediated transcriptional regulation [20,21]. Consequently, receptor turnover is tightly linked to receptor-mediated transcription.

Two main observations led us to the current study. First, our laboratory and others showed that proteasome inhibitors, such as MG132, increase GR mediated transcriptional activation of the mouse mammary tumor virus promoter (MMTV) in breast cancer cells [22,23]. Secondly, other groups showed that proteasome inhibitors were inhibitory to nuclear receptor function particularly that of the ER [19,24]. These findings suggested that proteasome activity

differentially modulates gene transcription in a receptor dependent manner. This divergent role of the proteasome in receptor-dependent transcriptional regulation is not well understood. Since previous experiments suggesting a requirement for proteasome activity in ER, but not GR were conducted using specific model genes, we used microarray analysis to test the requirement for proteasome activity in the regulation of global gene expression mediated by these two receptors. Data from the global gene expression analysis show that inhibiting proteasome activity modulates gene expression mediated by GR and ER in a similar manner. Specifically, the requirement for proteasome activity is gene, but not receptor specific. Proteasome activity modulates receptor dependent genes involved in the etiology of a number of diseases, including leukemia, HIV/AIDS and neurodegenerative disorders. Intriguingly, proteasome inhibition modulates a subset of transcripts that encode factors that regulate RNA polymerase II and DNA/histone modifying enzymes. Our study provides a snapshot of global gene expression after proteasome inhibition in breast cancer cells treated with either dexamethasone or 17β-estradiol. These data provide a useful tool particularly since proteasome inhibitors are currently in clinical trials as potential therapeutics for various diseases.

Materials and Methods

Cell Culture

The generation of MCF-7 cells stably expressing the GR and endogenous ERα has been described previously [25]. Briefly, parental MCF-7 cells (American Type Culture Collection, Manassas, Va.) were co-transfected with pGR-NEO and a neomycin resistance plasmid, pRSV-NEO, using the calcium phosphate precipitation method (GIBCO-BRL Life Technologies, Grand Island, NY) [26]. The resulting cell line which expresses both GR and ER shows similar gene expression profiles in response to17β-estradiol compared to MCF-7 from other laboratories [27-29]. Similar to ER, the GR in MCF-7 cells activates known exogenous and endogenous GR target genes [25,30,31].

For the current study, cells were grown in a humidified incubator at 37°C with 5% CO_2 in MEM supplemented with 2 mM glutamine, 100 μg/mL penicillin/streptomycin, 10 mM HEPES, 10% FBS and 300 μg/mL G418. For glucocorticoid treatment, cells were seeded overnight in phenol red-free MEM supplemented with 5% charcoal-stripped calf serum and 2 mM glutamate. Cells treated with 17β-estradiol were cultured in MEM media with 5% charcoal stripped serum for 3 days and then seeded for experiments as described for microarray analysis.

Antibodies and Western Blotting

After washing twice with PBS, cells were pelleted by centrifugation. For whole cell extracts, cells were lysed as previously described [25]. Twenty to 50 μg of protein was resolved on 4-12 % SDS-PAGE and transferred to a PVDF membrane (Amersham). Proteins were immunoblotted using the following antibodies: anti-GR-BUGR2 (Dr. B. Gametchu, Medical College of Wisconsin, Milwaukee, WI), ERα-H-184 Santa Cruz Biotechnology, β-Actin (Sigma), GAPDH (Research Diagnostics Inc).

Gene Expression Profiling and Analysis

Gene expression analysis was performed using Agilent Human1A array (pattern id = 01152) (Agilent Technologies, Palo Alto, CA). Total RNA samples were prepared from two biological replicates of MCF-7 cells treated with vehicle, 1 nM dexamethasone or 10 nM 17β-estradiol (24 hr), 1 mM MG132 (24 hr) or MG132 and dexamethasone or 17β-estradiol (24 hr) using RNeasy Midi Kits (Invitrogen). Total RNA was labeled with Cyanine (Cy) 3- or Cy5-dCTP (Amersham, Piscataway, NJ) using the Agilent Fluorescent Direct Label Kit protocol with a slight modification in the starting amount (10 μg was used rather than 20 μg). Each RNA pair (vehicle and either dexamethasone, 17β-estradiol, MG132, MG132 and dexamethasone, or

17β-estradiol and dexamethasone) was mixed and hybridized to an array at two separate times employing fluor reversal. Hybridizations were performed for 17 hours in a rotating hybridization oven using the Agilent 60-mer oligo microarray processing protocol. Slides were washed as indicated in this protocol and then scanned with an Agilent Scanner.

Data were retrieved with the Agilent Feature Extraction software (v7.1), using defaults for all parameters, except the Ratio terms. To account for the use of the Direct Label protocol, error terms were changed as suggested by Agilent as follows: Cy5 multiplicative error = 0.15, Cy3 multiplicative error = 0.25, Cy5 additive error = 20, Cy3 additive error = 20. The Agilent Feature Extraction Software adjusted the data to account for additive and multiplicative noise in the array data acquisition process. The resulting ratio intensity value for each gene feature on the array was averaged across technical and biological replicates as follows: the log base 10 ratio values from all four arrays for each comparison [two biological replicates, each with a fluor reversal (technical replicate)] were averaged in the Rosetta Resolver® system (Rosetta Biosoftware, Kirkland, WA) using the error-weighted approach [32]. Briefly, letting $x(i)$ represent the ith log base 10 ratio value for a gene and $\sigma_x(i)$ the measurement error, the error-weighted average for a gene feature is

$$\bar{x}=\frac{\sum_i w(i)x(i)}{\sum_i w(i)}, \quad \text{where } w(i)=\frac{1}{\sigma_x^2}, \quad i=1{:}N, \text{ and } N \text{ is the number of replicates.}$$

A p-value for each gene feature is computed based upon the reproducibility of the expression measurements across the four arrays (biological and technical replicates). Gene features with $p < 0.001$ for a given comparison were considered significantly and differentially expressed.

Validation of microarray results by real-time RT-PCR

The microarray data trends were verified by examining a subset of representative classes of genes after treatment with hormone and proteasome inhibitor for 24 hr. To establish whether the genes were direct targets of the hormone or proteasome inhibitor, expression of select genes was monitored after treating the cells for 2 hr. Because MG132 is known to inhibit targets other than the 26S proteasome, expression of a subset of genes was also determined after a similar treatment with the highly specific proteasome inhibitor epoxomicin. After removing genomic DNA, total RNA (1-2 μg) from cells treated with the vehicle, hormone or the proteasome inhibitor (MG132 or epoxomicin) in the presence or absence of hormone were reverse transcribed using oligo-dt as described in the Superscript Kit (Invitrogen Corp.). The cDNA was treated with ribonuclease H (Invitrogen Corp.) to remove RNA:DNA hybrids. The cDNA was diluted 5-fold with DNAse-free water and used for real-time PCR analysis.

Real-Time PCR Analysis

cDNA levels were detected using the STRATAGENE, Mx3000P™ real time PCR system and SYBR Green I dye (STRATAGENE, Cedar Creek, TX). Primers were created using Applied Biosystems Primer Express Software version 2.0. For cDNA amplification, 2-5 μL of cDNA was combined with SYBR Green PCR mix as described by the manufacturer (STRATAGENE, Cedar Creek, TX). GAPDH mRNA expression was used as the endogenous control for normalization of initial RNA levels. Data is expressed as relative expression.

Chromosome Map

Genes that were found to be significant in Rosetta Resolver ($p < 0.001$) following treatment by MG132, MG132 + DEX, and MG132 + E2 were displayed in the Physical Position View for the Agilent Human 1A array (011521) in Agilent's GeneSpring GX software (version 7.3.1).

Microarray data accession number

The microarray data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE8383″ [33].

Results

Global transcriptional changes in glucocorticoid and estrogen receptor targets after proteasome inhibition

It is well known that cellular levels of steroid hormone receptors including those of the glucocorticoid (GR) and estrogen receptors (ER) are tightly regulated by proteosomal degradation. Consequently proteasome inhibition by widely used proteasome inhibitors such as MG132, block ligand dependent degradation and stabilize receptor levels (Figure 1A and 2A). However, previous studies using model reporter gene assays have shown that proteasome inhibition increased GR-mediated gene transcription, whereas ER-mediated gene transcription is decreased. Since receptor levels, should correlate with gene expression, the divergent effect of proteasome inhibition on gene expression mediated by the two receptors is not well understood. To examine the global role of proteasome activity, we turned to transcript profiling to provide genome wide view of gene expression in response to proteasome inhibitor and hormone in MCF-7 cells. We compared transcripts from RNA treated with vehicle (Con) vs. dexamethasone (D or DEX) or 17β-estradiol (E2) vs. those treated with proteasome inhibitor MG132 (MG) vs. MG132 plus dexamethasone (MD) or MG132 plus 17β-estradiol (ME2). Those genes differentially expressed were clustered and displayed in dendograms (Figure 1B and 2B). In all figures MD designates treatment with MG132 and dexamethasone (D), whereas ME2 designates treatment with MG132 and 17β-estradiol (E2).

Proteasome inhibition has a synergistic and antagonistic effect on glucocorticoid-induced gene expression—In the first set of analysis we concentrated on genes affected by treatment with DEX alone or with DEX and MG. Clustering analysis revealed 4 broad transcript categories. The first category represents genes affected by glucocorticoid treatment only. Of the over 20,000 genes on the Agilent human 1a array, 268 genes were up-regulated and 118 down-regulated when cells were treated with DEX alone (Figure 1B and C). In the second category, 209 genes (131 + 78) were similarly affected by DEX and MG treatment (Figure 1C); of these 131 genes were up-regulated and 78 were down-regulated. In a third category, although 48 transcripts were affected in common by DEX and MG, the effect of the treatment on a specific gene was antagonistic; e.g., treatment with MG blocked DEX induction or repression of the gene (Figure 1C). A fourth category consisting of a total of 2945 genes that were affected when cells were treated with MG and DEX in a hormone independent manner, 1290 and 1655 gene transcripts were increased and decreased, respectively. We further explored the transcripts in the 3 categories where the hormone response is affected by proteasome inhibition. Genes from the fourth category are primarily affected by proteasome inhibition and are discussed in section 3. It is important to note that transcript profiling resulting in microarray analysis, as carried out in this study, only deciphers ‘relative’ changes among genes and not genome wide gene expression. While validation of all the genes identified was not practical, we chose a representative sample that was subsequently analyzed by quantitative RT-PCR to verify the microarray trends.

Among the genes in the first category affected by DEX alone were *bona fide* GR targets. These include 11-β-hydroxysteroid dehydrogenase type 2 (HSD11β2), msh homeobox homolog 2 (MSX2), dual specificity phosphatase 6 (DUSP6) and sin 3A associated protein (SAP 30) (Figure 1D and Table 1-1). Some genes known to be repressed by GR like neurturin (NRTN), adhesion molecule with Ig like domain 1 (Amigo1), heterogeneous nuclear ribonucleoprotein

A2/B1 (HNRPA2B1) and melanoma antigen family D4 (MAGED4) were down-regulated by DEX alone (Figure 1D, Table 1-1). HSD11β2 is a well established target of GR mediated activation. As predicted from the microarray analysis, treatment with DEX (D) for 24 hr increases HSD11β2 expression over 100-fold (Figure 1D, 24hr), whereas treatment with MG132 alone (MG) or with dexamethasone (MD) had no significant effect HSD11β2 expression compared to control. Furthermore, the HSD11β2 mRNA expression increased (6-fold) within 2 hr after dexamethasone treatment, indicating direct regulation of this gene by the GR (Figure 1D, 2hr). In a similar manner, treatment with dexamethasone decreased NTRN expression by 90% compared to control as predicted from microarray analysis (Figure 1D, 24hr). Compared to DEX treatment, treatment with proteasome inhibitor did not significantly affect NTRN expression, suggesting DEX-dependent repression of this gene at 24hr. This repression was not detected at an earlier time point in which DEX treatment increased NTRN expression 2-fold (Figure 1D, 2hr). Notably, treatment with proteasome inhibitor does not significantly changed NTRN expression compared to DEX.

The second category of transcripts was synergistically altered by MG and DEX (Figure 1E, Table 1-2). As demonstrated previously for model genes *in vitro*, proteasome inhibition enhanced glucocorticoid-mediated gene expression [22,23]. Similar to the effect observed with MMTV-LUC and CAT reporter gene, proteasome inhibition enhanced expression of some well characterized GR target genes [34-38]. These include S100 calcium binding protein (S100P), regulator of G protein signaling (RGS2) also known as G0S8, RNA Pol II elongation factor 2 (ELL2) and dual specificity phosphatase 1 (DUSP1) (Figure 1E, Table 1-2). Among the genes in this category were genes not previously shown to be glucocorticoid inducible, such as alpha B crystallin (CRYAB) and N-Myc downstream regulated gene 1 (NDRG1) which are mildly activated by DEX, but highly up-regulated after proteasome inhibition. Other genes in this category include collagen type VI, alpha 1 (COL6A1), musculoaponeurotic fibrosarcoma oncogene B (MAFB) and annexin 1 (ANXA1) (Figure 1E, Table 1-2). For this class of genes we validated expression of S100 P after treatment with DEX (D) or inhibitor and DEX (MG, MD). At 24 hr, treatment with DEX (D) increased S100P expression by 30-fold, MG alone was not significantly different from control. Treatment with MG and DEX (MD) synergistically increased S100P expression 120-fold, an effect significantly larger than the sum of the individual effect of hormone or inhibitor alone. (Figure 1E-24hr). A similar effect is observed when the cells were treated with DEX or MG for 2 hrs. DEX induced S100P expression 3-fold at early time points and this effect was potentiated by proteasome inhibition (6-fold) (Figure 1E- 2hr).

Conversely, proteasome inhibition facilitates glucocorticoid-mediated repression as seen for the GR target adhesion molecule with an Ig-like domain 2 (AMIGO2), 2-5-oligoadenylate synthetase 2 (OAS2), interferon-responsive protein 28 or receptor transporting protein 4 (RTP4/IFRG28), androgen-induced basic leucine zipper (AIBZIP/CREB3L4), neuronal cell adhesion molecule (NCAM2) and other transcripts, such as fasciculation and elongation protein zeta 1 (FEZ1) and hedgehog acyltransferase (HHAT) and transforming growth factor beta 3 (TGFB3) (Figure 1E, Table 1-2). Expression of TGFB3 was validated as an example of those genes repressed. At 24 hr, treatment with DEX (D) decreased TGFB3 expression by 50 percent. Treatment with MG and DEX (MD) synergistically decreased TFGB3 expression by over 90%, an effect significantly larger than the sum of the individual effect of hormone or inhibitor alone. (Figure 1E-24hr). Significant TGFB3 repression did not occur at shorter time points under these experimental conditions, although a trend to decrease was observed (Figure 1E- 2hr).

For the third category, treatment with either proteasome inhibitor or hormone had an antagonistic effect on gene expression. An antagonistic response was viewed as one where the inhibitor blocks hormone induction or repression of a transcript and vise versa. This third

category of genes was different from that described in Figure 1D (Table 1-1). In the first category, the hormone exerts its main effect on gene expression, whereas in the third category the hormone or proteasome inhibitor have an independent effect on gene expression, which is reversed in the opposite manner by either agent; i.e. antagonism. Proteasome inhibition attenuates DEX induction of a number of *bona fide* GR targets including, galanin (GAL), baculoviral IAP repeat-containing 3 (BIRC3) and B-Cell CLL/lymphoma 6 (BCL6) (Figure 1F, Table 1-3). For some genes DEX-induced changes in the levels of certain transcripts, but these transcripts were completely repressed by proteasome inhibition. These included transcripts for calcium binding protein A8 (S100A8), prolactin inducible protein (PIP), TAR (HIV) RNA binding protein (TARBP1) and transcripts encoding interferon genes IFIH1 and IFIT2 (Figure 1F, Table 1-3). The results from the microarray analysis were confirmed by RTPCR using GAL and IFIT2 as a representative gene for this class (Figure 1F). GAL expression increased 26-fold after treatment with DEX (D) for 24 hr, and this effect was reduced 7-fold by MG, which was very similar to microarray analysis (Table 1-3). A short time treatment with DEX induced GAL expression only 2-fold, and proteasome inhibition did not affect this induction, suggesting an indirect effect of inhibitor observed at 24 hr. A second example of antagonistic response was detected when DEX-mediated repression was abrogated by proteasome inhibition. Treatment with dexamethasone reduced IFIT2 expression by 85%, whereas treatment with MG alone increased IFIT2 expression 4-fold compared to control (Figure 1F). Co-treatment with dexamethasone and inhibitor reversed DEX-mediated repression by 8-fold as predicted by microarray analysis (Table 1-3). A short treatment time with DEX decreased IFIT2 expression by 60% with a smaller but consistent effect of the proteasome inhibitor compared to 24 hr treatment (Figure 1F-2hr).

Because MG132 has targets other than the 26S proteasome, we validated a select number of gene targets after treatment with a second proteasome inhibitor, epoxomicin. Gene expression profiles for HSD11B2, S100P and GAL following epoxomicin exposure were similar to those observed after MG132 treatment (Figure S1 A-C).

Proteasome inhibition has a synergistic and antagonistic effect on estrogen response

Previous studies suggested that proteasome inhibition repressed ER-mediated gene expression [19,24]. We therefore examined the effect of proteasome inhibition on estrogen response (Figure 2B, Table 2-1-4). We compared transcripts treated with E2 to those from cells treated with MG alone or MG plus E2. Genes were classified into 4 categories as carried out for the glucocorticoid response. The first category of genes was specifically altered by E2 treatment; 272 transcripts were up-regulated and 126 down-regulated, respectively (Figure 2C). Among those transcripts up-regulated by E2 were *bona fide* ER targets including early growth response 3 (EGR3), retinoblastoma binding protein 8 (RBBP8) and low density lipoprotein receptor related 8 (LRP8) (Figure 2D, Table 2-1). Transcripts repressed included grainyhead like protein 1 (GRHL1) or leader-binding protein 32 (LBP-32), transcripts encoding histone H2A (H2AFA) and H2B (H2BFQ) (Figure 2 D, Table 2-1). EGR3 is a well established target of ER. As predicted from the microarray analysis, treatment with E2 for 24 hr increased EGR3 expression 65-fold (Figure 2 D, 24hr), whereas treatment with MG132 alone (MG) led to a significant increase in expression compared to control. However, co-administration of drug and hormone (ME2) resulted in a smaller increase than seen with E2 alone. EGR3 mRNA expression increased (52-fold) within 2 hr after E2 and the inhibitor had no significant effect alone (MG) or on the ER-mediated induction (ME2), confirming EGR3 is primarily an ER target gene (Figure 2D, 2hr), In contrast to EGR3, LBP-32 was repressed (70%) by E2 at both time points (Figure 2D). Treatment with MG132 alone or with MG132 and E2 did not lead to a significant change in expression compared to control or E2.

The second category of genes were those synergistically up-regulated (66) or down-regulated (122) by treatment with MG and E2 (Figure 2E, Table 2-2). Among ER targets up-regulated after E2 and MG treatment was a GTP binding protein over expressed in skeletal muscle (GEM), tubulin beta 2 (TUBB2A), DEAD (Asp-Glu-Ala-Asp) box polypeptide 10 (DDX10) and cofilin 2 (CFL2). Proteasome inhibition also synergistically repressed ER targets including the well characterized ER target, thioredoxin interacting protein (TXNIP), calcium/calmodulin dependent kinase II inhibitor 1 (CANK2N1), SRY (sex determining region Y) box 13 (Sox 13), neuronal cell adhesion molecule (NCAM2), cadherin 10 type 2 (CDH10) CREB3L4/AIBZIP, AMIGO2 and S100 A8 (Figure 2E, Table 2-2). For this class of genes DDX10 and AMIGO2 expression were validated as representative genes. Treatment with E2 or inhibitor MG and E2 (MG, ME2) for 24 hr increased DDX10 expression by 2-fold; MG alone was only 6-fold. Treatment with MG and E2 (ME2) increases DDX10 expression 7.5-fold (Figure 2E-24hr). The synergistic action of proteasome inhibition of E2-mediated increase in DDX10 expression was more evident at 2 hr, whereas treatment with E2 induced DDX10 (13-fold) and treatment with MG and E2 led to a 26-fold induction (Figure 2E-2hr). As an additional positive control, we observed that proteasome inhibition increased E2 induction of pS2, a known ER target gene (Figure S2 A-B).

In the third category, as shown for the glucocorticoid response, proteasome inhibition antagonized the effects of estrogen response. Proteasome inhibition abrogated the effect of E2 on amphiregulin (AREG), epiregulin (EREG) and retinol binding protein 7 (RBP7) (Figure 2F, Table 2-3). A classic example of the previously reported repression of proteasome inhibition on ER-mediated regulation is the effect on the progesterone receptor (PGR), which is increased by E2, but repressed by MG (validation data not shown). Additionally, other ER targets including stromal derived factor 1 (SDF-1/CXCL12), collagen, type XII, alpha 1 (COL12A1), minichromosome maintenance deficient 6 (MCM6), DNA (cytosine-5) methyltransferase 1 (DNMT1) are induced by E2, but significantly repressed by MG (Figure 2F, Table 2-3). Other targets were repressed by E2, but up-regulated by proteasome inhibition (Figure 2F, Table 2-3). These included the lipocalin-2 (LCN2), a putative *in vivo* estrogen target gene and paracrine factor that mediates the growth regulatory effects of estrogen in normal breast epithelium. Additionally, tribbles homolog 3 (TRIB3), a negative regulator of NF-kappaB, interferon–induced protein with tetrapeptide repeats 2 (IFIT2) and sel-1-suppressor of lin-12 like (SEL1L), which plays a role in pancreatic carcinoma and breast cancer (Figure 2F, Table 2-3). There were also transcripts repressed by E2, but the repression dampened by proteasome inhibition, for example the immunoglobin-like domain counter receptor 1 (ILDR1) (Figure 2F, Table 2-3). Expression of SDF-1 was validated as example a gene that was activated by E2, but repressed by inhibitor (Figure 2F). SDF-1 expression increased 12-fold after treatment with E2 for 24 hr, and this effect is inhibited 3-fold by MG, very similar to what was observed in the microarray analysis (Figure 2F-24 hr, Table 2-3). SDF-1 is a direct target of ER and a short treatment time with E2 induces SDF-1 expression 8-fold. The impact of proteasome inhibition is observed at 24 hr suggesting an indirect effect of the inhibitor (Figure 2F-2 hr). In another characteristic antagonism, treatment with E2 for 24 hr decreased expression 30%, whereas treatment with MG alone increased IFIT2 expression 4-fold compared to control (Figure 1F). Co-treatment with E2 and inhibitor reversed E2-mediated repression, thereby increasing IFT2 expression by 7-fold, which was similar to that observed in microarray analysis (Table 2-3). A short treatment time with E2 induced IFIT2 repression by 30% with a smaller, but consistent antagonistic effect of the proteasome inhibitor (Figure 2F-2 hr). Interestingly, the effect of proteasome inhibition on ER-mediated induction and repression of SDF-1 and IFIT2, respectively, was very similar to that observed for the GR targets GAL and IFIT2 (Figure 1F). Furthermore IFIT2 is a target of both hormones and proteasome inhibition has similar inhibition effect on DEX and E2 mediated repression (Figure 1F and 2F). This observation solidifies the idea that the two receptors behave in a similar manner when the proteasome is inhibited. We further show that proteasome inhibition by

epoxomicin on ER-dependent gene expression is similar to that observed with MG132 treatment (Figure S3, A-C).

Specific effect of proteasome inhibitor on gene expression—The fourth category of genes represents those primarily affected by proteasome inhibition (MG). The transcripts activated in this class presumably do not require proteasome activity, while it may be required for the repressed transcripts. Some genes in this category were not significantly changed by either hormone acting alone, but significant changes in gene expression were observed after treatment with proteasome inhibitor and hormone. To pinpoint transcripts only affected by MG, we compared transcripts from MG alone with those affected by MG plus DEX or MG plus E2 (Figure 3A). A total of 583 genes were altered by MG alone. Of these genes, 294 were up-regulated and 289 down-regulated. Among the specific genes increased by proteasome inhibitor exclusively were replication factor C1 (activator 1) (RFC1), 5-azacystidine induced gene 2 (AZI2), proteasome subunits PSMB1 and PSMD12, CD44, DNA damage inducible beta GADD45B, p300/CBP associated factor (PCAF), SET and MYD domain containing (SMYD1), and TAF7 RNA polymerase II TATA box binding protein (TAF7). A number of transcripts were repressed by proteasome inhibition, including breast cancer 1 (BRCA1), jumonji containing 2D (JMJD2D) and jumonji AT rich interactive domain 2 (JARID2) (Figure 3B, Table 3-1).

A total of 913 transcripts were changed by MG and DEX, 487 up-regulated and 426 down-regulated. Key transcripts regulated in this manner are heat shock protein 70 (HSPA6), Kruppel-like factor 6 (KLF6) also known as core promoter element binding protein (COPEB), activating transcription factor 3 (ATF3), growth differentiation factor 15 (GDF15) also known as placental bone morphogenetic protein (PLAB) or nonsteroidal anti-inflammatory drug-activated gene (NAG-1), myeloid/lympoid or mixed lineage leukemia translocation 11 (AF1Q), GTP binding protein or gene expressed in mitogen stimulated T cells (GEM), and DNA damage inducible transcript 1 (GADD45A) (Figure 3C, Table 3-2). Conversely, some transcripts were repressed by MG plus DEX, including chloride intracellular channel 3 (CLIC3), lin-28 homolog of C elegans (lin 28), interferon induced transmembrane protein 2 (IFITM2), SOX 13, nuclear receptor type 1 (COUPTF11), S100 calcium binding protein A4 (S100A4) and transcription elongation factor A (SII) 2 and 3 (TCEA2 and 3). The microarray analyses were confirmed by RT-PCR of a representative genes, HSPA6 and S100A4 (Figure 3C). Treatment with proteasome inhibitor alone induced HSPA6 gene expression at both 2 hr and 24 hr, indicating HSPA6 is a direct target of proteasome inhibitor. Conversely, treatment with proteasome inhibitor results in the repression of S100A4 transcript at 24 hr, but not at 2 hr suggesting the effect of inhibitor on S100A4 gene is mediated in the long term (Figure 3C). To verify the effect of the inhibitor we demonstrated that treatment with epoxomicin increased expression of HSPA6 (Figure S1-D).

A total of 618 genes were altered by MG and E2, 290 were up-regulated and 328 down-regulated. The key transcripts activated by MG and E2 were HSPA6, KLF6/COPEB, ATF3, GDF15, AF1Q and GADD45A. Some transcripts were repressed by MG and E2, including CLIC3, lin 28, IFITM2, SOX 13, NR2F1 and 2, S100A4, TCEA2 and 3, zinc finger protein 467 (ZNF467), solute carrier family 40 (SLC40A1) and prolactin induced protein (PIP). Most these genes are also changed by MG and DEX; however, a number were specifically changed after treating with MG plus E2, including dehydrogenase/reductase (SDR family) member 10 (DHRS10), DNA damage inducible transcript 3 (DDIT3), DEAD (Asp-Glu-Ala-Asp) box polypeptide 43 (DDX43) and interleukin 8 (IL8) (Figure 3 D, Table 3-3). The microarray analyses were confirmed by RT-PCR of representative genes, ATF3 and Lin 28 (Figure 3D). Treatment with proteasome inhibitor alone induces ATF3 gene expression at both time points, indicating ATF3 is a direct target of proteasome inhibitor, but not E2. Treatment with proteasome inhibitor leads to decreased expression of Lin28 at 2 hr and 24 hr (Figure 3D). E2

alone, independent of inhibitor, led to a diminution in Lin 28 after 2 hr treatment (a result to be further investigated). For each category of genes the effect of the proteasome inhibitor on gene expression was verified by gene expression after treating with epoxomicin (Figure S3-D).

Approximately 1700 genes were common between MG plus DEX and MG plus E2, 699 transcripts up-regulated and 988 repressed, whereas 10 genes were differentially expressed. Common activated genes include CRYAB, NDRG1, GADD45A, DUSP1, KLF6/COPEB, HSPA6, GEM, TUBB2A, ATF3 and AF1Q; and examples of genes repressed include S100A8, COL12A1, CLIC3, AMIGO2, NR2F1, NCAM2, cAMP responsive element binding protein 3-like 4 (CREB3L4/AIBZIP), PIP, CXXC finger 4 (CXXC4/IDAX), SOX13 and lin 28 (Figure 3E, Table 3-4). The microarray analyses were confirmed by RT-PCR of a representative gene, CRYAB (Figure 3E). Treatment with proteasome inhibitor alone induces CRYAB gene expression at both 2 hr and 24 hr, indicating CRYAB is a direct target of proteasome inhibitor, but not DEX; however, treatment with DEX and MG132 highly induced CRYAB (Figure 3-MD). In contrast to DEX, treatment with E2 and inhibitor did not affect CRYAB expression (Figure 3E- ME2). In addition, prolactin-induced protein (another gene in this class) is repressed by inhibitor alone and with hormone (Figure 3E-PIP). The observation that CRYAB expression increases after treatment with proteasome inhibitor was confirmed after treatment with another inhibitor, epoxomicin (Figure S3-E).

Proteasome inhibition modulates transcripts encoding RNA polymerase II transcriptional regulators—To better understand the biological and molecular functions of the transcripts regulated after proteasome inhibition and hormone, we performed gene ontology classification. The analysis revealed that many of the transcripts changed after proteasome inhibition and hormone are characteristic of genes involved in transcription and transcription factor activity (Figure 4). Apart from transcripts encoding transcription factors, such as ATF3 and zinc finger-binding proteins, two prominent classes of transcripts emerged from further analysis. These included transcripts encoding factors that drive RNA polymerase II transcription and modify chromatin. Among transcripts changed by proteasome inhibitor that regulate RNA polymerase II transcription included PTEFb complex Cdk9 and cyclin K that regulates RNA polymerase carboxy-terminus phosphorylation. We note that treatment with DEX alone repressed CDK9 transcript, but treatment with MG and DEX increased Cdk9, whereas the treatment with E2 increased CDK9 transcript (2-fold) and MG plus E2 decreased Cdk9 transcript (Figure 4C). Transcripts encoding carboxy terminus phosphatase (CTD) including SSU72, CTDSP1 and CTDSPL were repressed by proteasome inhibition except CTDP1 (FCP1), which increased with proteasome inhibition (Figure 4C, Table 4-1).

Proteasome inhibition had significant effects on other RNA polymerase II regulators. Transcripts that encode the TATA box binding protein (TBP)-associated factors, TAF10 and TAF1B (TAFI63) were repressed by proteasome inhibition, whereas TAF1A, TAF2, TAF7, TAF9 and TAF 13 increased with proteasome inhibition (Figure 4C, Table 4-2). Transcripts that encode mediator subunits, MED10, MED28 and MED6 increased with proteasome inhibition (Figure 4C, Table 4-1). Genes that regulate the elongation rate of RNA polymerase II, RNA polymerase II elongation factor 2 (ELL2), which is also a GR target,, ELL and cell division cycle 73 (CDC73/PAF1) increased, whereas RNA polymerase II elongation factor-like 3 (ELL3) decreased.

Further analysis showed that proteasome inhibition had a substantial effect on transcripts encoding transcription elongation and translation initiation factors (Figure 4D, Table 4B). Transcription elongation factor A (SII) (TCEA) factors were all repressed by proteasome inhibition. MG plus DEX significantly decreased transcription elongation factor A (SII) like 1 (TCEAL1) and TCEAL4, while TCEA1 remained unchanged (Figure 4D). Proteasome

inhibition alone or in addition to either dexamethasone or E2 significantly repressed TCEA2, TCEA3, TCEAL8 and TCEAL5. A number of transcripts encoding eukaryotic translation factors were significantly increased by proteasome inhibition including EIF1, EIF1B, EIF2A and EIF2C3 (Argonaute3), whereas those transcripts that encode negative regulators of the translation factors, such as eukaryotic translation initiation factor 2- alpha kinase (EIF2AK2) an interferon induced kinase that phosphorylates EIF2A and eukaryotic translation initiation factor 4E binding protein 2 (EIF4EBP2) a protein that binds to EIFE to inhibit protein translation, are repressed by proteasome inhibition (Figure 4D, Table 4-2).

Proteasome inhibition modulates expression of chromatin regulators including histone and DNA modifying enzymes—Proteasome inhibition alters transcripts encoding enzymes or factors that modify DNA and histones. Nuclear receptors utilize a number of coregulators to modulate transcription. To date the best characterized histone modifying enzymes are those that mediate histone acetylation (HATs) and de-acetylation (HDACs), activating and repressing transcription, respectively. Proteasome inhibition increased some common nuclear receptor coactivators including NCOA6 also known as activating signal cointegrator (ASC2), NCOA7 also known as estrogen receptor activation protein 140 (ERAP140), thyroid interacting protein 4 (TRIP4) also known as ASC-1 and TRIP12. Conversely transcripts encoding co-repressors were decreased by proteasome inhibition including nuclear receptor co-repressor 2 (NCOR2 or SMRT) and histone deacetylases, HDAC1 and 8, although HDAC3 transcript was significantly increased when proteasome is inhibited in the presence of dexamethasone. Most strikingly, sin 3A associated protein (SAP30) is induced by DEX, but inhibited by MG alone and in the presence of DEX (Figure 4E and Table 4-3).

Apart from acetylation and deacetylation of histone N-terminal tails, another modification gaining interest with respect to gene regulation by a nuclear receptor is histone methylation. Examination of transcripts changes by proteasome inhibition revealed a number of histone methyltransferases and recently discovered demethylases were altered by proteasome inhibition. Transcripts encoding histone methyltransferases particularly associated with histone H3-Lysine 4 were increased by proteasome inhibition, including MLL and MLL translocation partners namely, MLLT2/AFF1/AF4/FMR2, MLLT11/AF1Q, SETD1A and SMYD1. Transcripts encoding other MLL translocation partners, MLLT3/AF9 and MLLT1/ENL decreased (Figure 4F and Table 4-4). Transcripts encoding histone methyltransferases specific for histone H3-lysine 9, euchromatin-lysine N-methyltransferase 1 (EHMT1 or G9 like protein, GLP) and EHMT2 (G9a), and the testis specific H3K9 methyltransferase SUV39H2 decreased, whereas the KAP-1 associating SET domain bifurcated 1 also known as ERG associated protein (ESET) increased after proteasome inhibition. Of note, EHMT1 increased by DEX, but repressed by MG and DEX, whereas SETDB1 is repressed by E2, but increased after MG and E2. In addition proteasome inhibition alters transcripts encoding methyltransferases targeting histone H3 lysine 36. These include Wolf-Hirschhorn syndrome candidate 1 (WHSC1) also known as multiple myeloma SET domain protein (MMSET) or nuclear SET domain-containing protein 2 (NSD2), Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1/NSD3) and SMYD2 which decreased by proteasome inhibition. In a number of cases the hormone component is involved, for example SMYD2 increased by hormone but decreased by proteasome inhibition. Transcripts encoding recently identified Jumonji-containing histone demethylases were also affected by proteasome inhibition including JARID2, JMJD2D and RBP2, which were repressed by proteasome inhibition whereas JMD1A transcript increased (Figure 4F and Table 4-4).

Protein arginine methylation has an important role in hormone regulated transcription [39] Proteasome inhibition alters expression of protein arginine methyltransferases (PRMT), including PRMT3 a ribosomal protein arginine methyltransferase that regulates ribosome

biosynthesis, PRMT8 a membrane-associated and tissue-specific arginine methyltransferase and PRMT6 a methyltransferase shown to possess auto-methylation activity and methylated the non-histone chromatin protein HMGA1 (Figure 4F, Table 4-4). Finally DNA methyltransferase, DNMT1, DNMT3B and 3L were significantly repressed by proteasome inhibition (Figure 4F, Table 4-4).

Among chromatin factors that are affected by proteasome inhibition were transcripts encoding various histone proteins. The major histone transcripts affected were those encoding histone H2A and H2B family members. These family members were all decreased by proteasome inhibition (Figure 4G, Table 4-5). Transcripts for histone H2AFL, H2AFY2, H2AFA, H2BFF, H2BFD, H2BFH, H2BFQ, H2BFE, H2BFB and H2BFK were repressed 2- to 4-fold by proteasome inhibition. Interestingly, histone H2AFY2 increased by E2 was inhibited by proteasome inhibition. Histone H2BFQ is highly down-regulated by E2, but this effect is reversed by proteasome inhibition. Variants of histone H3, H3FT and H3F1 were also down-regulated by proteasome inhibition. Histone H1F4 (H1.2), which is predicted to maintain low methylation state, was repressed up to 4-fold. Histone H1F0 (H1.0) was up-regulated by DEX, but repressed by MG and DEX (Figure 4G, Table 4-5).

Effect of proteasome inhibition on transcription of developmental genes, proteasome subunits and stress proteins—Because there were very significant changes in transcripts encoding MLL and MLL translocation partners, we investigated whether transcripts encoding clustered homeobox (Hox) genes were affected by proteasome inhibition. Knockout experiments have previously identified Hox genes as targets of MLL. Of the transcripts encoding HOX genes, HOXA1 which was down regulated by hormone alone (DEX or E2) was highly up-regulated by MG and either hormone. Other Hox genes were down-regulated by proteasome inhibition including those of HOXC8, HOXA10, HOX D9, B2, C13 and C9 (Figure 5A, Table 5-1).

Analysis of transcripts regulated by proteasome inhibition showed an increase in transcripts encoding lin-7 homolog A and C (Lin7A and C), but a decrease in Lin 7B was seen. Lin-28 was highly repressed by proteasome inhibition, where as sel-1 suppressor of lin-12-like increased by proteasome inhibition (Figure 5A, Table 5-1).

Among other targets of the proteasome are the proteasome subunits themselves. Our transcript profiling analysis shows that proteasome inhibition up-regulated 19S proteasome ATPase subunits PSMC1, -4, -5, and -6, but not PSMC2 and non-ATPase subunits, PSMD1, -2, -8, -9, -11, -12 and -14. Proteasome inhibition also increases transcripts encoding the 20S subunits, alpha subunits PSMA1, -3, -4, -5, and -7 and beta subunits 1, 2,3,4,5, 6 and 7. On the other hand, proteasome inhibition repressed transcripts encoding antigen presenting, immunoassembly proteasomes PSMB10, PSME1 and -2 (Figure 5B, Table 5-2).

Previous studies have shown that proteasome inhibition increased stress response factors, particularly heat shock proteins. Proteasome inhibition induced a global increase heat shock protein transcripts, including hsp90, -70 and -40 families. These changes are among the most pronounced changes of proteasome inhibition; for example, proteasome inhibition induced HSPA6 transcript (Hsp70B) up to 40-fold and DNAJB1 (Hsp40, subfamily B) up to 14-fold, whereas another member of this family DNAJC19 (Hsp40, subfamily C) is repressed (Figure 5C, Table 5-3).

Proteasome inhibition affects transcription of genes associated in the pathogenesis of neurodegenerative diseases, leukemia, multiple myeloma, breast/prostate cancer and HIV/AIDS—Proteasome inhibitors, such as bortezomib, are currently in clinical trials as potential therapeutic agents. In particular, protein inhibitors plus

DEX have been used to treat relapsed multiple myeloma. Using a chromosome tool, we aligned the 1697 genes affected in common by MG, MG plus DEX and MG plus E2 to chromosome loci (Figure 6, see also Figure 3A). Examination of chromosome loci showed specific clustering of genes or hot spots on chromosomes 1, 6, 11, 19 and on the X chromosome. Genes clustered on the hot spots marked in a black line on the specific chromosome are associated with leukemia, Kaposi sarcoma, severe combined immunodeficiency, non-Hodgkin's B-cell lymphoma, acute myeloid leukemia, breast cancer and Sjogren syndrome antigen among other diseases. Genes clustered in chromosome 19 encode a number of zinc finger proteins. This observation is interesting, considering that 50% of all human KRAB-ZNF genes are located on chromosome 19 and recent data shows that the specific domain harboring these genes is heterochromatic and marked by elevated binding of heterochromatin protein 1 (HP1) [40].

Discussion

A number of studies indicated that inhibiting proteasome degradation increased transcriptional activity of some, but not all nuclear receptors suggesting a receptor specific effect of proteasome inhibition [19,22,24,41,42]. Specifically blocking proteasome degradation with the proteasome inhibitor MG132 elevated GR, but diminished ER-mediated gene activation, suggesting that proteasome degradation is required for transactivation at least by the estrogen receptor [19,22-24]. However, these studies were based on either reporter gene constructs or limited individual receptor target genes [19,22-24]. We have taken a genomic approach to show that the requirement for proteasome activity is gene specific rather than receptor specific. Our data provides new information indicating that proteasome inhibition has both synergistic and antagonistic effects on GR and ER-mediated gene expression. Proteasome inhibition enhances GR-mediated gene expression of endogenous targets (S100P), but other known GR targets like galanin, BCL6 and TGFB3 are repressed [35-38].

We confirm previous reports that proteasome inhibition decreases E2-mediated progesterone receptor gene expression, but also show that E2 targets, such as DDX10, are synergistically induced by E2 and a proteasome inhibitor, whereas TXNIP, SOX13 and IFIT2 were synergistically repressed.

Gene expression profiles observed in this study are similar to those reported by others in MCF-7 cells treated with E2 [27-29]. With respect to the GR response, the gene profiling signature from the GR/ER positive MCF-7 cell line is similar to that observed in other cell lines in response to dexamethasone [35-38].

Present analysis suggests some negative cross-talk between GR and ER [25]. A number of gene transcripts are differentially regulated by GR and ER, when proteasome activity is inhibited. For example, the gene NDRG1 is activated by DEX and MG, but repressed by E2 and inhibitor. A similar trend follows for KLF6, SMYD2 and S100A8 genes. NDRG1 is markedly expressed in the placenta and it is the most ubiquitous member of the NDRG family genes (NDRG 1-4) [43]. Over expression of NDRG1 in colon, breast or prostate cell lines decreases proliferation rate, enhances differentiation and suppresses the metastatic potency of the tumor [44,45]. KLF6 or core promoter element binding protein is a Krüppel family of C2H2-type zinc finger protein involved in regulation and maintenance of the basal expression of TATA box-less genes. It is highly expressed in the placenta [46]. KLF6 is an inhibitor of cell proliferation, suggesting a role of KLF6 as a potential tumor suppressor [47]. SYMD2 has a role in cell proliferation since it was shown recently to methylate p53 [45,47,48]; S100 A8 is strongly up-regulated only in ductal carcinoma *in situ* [49]. For these genes, repression by E2 favors cell proliferation, whereas activation by DEX inhibits proliferation. It is of particular interest that some the genes differentially expressed after proteasome inhibition and hormone treatment are highly expressed in various type of breast tumors [50-52]. Proteasome inhibitors

are currently applied in the therapy of hormone responsive cancers; however, the negative crosstalk between GR and ER can influence the outcome of therapeutic application.

A novel finding from the genomic profiling is the regulation of transcripts encoding genes for RNA polymerase II transcriptional regulators (transcription elongation/translation initiation factors) and chromatin modifying enzymes (DNA and histone methyltransferases/histone demethylases/acetyltransferases/deacetylases). The profound impact of proteasome inhibition on transcriptional regulators suggests that proteasome activity can regulate transcription at multiple steps, initiation, elongation and even mRNA processing. Key molecules, such as TAFs, mediator subunits and KLF6 that impact transcriptional initiation/activation and confer gene specific activation, are altered by proteasome inhibition. TAFs and KLF6 play a role in regulation of TATA less promoters [46,53]. Perhaps these factors can account for differential regulation of receptor target genes after proteasome inhibition.

Additionally, proteasome inhibition alters transcripts encoding RNA polymerase II CTD phosphatases and transcriptional elongation factors (TCEA (SII), ELL). These factors can enhance or repress RNA Pol II elongation rate, supporting a role of the proteasome in transcriptional elongation. We have reported recently that proteasome activity at least in part regulates transcription by modulating the phosphorylation of RNA polymerase II, a hallmark of the elongating polymerase [31]. Other gene transcripts, such as transcriptional translation initiation factors and genes regulated by micro-RNAs (Lin 28, Lin 7), suggest proteasome activity might be required in the regulation of mRNA processing and translation [54-56].

DNA methylation and histone modifications have crucial roles in the control of gene activity. Changes in expression of enzymes that modify DNA or histones after proteasome inhibition can impact on gene expression. Proteasome inhibition alters expression of transcripts that encode DNA methyltransferases (DNMT1, 3L and 3B). DNA methylation is normally associated with gene silencing, but also provides multiple layers of gene control; for example, tissue specific gene expression. Proteasome activity may impact on genes tightly regulated by DNA methylation: for example, the melanoma antigen (MAGE) family of cancer testis genes and the S100 calcium binding protein A4 (S100A4), which is over-expressed in colon cancer, are tightly regulated by DNA methylation and in this study they are altered by proteasome inhibition [57,58](Figure 1C and 1 F).

Another level of transcriptional regulation by proteasome activity can be achieved by modification of chromatin architecture. Several gene transcripts encoding histone proteins and histone modifying enzymes are changed after proteasome inhibition. Histones are no longer considered to be simple DNA-packaging proteins: they are recognized as dynamic regulators of chromatin architecture and gene transcription. In this study we found changes in transcripts encoding specific histones and histone variants, providing an opportunity for proteasome activity in the regulation of chromatin architecture. We demonstrated that the H1.2 (H1F4) isoform, which is proposed to maintain low DNA methylation state, is significantly repressed by proteasome inhibition. In mammals, histone H1 is expressed in at least 8 isoforms. Though we do not know the direct effect of this isoform on receptor mediated-transcription, we have previously showed that prolonged DEX treatment effectively dephosphorylated the H1.3, H1.4, and H1.5 isoforms to repress MMTV transcription indicating that histone H1 isoforms directly influence the transcriptional activation/repression of specific genes [59].

Proteasome inhibition results in changes in expression of transcripts encoding a number of histone modifying enzymes, especially those resulting in arginine and lysine methylation. Transcripts encoding histone methyltransferases targeting histone H3-K4 and H3-K36 previously associated with active chromatin are significantly changed by proteasome inhibition. The changes in histone modifying enzymes, methyltransferases and demethylases

seen after proteasome inhibition offer an exciting mechanism to explain differential regulation of hormone mediated gene expression. Indeed, recent studies have shown that specific histone methyltransferases can regulate hormone response and impose gene specific functions [60].

Apart from transcriptional regulation, a number of the transcripts encoding histone modifying enzymes are particularly interesting because of their established or putative roles in human diseases. Proteasome inhibition alone or in the presence of either DEX or E2 leads to an increase mixed lineage leukemia (MLL) specific methyltransferase for histone H3 Lys4 (H3K4). In addition a number of MLL translocation partners, for example RNA polymerase II elongation factor 2 (ELL2) and AF1Q, are increased when cells are treated with proteasome inhibitor. Mixed-lineage leukemia 1 (MLL1) gene is disrupted by chromosomal translocation in acute leukemia and is a master regulator of Hox genes [61], which have been recognized as oncogenes in leukemia. Additionally, the oncogenic potential of Hox genes is implicated in various cancers [62]. For example, HOX A1 is up-regulated in cervical cancer and we found that it is altered by proteasome inhibition and estradiol [63]. The Hox cluster, C10, -11 and -13 are implicated in metastatic melanoma [64]. Hox C8 is over expressed in prostate cancer [65]. Interestingly, proteasome inhibition decreases most of Hox gene expression perhaps offering a clue on how proteasome inhibitors act as a therapeutic application in leukemia. Our studies reveal an interesting avenue to pursue as both the proteasome and steroid hormone receptors are targets for therapy in the treatment of leukemia [66,67].

Disruption of MLL function by translocation is recently implicated in the promiscuous regulation of cell cycle regulators (cyclin dependent kinases and kinase inhibitors) and a cluster of miRNAs involved in cancer, supporting a role of MLL in tumor formation and suppression [61,68,69]. Our cluster analysis after proteasome inhibition reveals a set of developmental genes that are regulated by miRNAs are altered by proteasome inhibition. Lin 28 encodes a RNA binding protein of which functional mutations results in abnormal development of various cell lineages [70]. Lin 28 is regulatory target of mir-125 cluster which function in neuronal development [56]. Lin7A, Lin7B, and Lin7C, which each encodes a protein that is required for generation and maintenance of neuroepithelial cell junctions is a proposed target for mir22 and mir365 (http://microrna.sanger.ac.uk/).

The genes encoding Wolf-Hirschhorn syndrome candidate 1 (WHSC1) also known as multiple myeloma SET domain (MMSET) or nuclear receptor-binding SET domain-containing protein 2 (NSD2) and Wolf-Hirschhorn syndrome candidate 1-like 1 (WHSC1L1) also known as NSD3, the putative histone methyltransferase targeting both histone H3-K36 and H4-K20 are down-regulated in the cells treated with proteasome inhibitor and hormone. Translocations between multiple myeloma SET domain (MMSET) and fibroblast growth factor receptor 3 (FGFR3) result in multiple myeloma [71]. Additionally a set of recently discovered histone demethylases in the Jumonji and Jarid family are altered by proteasome inhibition. These changes in molecules that impact on multiple myeloma are interesting especially since in clinical trials proteasome inhibitors are used to treat multiple myeloma patients with glucocorticoid resistance who have undergone relapse, where treatment with dexamethasone and proteasome inhibitor restores clinical outcome [72].

Finally, given the potential of proteasome inhibitors in antiviral therapy, an interesting candidate in this regard is the estrogen-dependent gene stromal cell-derived factor (SDF-1 or CXCL12) a ligand of CCRX4 chemokine receptor, which is involved in diseases including AIDS and cancer cell metastasis [73,74]. Other molecules involved in HIV transcription are altered by proteasome inhibition include NR2F1, the proteasome subunit PSMC4 which interacts with HIV TAT and the protein arginine methyltransferase PRMT6 which methylates and modulates TAT-mediated transactivation [75-77].

Proteasome inhibition modulates transcripts encoding genes involved in protein folding, cell migration, cell cycle regulation, apoptosis, inflammatory responses, cell adhesion, antigen presentation and ion transport to name a few. Importantly, our genome-wide transcript profiling analysis and chromosome mapping shows that proteasome inhibition impacts on expression of many genes involved in the pathogenesis of various human diseases including many cancers, HIV/AIDs and neurodegenerative disorders, Alzheimer's, Parkinson's and Huntington's [17, 78]. Many proteasome targets, such p53, MDM2 and ER, play critical roles in cell growth and proliferation and can contribute to survival of tumor cells. Not surprisingly, inhibitors of the proteasome, such as Velcade/Bortezomib have been showed to inhibit tumor growth in clinical trials of multiple myeloma, breast, pancreatic, lung, and ovarian cancers [79,80]. The precise mechanisms of how proteasome inhibitors, such as Velcade, work as anti-tumor agents are unknown. The predominant view attributes the outcome of the therapy to the degradation of specific tumor suppressors or cell cycle regulators or in-activation of the NFkB due to its anti-apoptotic activity [81]. Our analysis of proteasome/hormone receptor mediated gene transcription suggests alternative pathways that may provide a mechanistic explanation for therapeutic outcomes of proteasome inhibitors. Our studies imply that proteasome activity modulates NR function via changes in chromatin enzymes, there by implicating the proteasome in epigenetic contribution to human disease. Presently, there is evidence to show that disruption in the balance of epigenetic networks can cause pathological disease states, such as leukemia and inhibitors for chromatin modifying enzymes, offer future prospects for epigenetic therapy [82,83]. Proteasome inhibitors join other classes of therapy, such as DNA demethylating agents and HDACs that change epigenetic marks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Mangelsdorf DJ, Thummel C, Beato M, et al. The nuclear receptor superfamily: the second decade. Cell 1995;83(6):835–839. [PubMed: 8521507]
2. Yamamoto KR. Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 1985;19:209–252. [PubMed: 3909942]
3. Kinyamu HK, Archer TK. Modifying chromatin to permit steroid hormone receptor-dependent transcription. Biochim Biophys Acta 2004;1677(1-3):30–45. [PubMed: 15020043]
4. Kishimoto M, Fujiki R, Takezawa S, et al. Nuclear receptor mediated gene regulation through chromatin remodeling and histone modifications. Endocr J 2006;53(2):157–172. [PubMed: 16618973]
5. Kinyamu HK, Chen J, Archer TK. Linking the ubiquitin-proteasome pathway to chromatin remodeling/modification by nuclear receptors. J Mol Endocrinol 2005;34(2):281–297. [PubMed: 15821097]
6. Nawaz Z, O'Malley BW. Urban renewal in the nucleus: is protein turnover by proteasomes absolutely required for nuclear receptor-regulated transcription? Mol Endocrinol 2004;18(3):493–499. [PubMed: 14673136]
7. Hoang T, Fenne IS, Cook C, et al. cAMP-dependent protein kinase regulates ubiquitin-proteasome-mediated degradation and subcellular localization of the nuclear receptor coactivator GRIP1. J Biol Chem 2004;279(47):49120–49130. [PubMed: 15347661]

8. Li X, Lonard DM, Jung SY, et al. The SRC-3/AIB1 coactivator is degraded in a ubiquitin- and ATP-independent manner by the REGgamma proteasome. Cell 2006;124(2):381–392. [PubMed: 16439211]
9. Yan F, Gao X, Lonard DM, Nawaz Z. Specific ubiquitin-conjugating enzymes promote degradation of specific nuclear receptor coactivators. Mol Endocrinol 2003;17(7):1315–1331. [PubMed: 12663742]
10. Baker SP, Grant PA. The proteasome: not just degrading anymore. Cell 2005;123(3):361–363. [PubMed: 16269325]
11. Collins GA, Tansey WP. The proteasome: a utility tool for transcription? Curr Opin Genet Dev 2006;16(2):197–202. [PubMed: 16503126]
12. Goldberg AL. Protein degradation and protection against misfolded or damaged proteins. Nature 2003;426(6968):895–899. [PubMed: 14685250]
13. Glickman MH, Ciechanover A. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 2002;82(2):373–428. [PubMed: 11917093]
14. Pickart CM. Back to the future with ubiquitin. Cell 2004;116(2):181–190. [PubMed: 14744430]
15. Fang S, Weissman AM. A field guide to ubiquitylation. Cell Mol Life Sci 2004;61(13):1546–1561. [PubMed: 15224180]
16. Ciechanover A. Intracellular Protein Degradation: From a Vague Idea thru the Lysosome and the Ubiquitin-Proteasome System and onto. Human Diseases and Drug Targeting Hematology. Am Soc Hematol Educ Program 2006:1–12.
17. Schwartz AL, Ciechanover A. The ubiquitin-proteasome pathway and pathogenesis of human diseases. Annu Rev Med 1999;50:57–74. [PubMed: 10073263]
18. Lipford JR, Smith GT, Chi Y, Deshaies RJ. A putative stimulatory role for activator turnover in gene expression. Nature 2005;438(7064):113–116. [PubMed: 16267558]
19. Reid G, Hubner MR, Metivier R, et al. Cyclic, proteasome-mediated turnover of unliganded and liganded ERalpha on responsive promoters is an integral feature of estrogen signaling. Mol Cell 2003;11(3):695–707. [PubMed: 12667452]
20. Lee JW, Ryan F, Swaffield JC, Johnston SA, Moore DD. Interaction of thyroid-hormone receptor with a conserved transcriptional mediator. Nature 1995;374(6517):91–94. [PubMed: 7870181]
21. Zhang H, Sun L, Liang J, et al. The catalytic subunit of the proteasome is engaged in the entire process of estrogen receptor-regulated transcription. Embo J 2006;25(18):4223–4233. [PubMed: 16957778]
22. Deroo BJ, Rentsch C, Sampath S, Young J, DeFranco DB, Archer TK. Proteasomal inhibition enhances glucocorticoid receptor transactivation and alters its subnuclear trafficking. Mol Cell Biol 2002;22(12):4113–4123. [PubMed: 12024025]
23. Wallace AD, Cidlowski JA. Proteasome-mediated glucocorticoid receptor degradation restricts transcriptional signaling by glucocorticoids. J Biol Chem 2001;276(46):42714–42721. [PubMed: 11555652]
24. Lonard DM, Nawaz Z, Smith CL, O'Malley BW. The 26S proteasome is required for estrogen receptor-alpha and coactivator turnover and for efficient estrogen receptor-alpha transactivation. Mol Cell 2000;5(6):939–948. [PubMed: 10911988]
25. Kinyamu HK, Archer TK. Estrogen receptor-dependent proteasomal degradation of the glucocorticoid receptor is coupled to an increase in mdm2 protein expression. Mol Cell Biol 2003;23(16):5867–5881. [PubMed: 12897156]
26. Fryer CJ, Nordeen SK, Archer TK. Antiprogestins mediate differential effects on glucocorticoid receptor remodeling of chromatin structure. J Biol Chem 1998;273(2):1175–1183. [PubMed: 9422784]
27. Frasor J, Danes JM, Komm B, Chang KC, Lyttle CR, Katzenellenbogen BS. Profiling of estrogen up- and down-regulated gene expression in human breast cancer cells: insights into gene networks and pathways underlying estrogenic control of proliferation and cell phenotype. Endocrinology 2003;144(10):4562–4574. [PubMed: 12959972]
28. Lin CY, Strom A, Vega VB, et al. Discovery of estrogen receptor alpha target genes and response elements in breast tumor cells. Genome Biol 2004;5(9):R66. [PubMed: 15345050]
29. Lobenhofer EK, Bennett L, Cable PL, Li L, Bushel PR, Afshari CA. Regulation of DNA replication fork genes by 17beta-estradiol. Mol Endocrinol 2002;16(6):1215–1229. [PubMed: 12040010]

30. Hebbar PB, Archer TK. Chromatin-dependent cooperativity between site-specific transcription factors in vivo. J Biol Chem 2007;282(11):8284–8291. [PubMed: 17186943]

31. Kinyamu HK, Archer TK. Proteasome Activity Modulates Chromatin Modifications and RNA Polymerase II Phosphorylation To Enhance Glucocorticoid Receptor-Mediated Transcription. Mol Cell Biol 2007;27(13):4891–4904. [PubMed: 17438138]

32. Weng L, Dai H, Zhan Y, He Y, Stepaniants SB, Bassett DE. Rosetta error model for gene expression analysis. Bioinformatics 2006;22(9):1111–1121. [PubMed: 16522673]

33. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res 2002;30(1):207–210. [PubMed: 11752295]

34. Rhen T, Grissom S, Afshari C, Cidlowski JA. Dexamethasone blocks the rapid biological effects of 17beta-estradiol in the rat uterus without antagonizing its global genomic actions. Faseb J 2003;17 (13):1849–1870. [PubMed: 14519664]

35. Rogatsky I, Wang JC, Derynck MK, et al. Target-specific utilization of transcriptional regulatory surfaces by the glucocorticoid receptor. Proc Natl Acad Sci U S A 2003;100(24):13845–13850. [PubMed: 14617768]

36. Stojadinovic O, Lee B, Vouthounis C, et al. Novel genomic effects of glucocorticoids in epidermal keratinocytes: inhibition of apoptosis, interferon-gamma pathway, and wound healing along with promotion of terminal differentiation. J Biol Chem 2007;282(6):4021–4034. [PubMed: 17095510]

37. Wan Y, Nordeen SK. Overlapping but distinct gene regulation profiles by glucocorticoids and progestins in human breast cancer cells. Mol Endocrinol 2002;16(6):1204–1214. [PubMed: 12040008]

38. Wang JC, Derynck MK, Nonaka DF, Khodabakhsh DB, Haqq C, Yamamoto KR. Chromatin immunoprecipitation (ChIP) scanning identifies primary glucocorticoid receptor target genes. Proc Natl Acad Sci U S A 2004;101(44):15603–15608. [PubMed: 15501915]

39. Lee DY, Teyssier C, Strahl BD, Stallcup MR. Role of protein methylation in regulation of transcription. Endocr Rev 2005;26(2):147–170. [PubMed: 15479858]

40. Vogel MJ, Guelen L, de Wit E, et al. Human heterochromatin proteins form large domains containing KRAB-ZNF genes. Genome Res 2006;16(12):1493–1504. [PubMed: 17038565]

41. Blanquart C, Barbier O, Fruchart JC, Staels B, Glineur C. Peroxisome proliferator-activated receptor alpha (PPARalpha) turnover by the ubiquitin-proteasome system controls the ligand-induced expression level of its target genes. J Biol Chem 2002;277(40):37254–37259. [PubMed: 12118000]

42. Lin HK, Altuwaijri S, Lin WJ, Kan PY, Collins LL, Chang C. Proteasome activity is required for androgen receptor transcriptional activity via regulation of androgen receptor nuclear translocation and interaction with coregulators in prostate cancer cells. J Biol Chem 2002;277(39):36570–36576. [PubMed: 12119296]

43. Zhou RH, Kokame K, Tsukamoto Y, Yutani C, Kato H, Miyata T. Characterization of the human NDRG gene family: a newly identified member, NDRG4, is specifically expressed in brain and heart. Genomics 2001;73(1):86–97. [PubMed: 11352569]

44. Bandyopadhyay S, Pai SK, Gross SC, et al. The Drg-1 gene suppresses tumor metastasis in prostate cancer. Cancer Res 2003;63(8):1731–1736. [PubMed: 12702552]

45. Lachat P, Shaw P, Gebhard S, van Belzen N, Chaubert P, Bosman FT. Expression of NDRG1, a differentiation-related gene, in human tissues. Histochem Cell Biol 2002;118(5):399–408. [PubMed: 12432451]

46. Koritschoner NP, Bocco JL, Panzetta-Dutari GM, Dumur CI, Flury A, Patrito LC. A novel human zinc finger protein that interacts with the core promoter element of a TATA box-less gene. J Biol Chem 1997;272(14):9573–9580. [PubMed: 9083102]

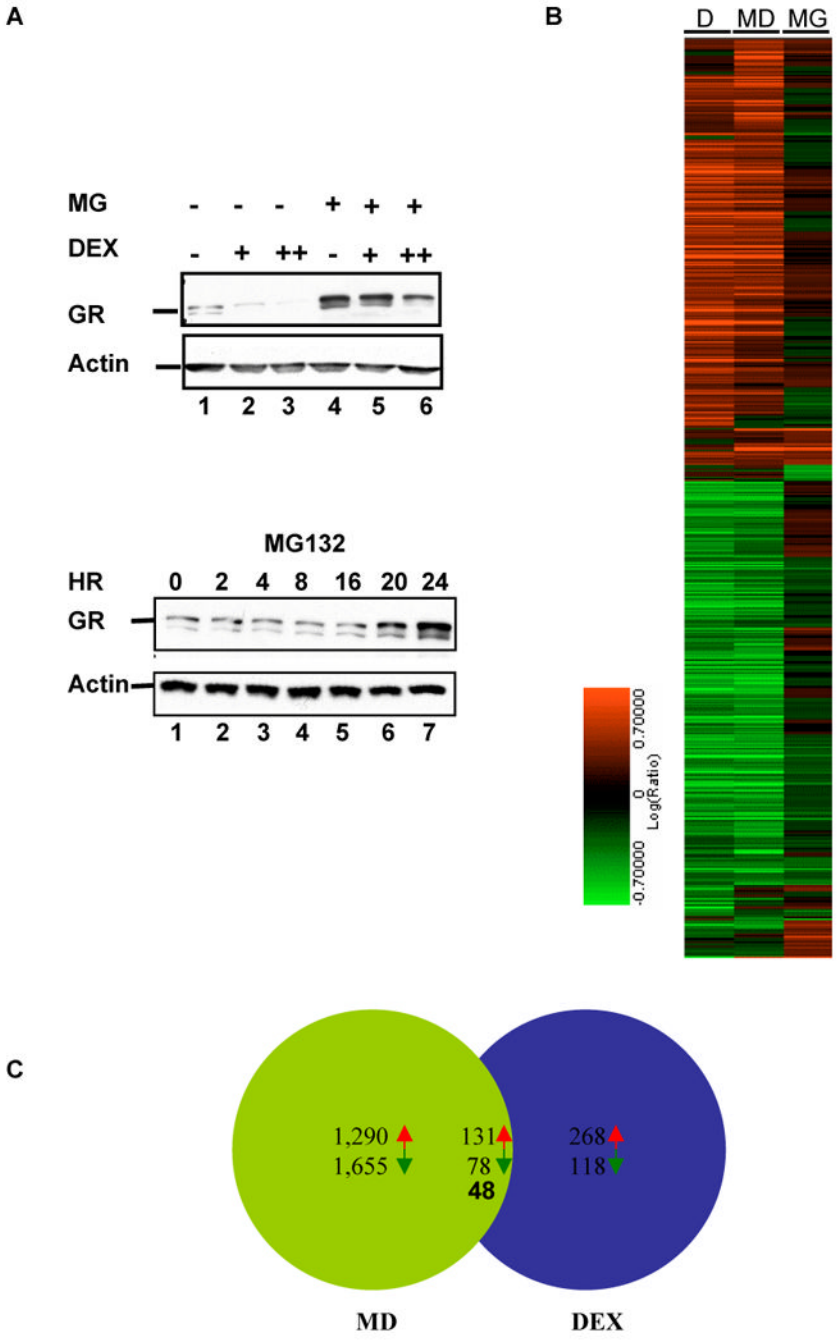
47. Narla G, Friedman SL, Martignetti JA. Kruppel cripples prostate cancer: KLF6 progress and prospects. Am J Pathol 2003;162(4):1047–1052. [PubMed: 12651597]

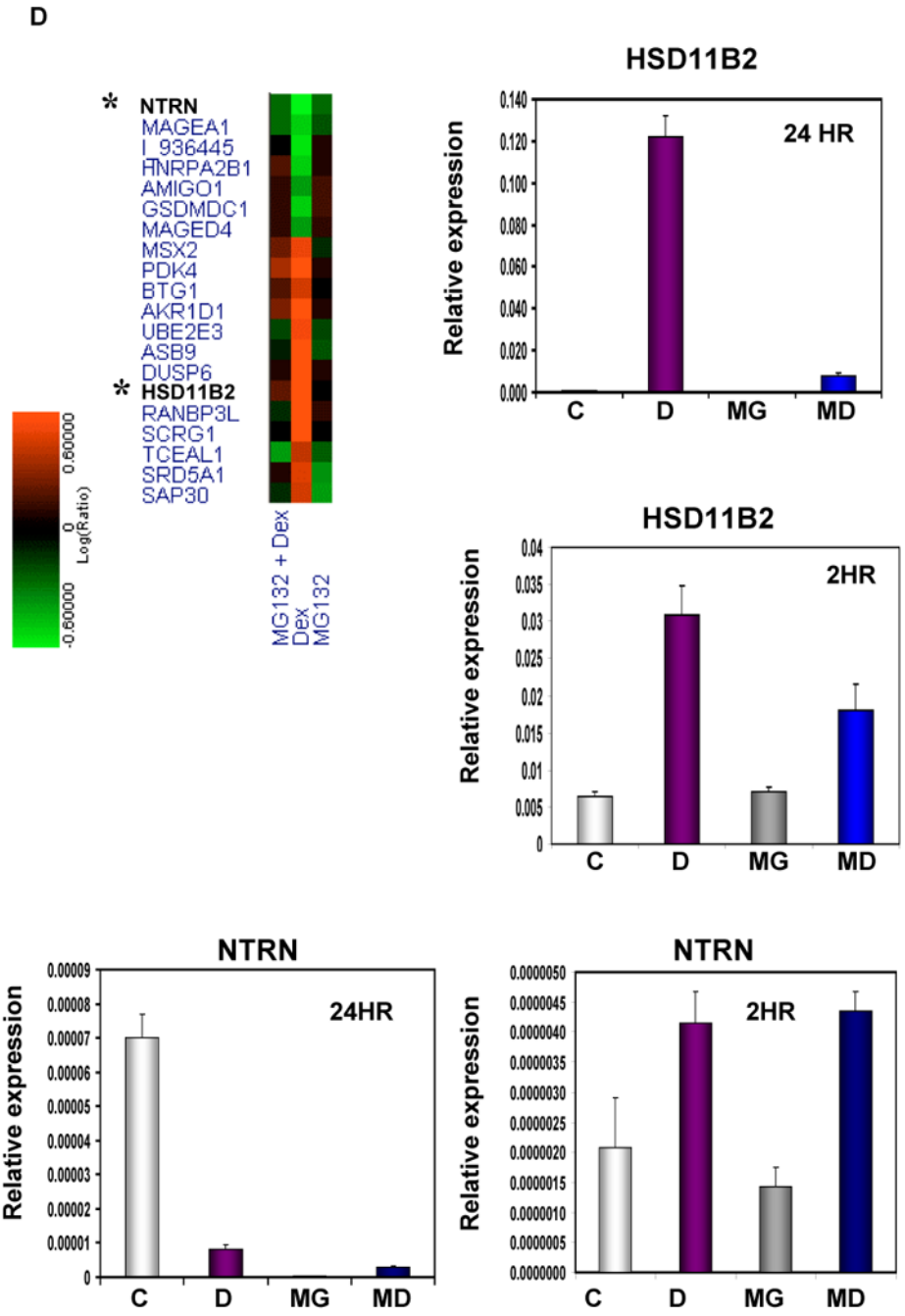
48. Huang J, Perez-Burgos L, Placek BJ, et al. Repression of p53 activity by Smyd2-mediated methylation. Nature 2006;444(7119):629–632. [PubMed: 17108971]

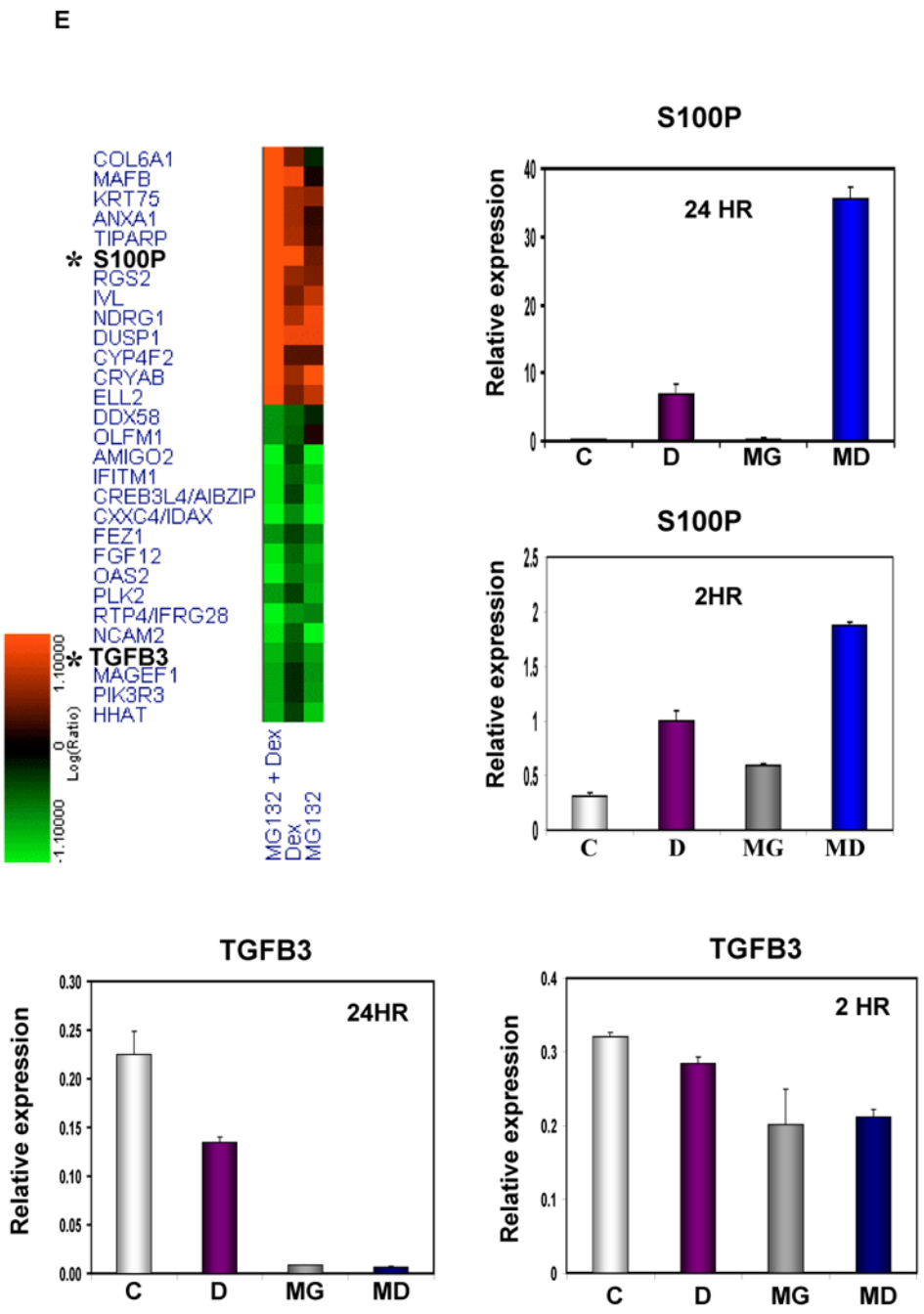
49. Carlsson H, Petersson S, Enerback C. Cluster analysis of S100 gene expression and genes correlating to psoriasin (S100A7) expression at different stages of breast cancer development. Int J Oncol 2005;27(6):1473–1481. [PubMed: 16273201]

50. Perou CM, Jeffrey SS, van de Rijn M, et al. Distinctive gene expression patterns in human mammary epithelial cells and breast cancers. Proc Natl Acad Sci U S A 1999;96(16):9212–9217. [PubMed: 10430922]
51. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. Nature 2000;406 (6797):747–752. [PubMed: 10963602]
52. Zhu Y, Wang A, Liu MC, et al. Estrogen receptor alpha positive breast tumors and breast cancer cell lines share similarities in their transcriptome data structures. Int J Oncol 2006;29(6):1581–1589. [PubMed: 17089000]
53. Wright KJ, Marr MT 2nd, Tjian R. TAF4 nucleates a core subcomplex of TFIID and mediates activated transcription from a TATA-less promoter. Proc Natl Acad Sci U S A 2006;103(33):12347–12352. [PubMed: 16895980]
54. Jiang HY, Jiang L, Wek RC. The eukaryotic initiation factor-2 kinase pathway facilitates differential GADD45a expression in response to environmental stress. J Biol Chem 2007;282(6):3755–3765. [PubMed: 17170114]
55. Stavreva DA, Kawasaki M, Dundr M, et al. Potential roles for ubiquitin and the proteasome during ribosome biogenesis. Mol Cell Biol 2006;26(13):5131–5145. [PubMed: 16782897]
56. Wu L, Belasco JG. Micro-RNA regulation of the mammalian lin-28 gene during neuronal differentiation of embryonal carcinoma cells. Mol Cell Biol 2005;25(21):9198–9208. [PubMed: 16227573]
57. De Smet C, Lurquin C, Lethe B, Martelange V, Boon T. DNA methylation is the primary silencing mechanism for a set of germ line- and tumor-specific genes with a CpG-rich promoter. Mol Cell Biol 1999;19(11):7327–7335. [PubMed: 10523621]
58. Nakamura N, Takenaga K. Hypomethylation of the metastasis-associated S100A4 gene correlates with gene activation in human colon adenocarcinoma cell lines. Clin Exp Metastasis 1998;16(5): 471–479. [PubMed: 10091942]
59. Banks GC, Deterding LJ, Tomer KB, Archer TK. Hormone-mediated dephosphorylation of specific histone H1 isoforms. J Biol Chem 2001;276(39):36467–36473. [PubMed: 11479299]
60. Garcia-Bassets I, Kwon YS, Telese F, et al. Histone methylation-dependent mechanisms impose ligand dependency for gene activation by nuclear receptors. Cell 2007;128(3):505–518. [PubMed: 17289570]
61. Hess JL. Mechanisms of transformation by MLL. Crit Rev Eukaryot Gene Expr 2004;14(4):235–254. [PubMed: 15663355]
62. Grier DG, Thompson A, Kwasniewska A, McGonigle GJ, Halliday HL, Lappin TR. The pathophysiology of HOX genes and their role in cancer. J Pathol 2005;205(2):154–171. [PubMed: 15643670]
63. Shim C, Zhang W, Rhee CH, Lee JH. Profiling of differentially expressed genes in human primary cervical cancer by complementary DNA expression array. Clin Cancer Res 1998;4(12):3045–3050. [PubMed: 9865919]
64. Cillo C, Cantile M, Mortarini R, Barba P, Parmiani G, Anichini A. Differential patterns of HOX gene expression are associated with specific integrin and ICAM profiles in clonal populations isolated from a single human melanoma metastasis. Int J Cancer 1996;66(5):692–697. [PubMed: 8647634]
65. Alami Y, Castronovo V, Belotti D, Flagiello D, Clausse N. HOXC5 and HOXC8 expression are selectively turned on in human cervical cancer cells compared to normal keratinocytes. Biochem Biophys Res Commun 1999;257(3):738–745. [PubMed: 10208853]
66. Harris HA. Estrogen receptor-beta: recent lessons from in vivo studies. Mol Endocrinol 2007;21(1): 1–13. [PubMed: 16556737]
67. Musto P, Rossini F, Gay F, et al. Efficacy and safety of bortezomib in patients with plasma cell leukemia. Cancer 2007;109(11):2285–2290. [PubMed: 17469169]
68. Guenther MG, Jenner RG, Chevalier B, et al. Global and Hox-specific roles for the MLL1 methyltransferase. Proc Natl Acad Sci U S A 2005;102(24):8603–8608. [PubMed: 15941828]
69. Milne TA, Hughes CM, Lloyd R, et al. Menin and MLL cooperatively regulate expression of cyclin-dependent kinase inhibitors. Proc Natl Acad Sci U S A 2005;102(3):749–754. [PubMed: 15640349]
70. Richards M, Tan SP, Tan JH, Chan WK, Bongso A. The transcriptome profile of human embryonic stem cells as defined by SAGE. Stem Cells 2004;22(1):51–64. [PubMed: 14688391]

71. Rasmussen T, Hudlebusch HR, Knudsen LM, Johnsen HE. FGFR3 dysregulation in multiple myeloma: frequency and prognostic relevance. Br J Haematol 2002;117(3):626–628. [PubMed: 12028033]
72. Jagannath S, Barlogie B, Berenson J, et al. A phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 2004;127(2):165–172. [PubMed: 15461622]
73. Vergote D, Butler GS, Ooms M, et al. Proteolytic processing of SDF-1alpha reveals a change in receptor specificity mediating HIV-associated neurodegeneration. Proc Natl Acad Sci U S A 2006;103(50):19182–19187. [PubMed: 17148615]
74. Wang Z, Ma Q. beta-Catenin is a promising key factor in the SDF-1/CXCR4 axis on metastasis of pancreatic cancer. Med Hypotheses. 2007
75. Frankel A, Yadav N, Lee J, Branscombe TL, Clarke S, Bedford MT. The novel human protein arginine N-methyltransferase PRMT6 is a nuclear enzyme displaying unique substrate specificity. J Biol Chem 2002;277(5):3537–3543. [PubMed: 11724789]
76. Rohr O, Schwartz C, Hery C, Aunis D, Tardieu M, Schaeffer E. The nuclear receptor chicken ovalbumin upstream promoter transcription factor interacts with HIV-1 Tat and stimulates viral replication in human microglial cells. J Biol Chem 2000;275(4):2654–2660. [PubMed: 10644726]
77. Xie B, Invernizzi CF, Richard S, Wainberg MA. Arginine methylation of the human immunodeficiency virus type 1 Tat protein by PRMT6 negatively affects Tat Interactions with both cyclin T1 and the Tat transactivation region. J Virol 2007;81(8):4226–4234. [PubMed: 17267505]
78. Reinstein E, Ciechanover A. Narrative review: protein degradation and human diseases: the ubiquitin connection. Ann Intern Med 2006;145(9):676–684. [PubMed: 17088581]
79. Orlowski RZ, Dees EC. The role of the ubiquitination-proteasome pathway in breast cancer: applying drugs that affect the ubiquitin-proteasome pathway to the therapy of breast cancer. Breast Cancer Res 2003;5(1):1–7. [PubMed: 12559038]
80. Richardson PG, Mitsiades C. Bortezomib: proteasome inhibition as an effective anticancer therapy. Future Oncol 2005;1(2):161–171. [PubMed: 16555986]
81. Roccaro AM, Hideshima T, Richardson PG, et al. Bortezomib as an antitumor agent. Curr Pharm Biotechnol 2006;7(6):441–448. [PubMed: 17168660]
82. Esteller M. Cancer epigenomics: DNA methylomes and histone-modification maps. Nat Rev Genet 2007;8(4):286–298. [PubMed: 17339880]
83. Jones PA, Baylin SB. The epigenomics of cancer. Cell 2007;128(4):683–692. [PubMed: 17320506]







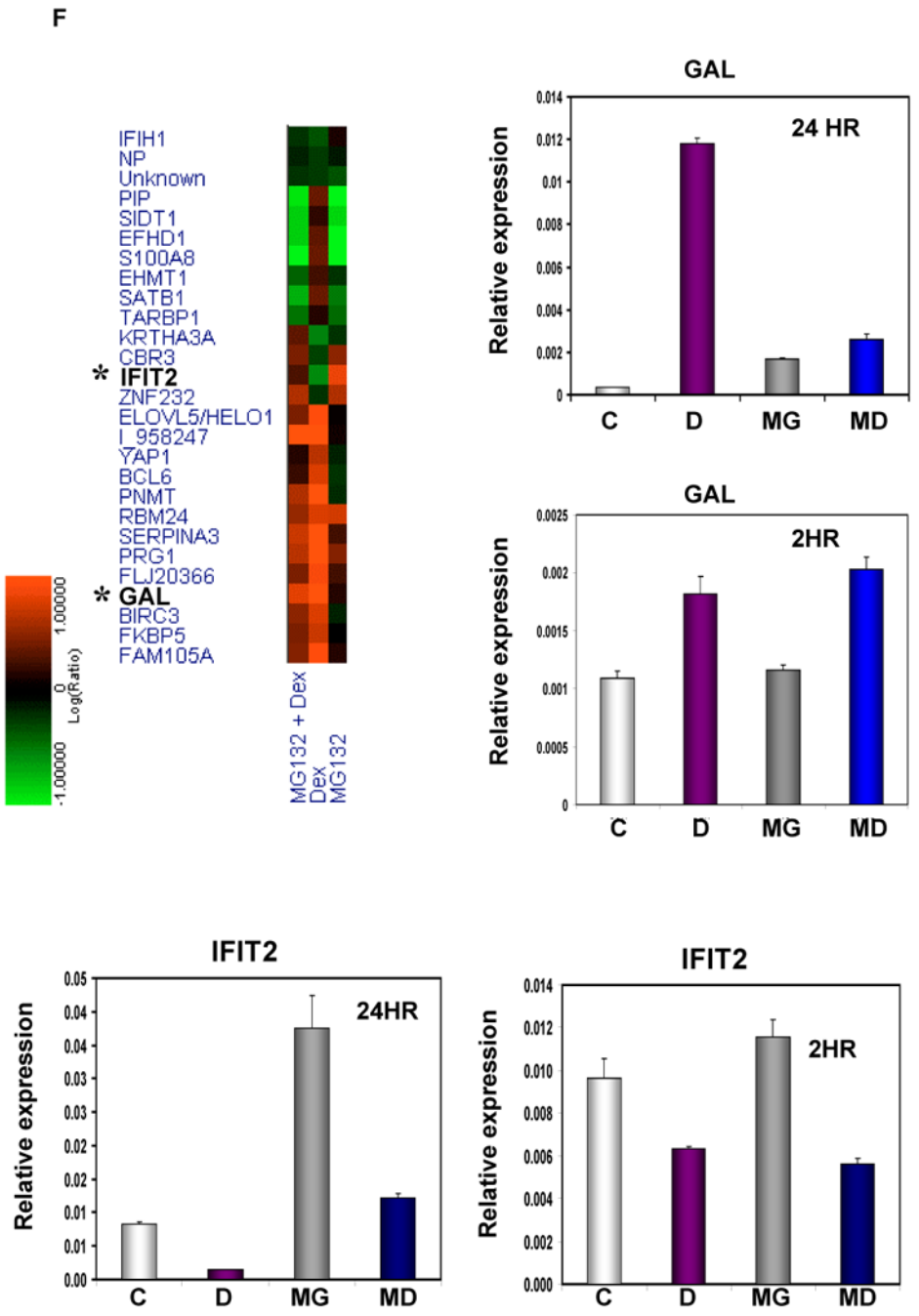
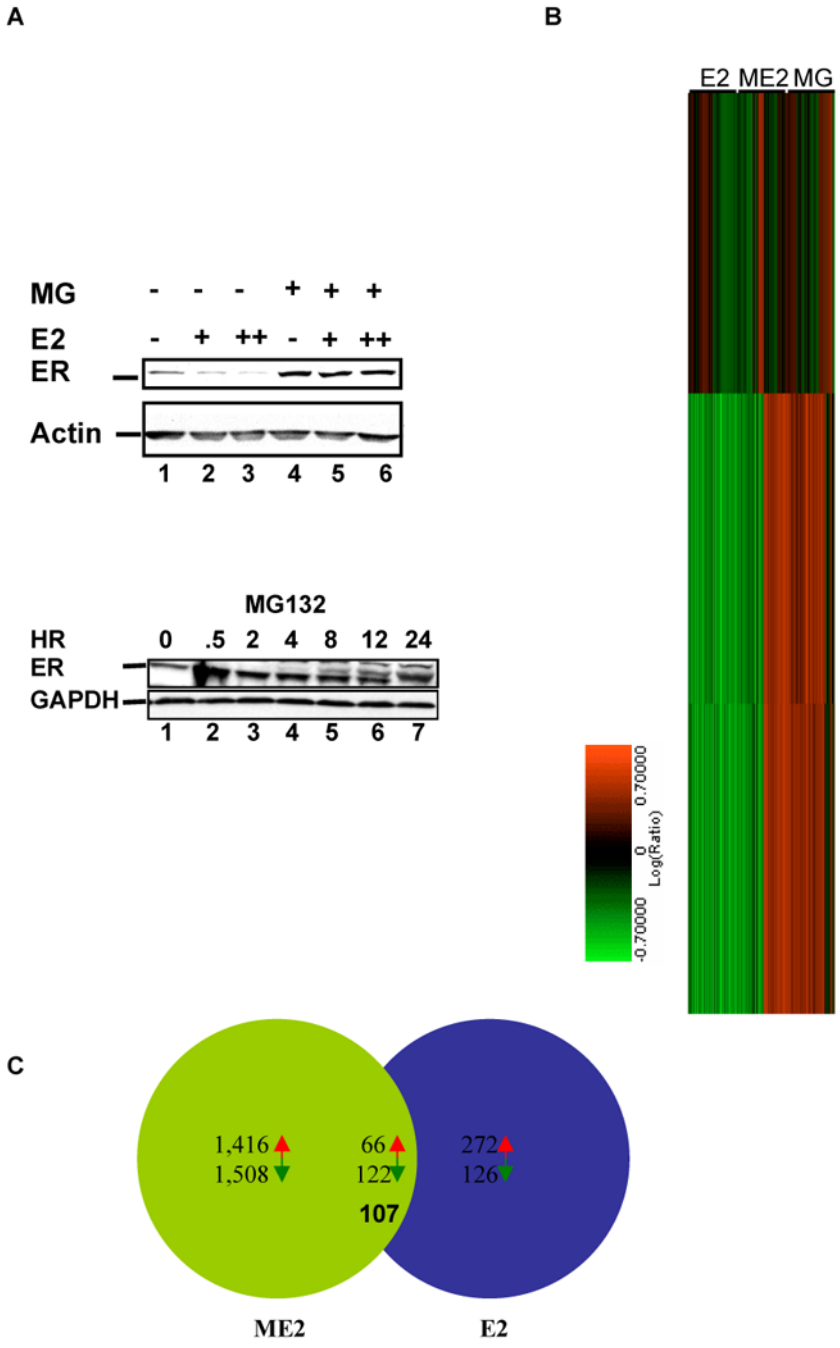
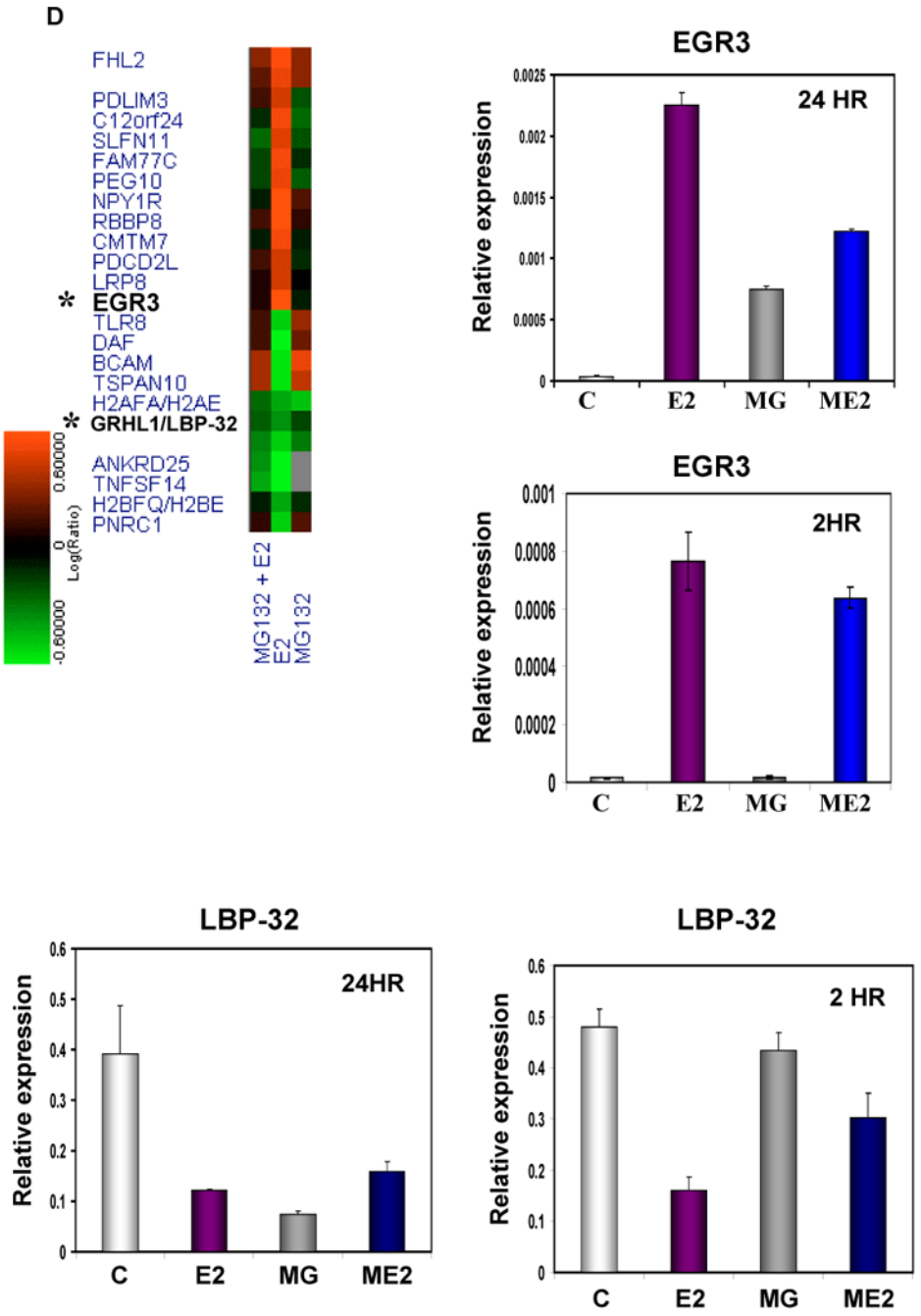


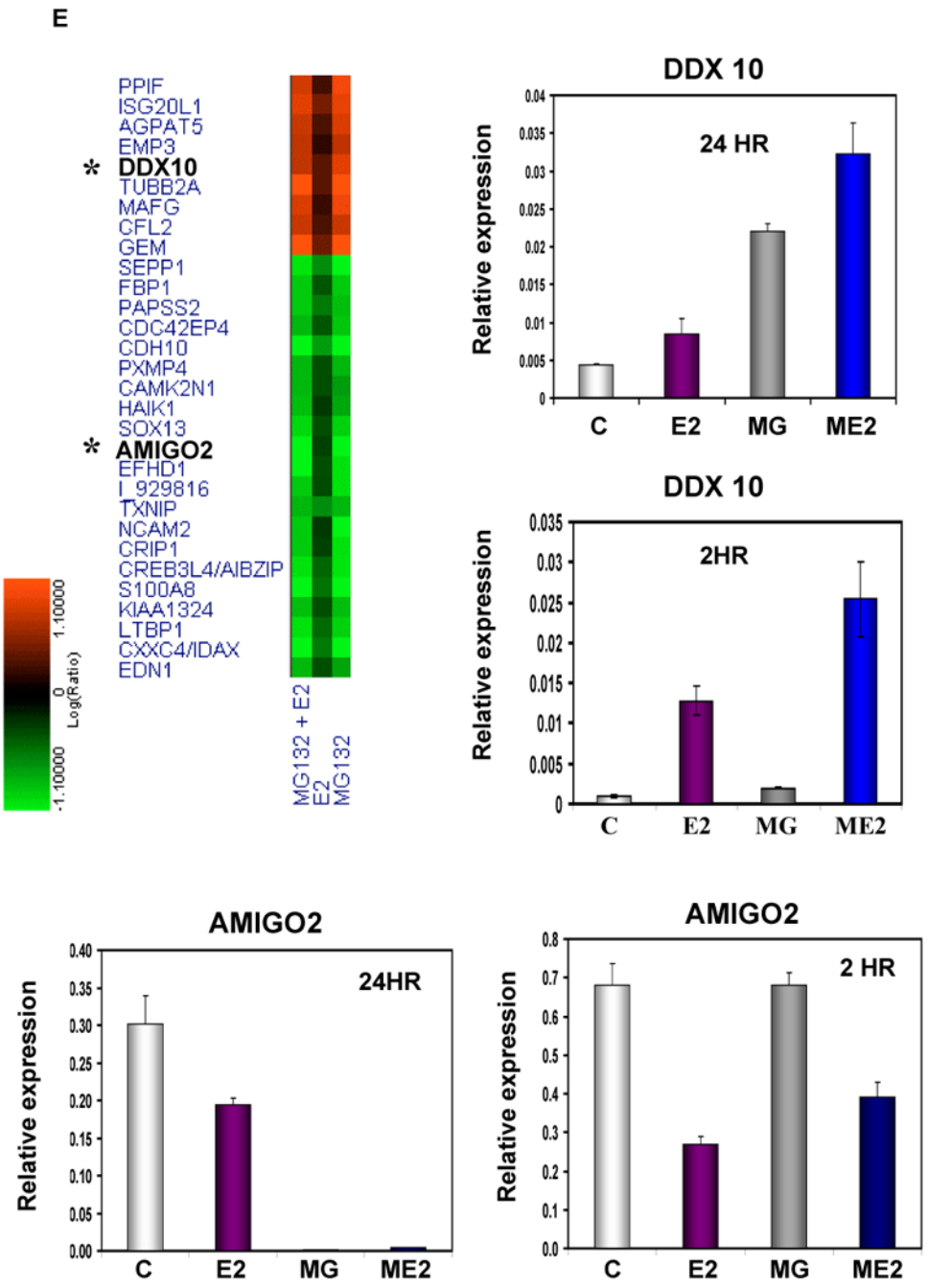
Figure 1.

Global transcriptional profile from MCF-7 cells treated with dexamethasone or proteasome inhibitor. (A) Proteasome inhibition blocks ligand dependent GR turnover. Whole cell extracts from untreated cells (lane 1), cell treated with DEX for 4 (+) or 24 hr (++, lanes 2 and 3), MG132 alone (24hr, lane 4) or MG132 and dexamethasone for 4 or 24 hr (lanes 5 and 6) were immunoblotted with antibodies against GR and actin as control (top). Proteasome inhibition stabilizes GR protein (bottom). (B) Cluster analysis of genes whose level of transcription changed ($p < 0.001$) in 4 replicate experiments after treating MCF-7 cells with dexamethasone alone (DEX), proteasome inhibitor alone (MG) or proteasome inhibitor and dexamethasone (MD) compared to cells treated with vehicle. Intensity of color correlates with the degree of

up-regulation (red) or down-regulation (green). (C) Venn diagram showing the number of genes up or down regulated by dexamethasone (D or DEX) alone or dexamethasone and proteasome inhibitor (MD). The common boundary represents genes regulated synergistically by dexamethasone and proteasome inhibitor. Bold letters represent antagonistic response between dexamethasone and proteasome inhibitor. (D) Cluster analysis of genes regulated by dexamethasone alone with HSD11B2 and NTRN are examples of genes in this class. RNA expression was determined by quantitative RT-PCR after 2 or 24 hr treatment. (E) Cluster analysis of common genes regulated by dexamethasone and proteasome inhibitor with S100P and TGFB3 as examples of genes increased or repressed in a synergistic response between dexamethasone and proteasome inhibitor. RNA expression was determined by quantitative RT-PCR after 2 or 24 hr treatment. (F) Cluster analysis of genes representing an antagonistic response between dexamethasone and proteasome inhibitor with galanin (GAL) and IFIT2 as examples. RNA levels were determined by quantitative RT-PCR after 2 or 24 hr treatment.







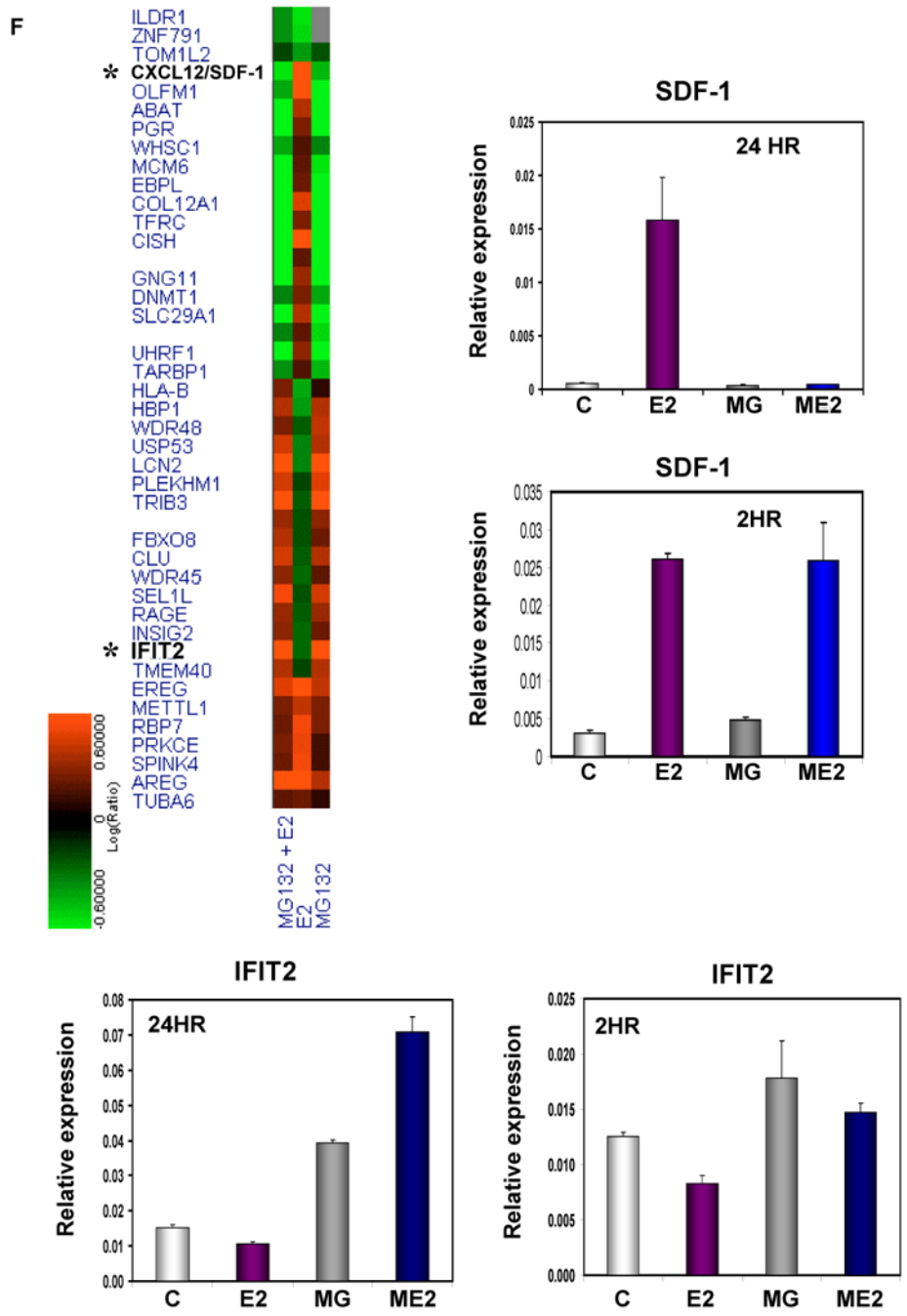
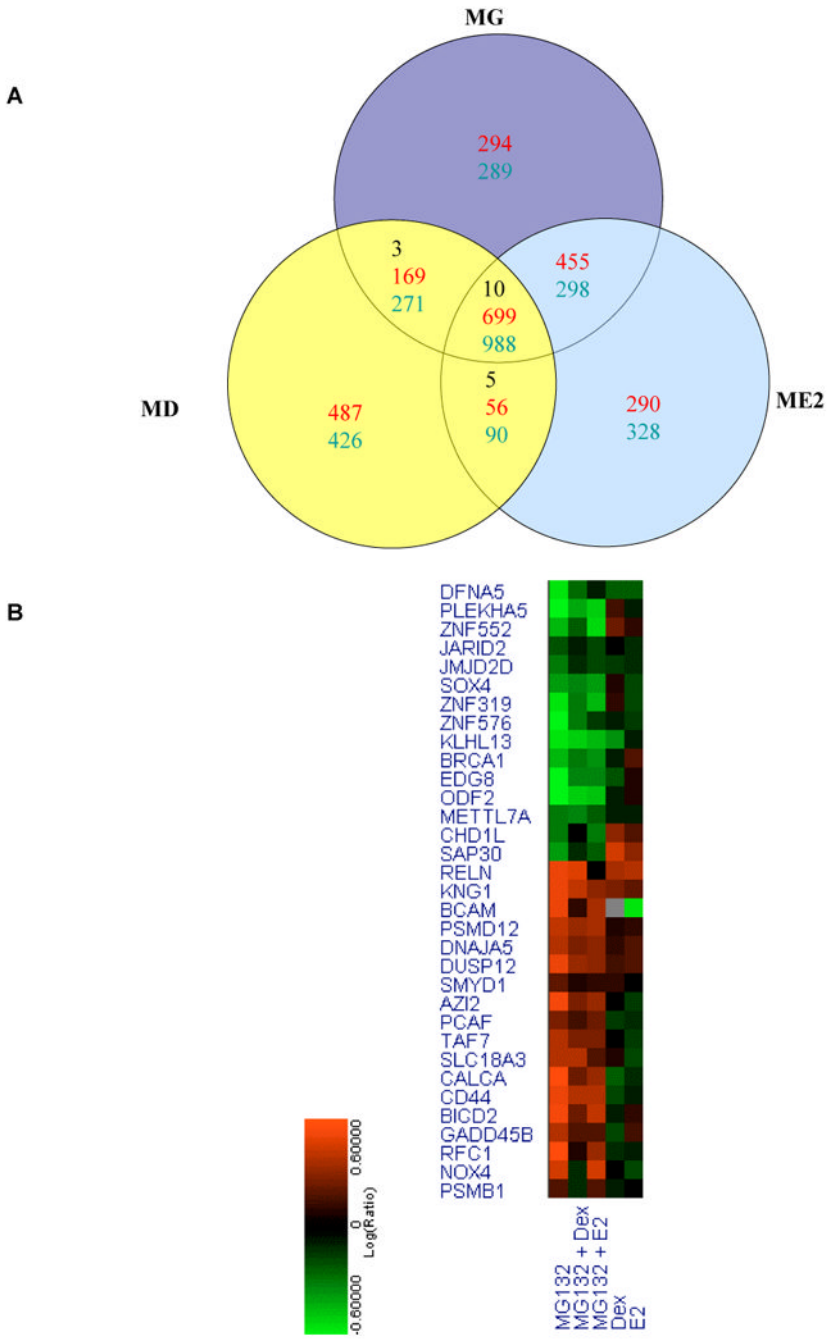
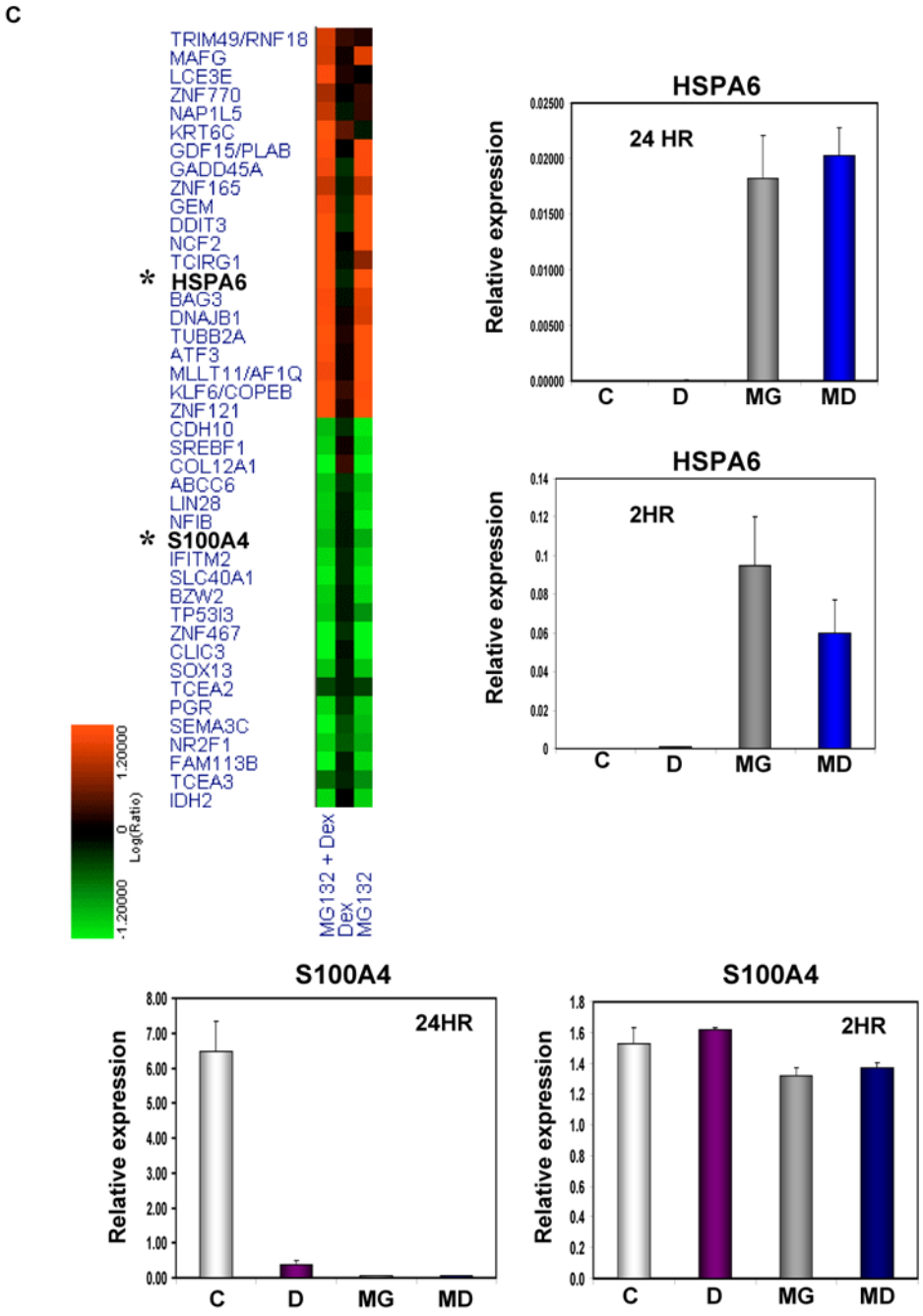


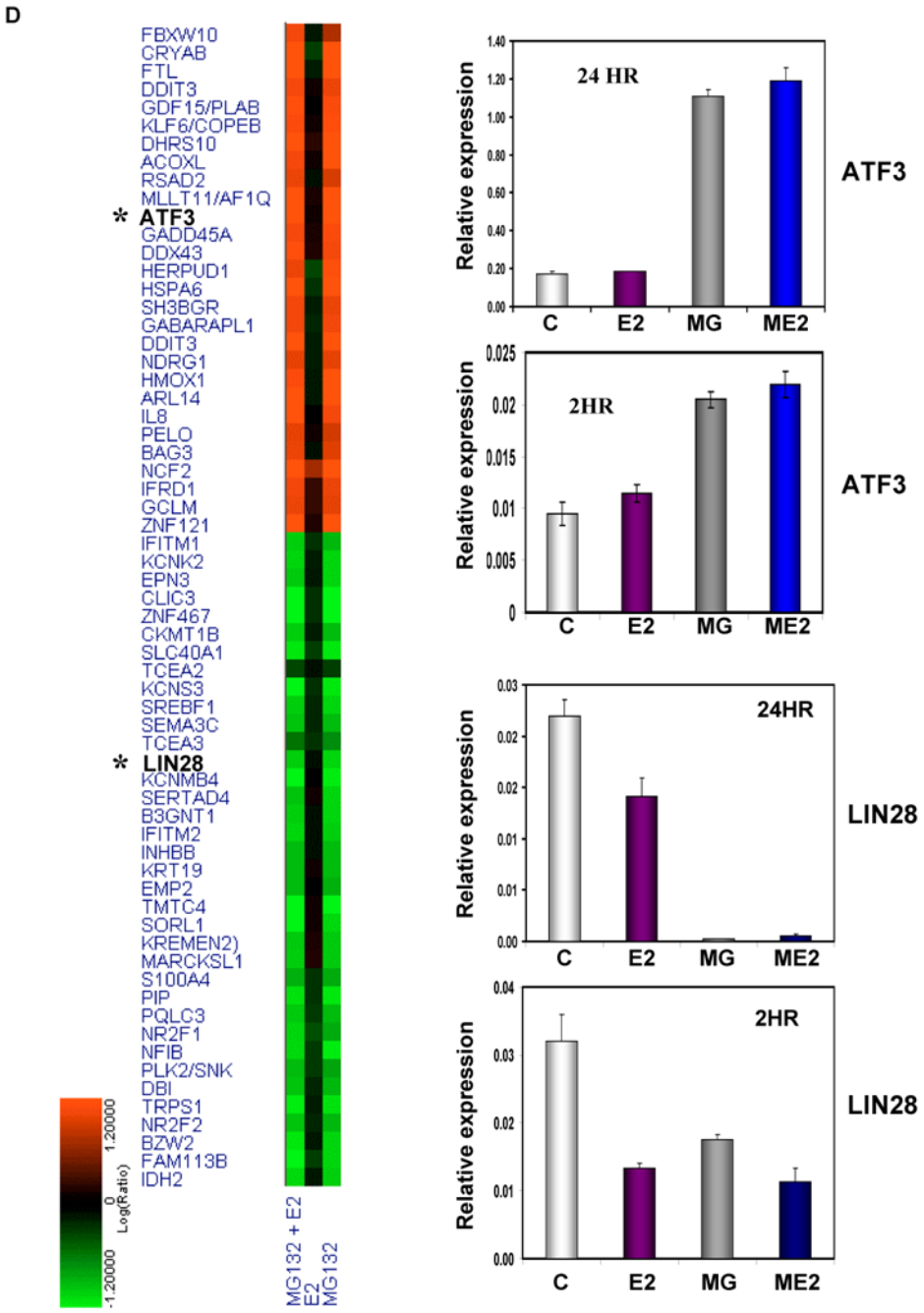
Figure 2.

Global transcriptional profile from MCF-7 cells treated with 17β-estradiol or proteasome inhibitor. (A). Proteasome inhibition blocks ligand dependent ER turnover. Whole cell extracts from untreated cells (lane 1), cells treated with E2 for 4 (+) or 24 hr (++, lanes 2 and 3), MG132 alone (24 hr, lane4) or MG132 and E2 for 4 or 24 hr (lanes 5 and 6) were immunoblotted with antibodies against ER and Actin as control (top). Proteasome inhibition stabilizes ER protein, GAPDH is a control (bottom). (B) Cluster analysis of genes whose level of transcription changed ($p < 0.001$) in 4 replicate experiments after treating MCF-7 cells with 17β-estradiol alone (E2), proteasome inhibitor alone (MG) or proteasome inhibitor and dexamethasone (ME2) compared to cells treated with vehicle. The weighted correlation between the two

biological replicates for all treatments was averaging r = 0.95. Intensity of color correlates with the degree of up-regulation (red) or down-regulation (green). (C) Venn diagram showing the number of genes up or down regulated by 17β-estradiol (E2) alone or 17β-estradiol and proteasome inhibitor (ME2). The common boundary represents genes regulated synergistically by E2 and proteasome inhibitor. Bold letters represent antagonistic response between E2 and proteasome inhibitor. (D) Cluster analysis of genes regulated by 17β-estradiol alone with EGR3 and LBP-32 as representative genes. RNA expression was determined by quantitative RT-PCR after 24 or 2 hr treatment. (E) Cluster analysis of common genes regulated by17β-estradiol and proteasome inhibitor with DDX10 and AMIGO2 as an example of genes exhibiting a synergistic response to E2 and proteasome inhibitor. RNA expression was determined by quantitative RT-PCR after 2 or 24 hr treatments. (F) Cluster analysis of genes representing an antagonistic response between 17β-estradiol and proteasome inhibitor, SDF-1 and IFIT2 are examples. RNA expression was determined by quantitative RT-PCR after 2 or 24 hr treatments.







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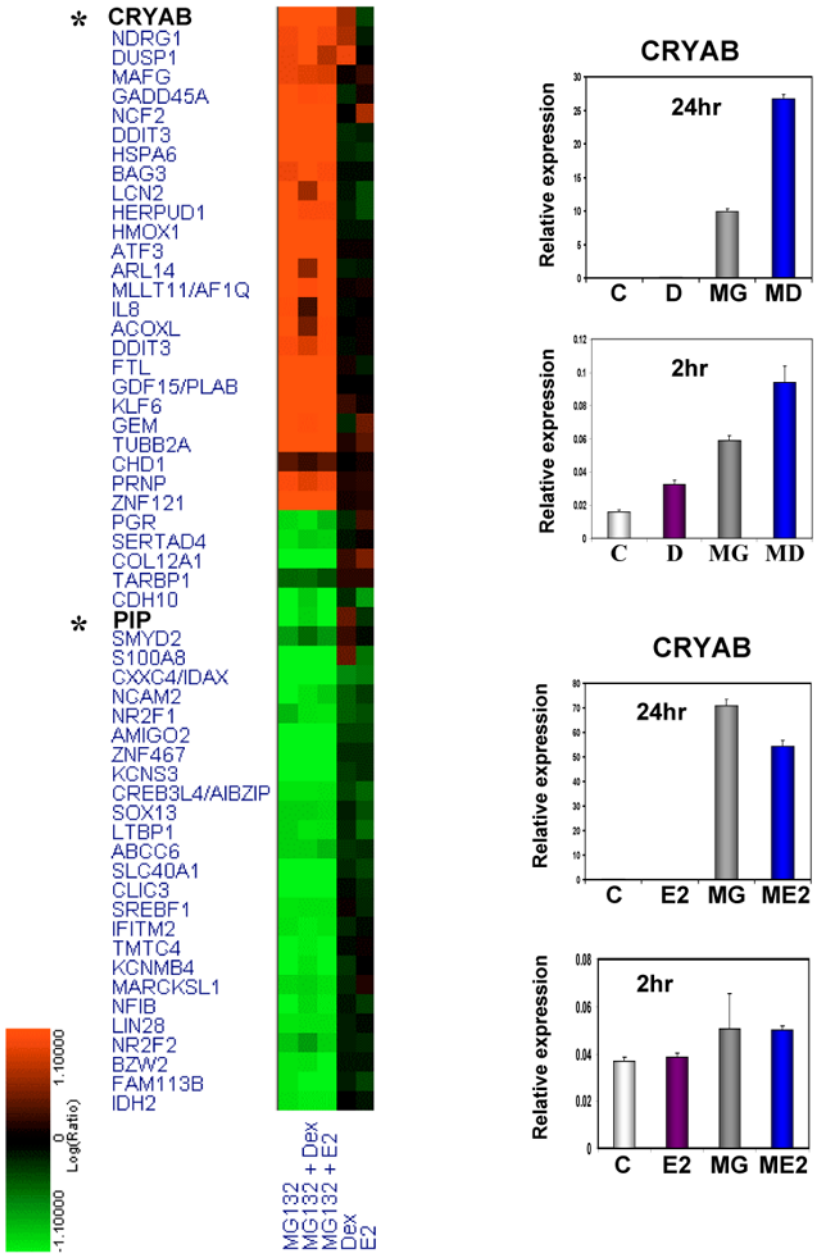


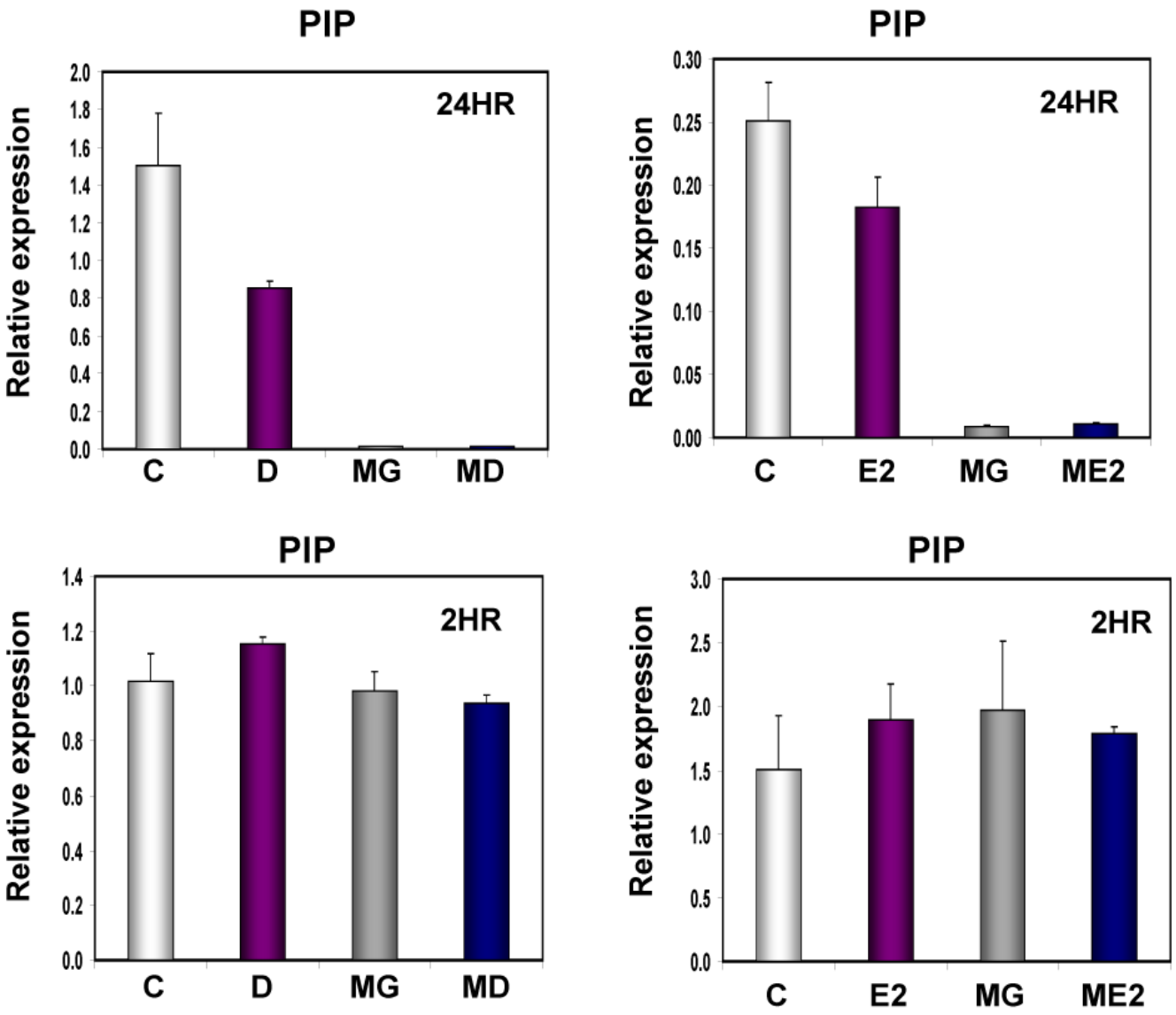
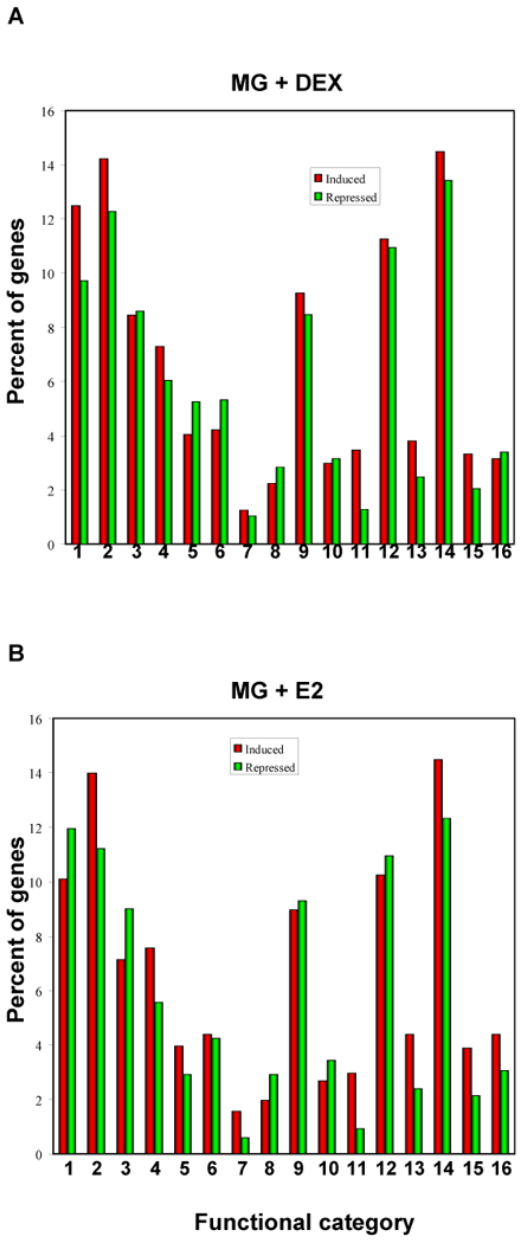
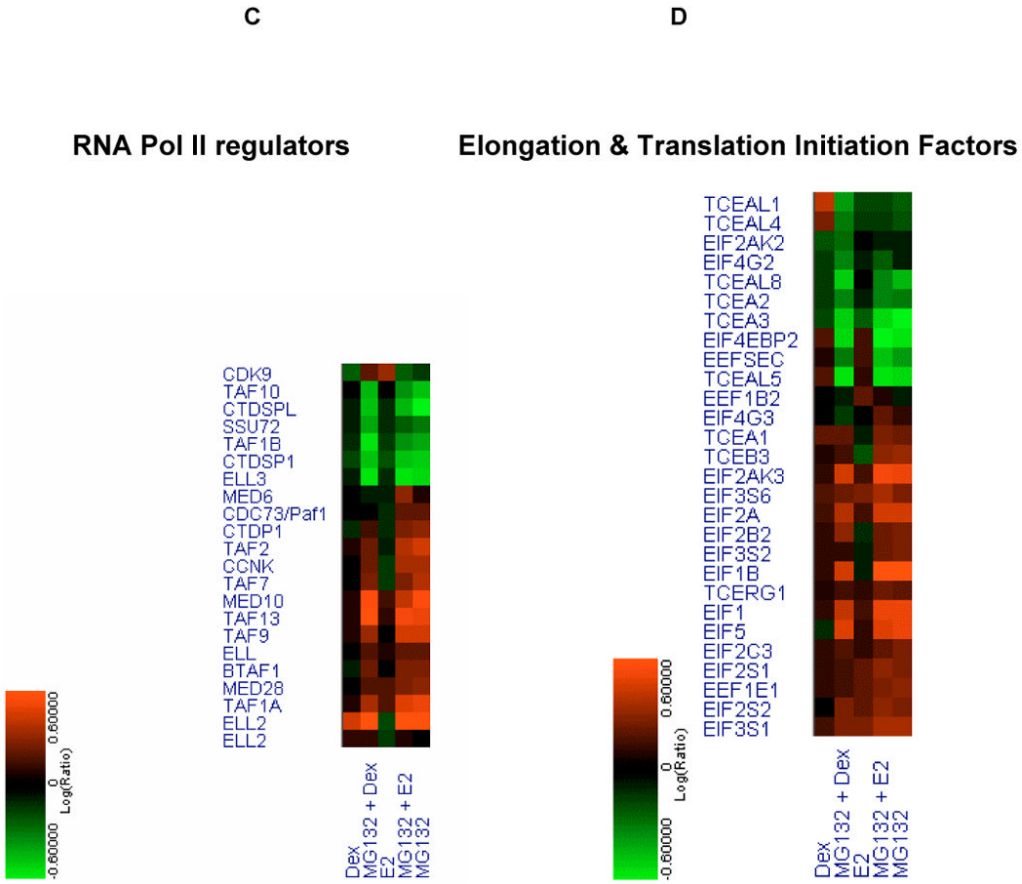


Figure 3.

Proteasome inhibition results in broad changes in gene expression. (A) Venn diagrams showing the number of genes up- or down-regulated by proteasome inhibitor alone and in common with either dexamethasone (MD) or 17β-estradiol (ME2). (B) Cluster analysis of genes changed by proteasome inhibitor alone. (C) Cluster analysis of genes mainly affected by proteasome inhibitor with additional effect by dexamethasone, HSPA6 and S100A4 are examples. RNA expression was determined by quantitative RT-PCR after 2 or 24 hr treatment. (D) Cluster analysis of genes mainly affected by proteasome inhibitor with additional effect by E2, ATF3 and Lin28 are examples. RNA expression was determined by quantitative RT-PCR after 2 or 24 hr treatment. (E) Cluster analysis showing genes changed by proteasome inhibitor with a differential effect of hormone, CRYAB and PIP as examples. RNA expression was determined by quantitative RT-PCR after 24 treatment.



Group	Functional categories	Gene Ontology
1	Signal transduction	GO:0007165
2	**Transcription**	**GO:0006350**
3	Ion transport	GO:0006811
4	Protein transport	GO:0015031
5	Cell cycle	GO:0007049
6	Cytoskeleton organization and biogenesis	GO:0007010
7	Ribosome	GO:0005840
8	Cell adhesion molecule activity	GO:0005194
9	Cell growth	GO:0016049
10	Cell proliferation	GO:0008283
11	Response to stress	GO:0006950
12	Transport	
13	Cell death	GO:0008219
14	Transcription factor activity	GO:0003700
15	Apoptosis regulator activity	GO:0016329
16	Protein biosynthesis	GO:00016412



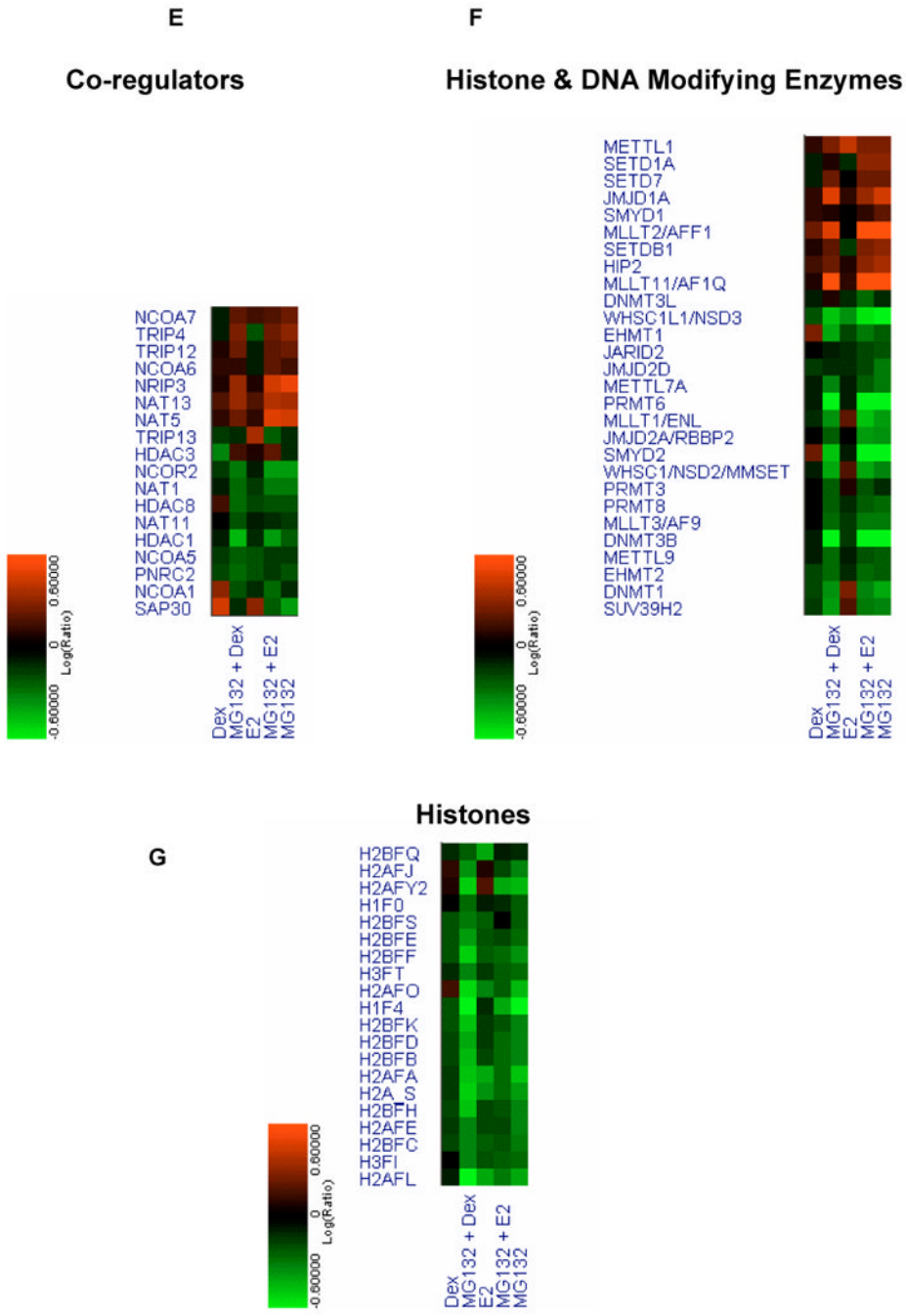


Figure 4.

Functional classification of genes changed after treatment of MCF-7 with proteasome inhibitor and dexamethasone (A) or 17β-estradiol (B), X- axis represents functional category shown on table, Y-axis represents percent of total genes in the category compared to total genes changed by the treatment. The genes affected by proteasome inhibitor and hormone categorized in functional groups according to their main known function based on LocusLink, OMIM, PubMed, GeneCards, and GenMAPP databases. (C). Cluster analysis of genes encoding RNA polymerase II regulators. (D) Cluster analysis of genes encoding transcriptional elongation and translation initiation factors. (E) Cluster analysis of genes encoding transcriptional co-

regulators. (F) Cluster analysis of genes encoding histone and DNA modifying enzymes. (G) Cluster analysis of genes encoding histones.

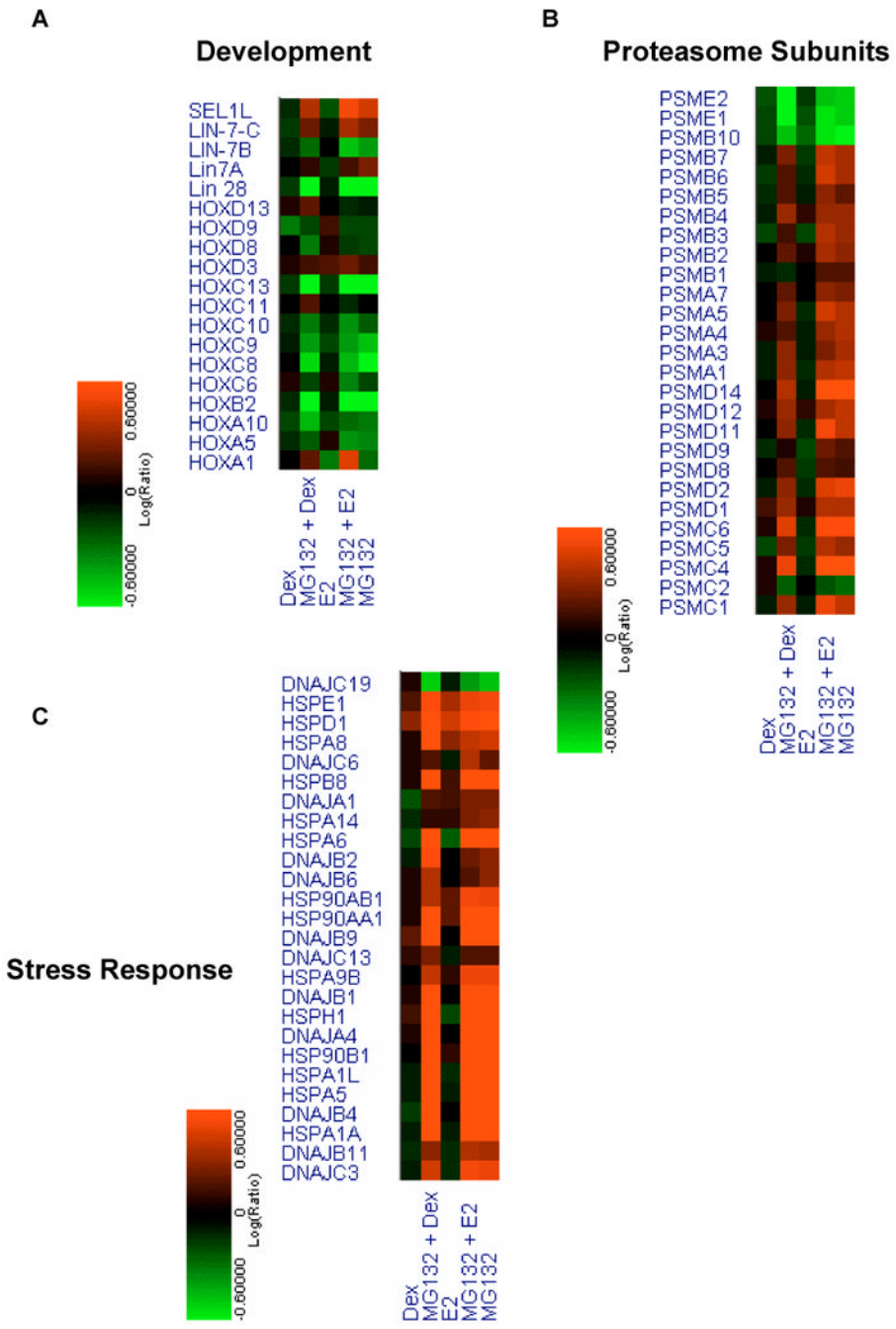


Figure 5.

Proteasome inhibition alters transcription of developmental, proteasome subunits and stress response genes. (A). Cluster analysis of genes encoding developmental genes. (B) Cluster analysis of genes encoding proteasome subunits. (C) Cluster analysis of genes encoding stress response proteins.

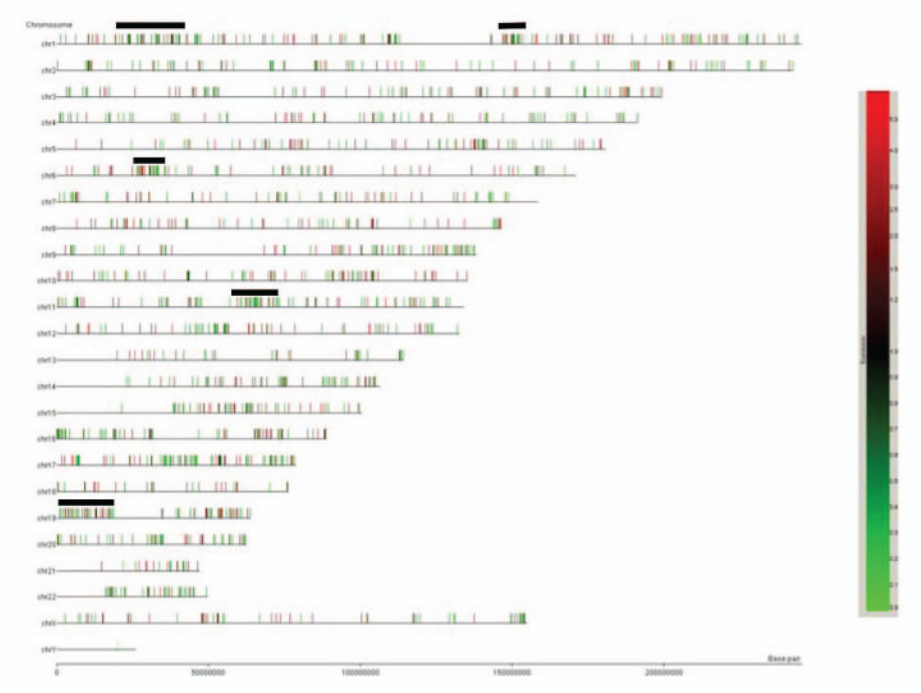


Figure 6.
Proteasome inhibition affects genes at specific chromosome loci. A chromogram showing genes affected by exclusively by proteasome inhibition (red up-regulated; green down-regulated). Proposed hot spots within chromosomes are indicated by a black line.

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Table 1

TABLE 1-1. Genes affected by treatment with dexamethasone only				
Gene	**Name**	**GenBank**	**DEX**	**MD**
HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2	NM_000196	14	2
AKR1D1	aldo-keto reductase family 1, member D1 (delta 4-3-ketosteroid-5-beta-reductase)	NM_005989	6	2
ASB9	ankyrin repeat and SOCS box-containing 9	NM_024087	5	-1
RANBP3L	RAN binding protein 3-like	NM_145000	5	-1
SCRG1	scrapie responsive protein 1	NM_007281	4	1
PDK4	pyruvate dehydrogenase kinase, isozyme 4	NM_002612	4	2
DUSP6	dual specificity phosphatase 6	NM_001946	4	1
UBE2E3	ubiquitin-conjugating enzyme E2E 3 (UBC4/5 homolog, yeast)	NM_006357	3	-1
MSX2	msh homeobox homolog 2 (Drosophila)	NM_002449	3	2
SRD5A1	steroid-5-alpha-reductase, alpha polypeptide 1 (3-oxo-5 alpha-steroid delta 4-dehydrogenase alpha 1)	NM_001047	3	1
BTG1	B-cell translocation gene 1, anti-proliferative	NM_00173	3	1
SAP30	Sin3A-associated protein, 30kDa	NM_003864	3	-1
TCEAL1	transcription elongation factor A (SII)-like 1	NM_001006640	3	-2
NRTN	neurturin	NM_004558	-5	-2
HNRPA2B1	heterogeneous nuclear ribonucleoprotein A2/B1	NM_002137	-3	1
MAGEA1	melanoma antigen family A, 1 (directs expression of antigen MZ2-E)	NM_004988	-3	-2
GSDMDC1	gasdermin domain containing 1	NM_024736	-3	1
I_936445	contains leucine repeat; Homo sapiens CAGH1 alternate open reading frame mRNA, complete cds.	U80760	-3	1
MAGED4	melanoma antigen family D, 4	NM_030801	-3	1
AMIGO1	adhesion molecule with Ig-like domain 1	NM_020703	-2	1

TABLE 1-2. Common genes changed synergistically by proteasome inhibitor and dexamethasone				
Gene	**Name**	**GenBank**	**DEX**	**MD**
CRYAB	Alpha B crystallin	NM_001885	4	81
KRT75	Cytokeratin type II	NM_004693	4	36
DUSP1	Dual Specificity Phosphatase 1	NM_004417	9	27
S100P	S100 calcium binding protein	NM_005980	12	26

TABLE 1-2. Common genes changed synergistically by proteasome inhibitor and dexamethasone				
Gene	**Name**	**GenBank**	**DEX**	**MD**
TIPARP	TCDD-inducible poly(ADP-ribose) polymerase	NM_015508	5	24
COL6A1	Collagen type VI, alpha 1	NM_001848	3	23
ANXA1	Annexin 1	NM_000700	4	21
NDRG1	N-myc downstream regulated gene 1	NM_006096	4	17
MAFB	Kreisler (musculoaponeurotic fibrosarcoma oncogene protein B	NM_005461	9	16
RGS2	Regulator of G protein	NM_002923	4	14
CYP4F2	cytochrome P450, family 4, subfamily F, polypeptide 2	NM_001082	2	13
IVL	involucrin	NM_005547	3	13
ELL2	RNA Pol II elongation factor 2	NM_012081	3	12
	Repressed by proteasome inhibitor and dexamethasone			
AMIGO2	adhesion molecule with Ig-like domain 2	NM_181847	-2	-57
CXXC4/IDAX	CXXC finger 4, Inhibition of the Dvl and Axin complex	NM_025212	-1	-36
OAS2	2-5-oligoadenylate synthetase 2	NM_016817	-3	-12
RTP4/IFRG28	Interferon-responsive protein 28 or Receptor transporting protein 4	NM_022147	-4	-11
CREB3L4/AIBZIP	Androgen-induced basic leucine zipper	NM_130898	-2	-9
FGF12	Fibroblast growth factor 12	NM_004113	-2	-10
IFITM1	Interferon-induced transmembrane protein 1	NM_003641	-2	-10
NCAM2	Neural cell adhesion molecule 2	U75330	-2	-10
TGFB3	Transforming growth factor-beta 3	NM_003239	-2	-6
HHAT	hedgehog acyltransferase	NM_018194	-2	-6
MAGEF1	Melanoma antigen F1	NM_022149	-1	-5
PIK3R3	Phosphoinositide-3-kinase regulatory subunit 3	NM_003629	-1	-5
PLK2	polo-like kinase 2 (Drosophila)	NM_006622	-1	-5
OLFM1	Olfactomedin 1	NM_006334	-2	-4
FEZ1	Fasciculation and elongation protein zeta 1	NM_005103	-2	-4
DDX58	Retinoic inducible gene 1, DDX58 (DEAD/DEAH) box, RNA helicase	NM_014314	-3	-4

TABLE 1-3. Common genes with an antagonistic response between proteasome inhibitor and dexamethasone				
Gene	**Name**	**GenBank**	**DEX**	**MD**
ZNF232	Zink-finger protein 232	NM_014519	-2	4

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TABLE 1-3. Common genes with an antagonistic response between proteasome inhibitor and dexamethasone				
Gene	**Name**	**GenBank**	**DEX**	**MD**
CBR3	Carbonyl reductase 3	NM_001236	-3	3
KRTHA3A	Keratin hair acidic 3A	NM_004138	-2	2
IFIT2	Interferon induced protein with tetra repeats 2	NM_001547	-2	2
S100A8	S100A8 calcium binding protein A8	NM_002964	2	-45
PIP	Prolactin-inducible protein	NM_002652	2	-8
SIDT1	SID1 transmembrane family, member 1	NM_017699	2	-7
EFHD1	EF-hand domain family, member D1	NM_025202	2	-6
SATB1	Special AT-rich sequence binding protein 1	NM_002971	2	-5
TARBP1	TAR (HIV) RNA binding protein 1	NM_005646	1	-3
EHMT1	Euchromatin histone methyl transferase 1	NM_024757	2	-2
GAL	galanin	NM_015973	23	7
I__958247	serine protease inhibitor	AK001520	19	13
PNMT	phenylethanolamine N-methyltransferase	NM_002686	18	4
SERPINA3	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	NM_001085	13	5
PRG1	proteoglycan 1, secretory granule	NM_002727	10	5
RBM24	RNA binding motif protein 24	NM_153020	6	3
BIRC3	baculoviral IAP repeat-containing 3	NM_001165	6	3
FAM105A	family with sequence similarity 105, member A	NM_019018	8	3
ELOVL5/HELO1	ELOVL family member 5, elongation of long chain fatty acids	NM_021814	9	3
FKBP5	FK506 binding protein 5	NM_004117	5	3
FLJ20366	hypothetical protein FLJ20366	NM_017786	8	3
BCL6	B-cell CLL/lymphoma 6 (zinc finger protein 51)	NM_138931	6	2
YAP1	Yes-associated protein 1, 65kDa	NM_006106	5	1
IFIH1	interferon induced with helicase C domain 1	NM_022168	-2	-1
NP	nucleoside phosphorylase	NM_000270	-2	-1
Unknown	Transcribed locus	BX113166	-2	-1

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Table 2

Table 2-1. Genes affected by treatment with 17β-estradiol only				
Gene	**Name**	**GenBank**	**E2**	**ME2**
EGR3	early growth response 3	NM_004430	7	1
NPY1R	neuropeptide Y receptor Y1	NM_000909	5	-1
RBBP8	retinoblastoma binding protein 8	NM_002894	4	1
FHL2	four and a half LIM domains 2	NM_201555	4	2
CMTM7	CKLF-like MARVEL transmembrane domain containing 7	NM_138410	3	-1
C12orf24	chromosome 12 open reading frame 24	NM_013300	3	-1
FAM77C	family with sequence similarity 77, member C	NM_024522	3	-1
PEG10	Homo sapiens MEF3L1 mRNA for MEF3 like 1, complete cds.	AB049150	3	-1
SLFN11	schlafen family member 11	NM_152270	3	-2
PDCD2L	programmed cell death 2-like	NM_032346	3	1
PDLIM3	PDZ and LIM domain 3	NM_014476	3	1
LRP8	low density lipoprotein receptor-related protein 8, apolipoprotein e receptor	NM_033300	3	1
DAF	Decay accelating factor	NM_000574.2	-4	1
TNFSF14	tumor necrosis factor (ligand) superfamily, member 14	NM_003807	-4	-2
ANKRD25	ankyrin repeat domain 25	AK000011	-3	-2
BCAM	basal cell adhesion molecule (Lutheran blood group)	NM_005581	-3	2
TSPAN10	tetraspanin 10	NM_031945	-3	2
PNRC1	proline-rich nuclear receptor coactivator 1	NM_006813	-3	1
TLR8	toll-like receptor 8	NM_016610	-3	1
H2AFA/H2AE	histone 1, H2ae	NM_021052	-2.3	-1.6
H2BFQ/H2BE	histone 2, H2be	NM_003528	-2.3	-1.0
GRHL1/LBP-32	grainyhead-like 1 (Drosophila)/Lamin Binding protein-32	NM_014552	-2.0	-1.5

Table 2-2 Common genes changed synergistically by proteasome inhibitor and 17β-estradiol				
Gene	**Name**	**GenBank**	**E2**	**ME2**
GEM	GTP binding protein overexpressed in skeletal muscle	NM_005261	3	24
TUBB2A	tubulin, beta 2A	NM_001069	2	16

Table 2-2 Common genes changed synergistically by proteasome inhibitor and 17β-estradiol				
Gene	**Name**	**GenBank**	**E2**	**ME2**
ISG20L1	interferon stimulated exonuclease gene 20kDa-like 1	NM_022767	3	7
MAFG	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	NM_002359	2	7
PPIF	peptidylprolyl isomerase F (cyclophilin F)	NM_005729	2	6
AGPAT5	1-acylglycerol-3-phosphate O-acyltransferase 5	NM_018361	2	6
CFL2	cofilin 2 (muscle)	NM_021914	2	6
DDX10	DEAD (Asp-Glu-Ala-Asp) box polypeptide 10	NM_004398	1	6
EMP3	epithelial membrane protein 3	NM_001425	2	6
	Repressed by proteasome inhibitor and 17β-estradiol			
AMIGO2	adhesion molecule with Ig-like domain 2	NM_181847	-2	-45
S100A8	S100 calcium binding protein A8	NM_002964	-3	-29
CXXC4/IDAX	CXXC finger 4	NM_025212	-3	-25
CDH10	cadherin 10, type 2 (T2-cadherin)	NM_006727	-4	-16
EFHD1	EF-hand domain family, member D1	NM_025202	-2	-11
SEPP1	selenoprotein P, plasma, 1	NM_005410	-4	-10
CREB3L4/AIBZIP	cAMP responsive element binding protein 3-like 4	NM_130898	-2	-10
LTBP1	latent transforming growth factor beta binding protein 1	NM_206943	-3	-9
SOX13	SRY (sex determining region Y)-box 13	NM_005686	-2	-9
PAPSS2	3′-phosphoadenosine 5′-phosphosulfate synthase 2	NM_001015880	-3	-8
FBP1	fructose-1,6-bisphosphatase 1	NM_000507	-2	-8
CRIP1	cysteine-rich protein 1 (intestinal)	NM_001311	-2	-8
NCAM2	neural cell adhesion molecule 2	U75330	-2	-7
KIAA1324	KIAA1324	NM_020775	-2	-6
HAIK1	Homo sapiens keratin 23, transcript variant 2, mRNA	NM_173213	-2	-6
EDN1	endothelin 1	NM_001955	-2	-6
PXMP4	peroxisomal membrane protein 4, 24kDa	NM_007238	-2	-6
CAMK2N1	calcium/calmodulin-dependent protein kinase II inhibitor 1	BC020630	-2	-6
CDC42EP4	CDC42 effector protein (Rho GTPase binding) 4	NM_012121	-2	-6
TXNIP	thioredoxin interacting protein	NM_006472	-4	-6

Table 2-3 Common genes with an antagonistic response between proteasome inhibitor and 17β-estradiol				
Gene	**Name**	**GenBank**	**E2**	**ME2**
LCN2	lipocalin 2 (oncogene 24p3)	NM_005564	-2	18
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	NM_001547	-2	8
TRIB3	tribbles homolog 3 (Drosophila)	NM_021158	-1	5
SEL1L	sel-1 suppressor of lin-12-like (C. elegans)	NM_005065	-1	3
USP53	ubiquitin specific peptidase 53	BC017382	-2	3
CLU	clusterin	NM_203339	-1	3
PLEKHM1	pleckstrin homology domain containing, family M (with RUN domain) member 1	NM_014798	-1	3
TMEM40	transmembrane protein 40	NM_018306	-1	2
FBXO8	F-box protein 8	NM_012180	-1	2
HBP1	HMG-box transcription factor 1	NM_012257	-2	2
RAGE	renal tumor antigen	NM_014226	-1	2
WDR45	WD repeat domain 45	NM_007075	-2	2
INSIG2	insulin induced gene 2	NM_016133	-2	2
WDR48	WD repeat domain 48	NM_020839	-1	2
HLA-B	major histocompatibility complex, class I, B	NM_005514	-2	2
AREG	amphiregulin (schwannoma-derived growth factor)	NM_001657	5	4
EREG	epiregulin	NM_001432	4	3
SPINK4	serine peptidase inhibitor, Kazal type 4	NM_014471	3	2
RBP7	retinol binding protein 7, cellular	NM_052960	3	2
PRKCE	protein kinase C, epsilon	NM_005400	3	2
METTL1	methyltransferase like 1	NM_005371	3	2
TUBA6	tubulin, alpha 6	NM_032704	2	1
COL12A1	collagen, type XII, alpha 1	NM_004370	3	-20
PGR	progesterone receptor	NM_000926	2	-6
SLC29A1	solute carrier family 29 (nucleoside transporters), member 1	NM_004955	2	-6
EBPL	emopamil binding protein-like	NM_032565	2	-5
UHRF1	ubiquitin-like, containing PHD and RING finger domains, 1	NM_013282	2	-5
ABAT	4-aminobutyrate aminotransferase	NM_020686	2	-4
TFRC	transferrin receptor (p90, CD71)	NM_003234	2	-4

Table 2-3 Common genes with an antagonistic response between proteasome inhibitor and 17β-estradiol				
Gene	**Name**	**GenBank**	**E2**	**ME2**
CISH	cytokine inducible SH2-containing protein	NM_145071	4	-4
MCM6	MCM6 minichromosome maintenance deficient 6 (MIS5 homolog, S. pombe)	NM_005915	2	-4
GNG11	guanine nucleotide binding protein (G protein), gamma 11	NM_004126	2	-4
CXCL12/SDF-1	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	NM_199168	12	-3
WHSC1	Wolf-Hirschhorn syndrome candidate 1	NM_133334	1	-2
OLFM1	olfactomedin 1	NM_006334	7	-2
TARBP1	Tar (HIV-1) RNA binding protein 1	NM_005646	1	-2
DNMT1	DNA (cytosine-5-)-methyltransferase 1	NM_001379	2	-2
TOM1L2	target of myb1-like 2 (chicken)	AK055959	-2	-1
ZNF791	zinc finger protein 791	NM_153358	-3	-2
ILDR1	immunoglobulin-like domain containing receptor 1	NM_175924	-3	-2

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Table 3

TABLE 3-1. Genes predominantly changed by proteasome inhibitor independent of either dexamethasone or 17β-estradiol					
Gene	**Name**	**GenBank**	**MD**	**ME2**	**MG**
RFC1	replication factor C (activator 1) 1, 145kDa	X75917	1.1	2.0	3.6
CALCA	Homo sapiens mRNA for calcitonin and calcitonin gene related peptide (CGRP).	X02330	1.7	2.0	3.5
AZI2	5-azacytidine induced 2	NM_022461	1.8	2.0	3.4
BICD2	bicaudal D homolog 2 (Drosophila)	NM_015250	1.6	2.5	3.3
KNG1	kininogen 1	NM_000893	2.5	1.8	3.2
RELN	reelin	NM_173054	2.9	1.0	3.2
CD44	CD44 molecule (Indian blood group)	NM_000610	2.3	2.4	3.2
DUSP12	dual specificity phosphatase 12	NM_007240	1.9	1.9	3.1
BCAM	basal cell adhesion molecule (Lutheran blood group)	NM_005581	1.2	2.1	3.1
NOX4	NADPH oxidase 4	NM_016931	-1.1	2.7	2.6
PSMD12	proteasome (prosome, macropain) 26S subunit, non-ATPase, 12	NM_002816	2.1	2.2	2.5
GADD45B	growth arrest and DNA-damage-inducible, beta	NM_015675	1.4	1.4	2.4
SLC18A3	solute carrier family 18 (vesicular acetylcholine), member 3	NM_003055	2.4	1.3	2.3
DNAJA5	DnaJ homology subfamily A member 5	NM_194283	1.8	1.8	2.3
TAF7	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa	NM_005642	1.7	1.7	2.3
PCAF	p300/CBP-associated factor	NM_003884	1.3	1.6	1.7
SMYD1	SET and MYND domain containing 1	NM_198274	1.1	1.2	1.5
PSMB1	proteasome (prosome, macropain) subunit, beta type, 1	NM_002793	-1.1	1.4	1.4
ZNF576	zinc finger protein 576	NM_024327	-1.8	-1.2	-4.0
ODF2	outer dense fiber of sperm tails 2	NM_002540	-2.8	-2.7	-3.9
EDG8	endothelial differentiation, sphingolipid G-protein-coupled receptor, 8	NM_030760	-1.8	-1.9	-3.9
PLEKHA5	pleckstrin homology domain containing, family A member 5	NM_019012	-2.3	-3.0	-3.7
DFNA5	deafness, autosomal dominant 5	NM_004403	-1.6	-1.0	-3.5
KLHL13	kelch-like 13 (Drosophila)	NM_033495	-2.9	-2.5	-3.4
ZNF319	zinc finger protein 319	NM_020807	-1.7	-2.6	-3.2
ZNF552	zinc finger protein 552	NM_024762	-1.5	-3.1	-2.5
BRCA1	breast cancer 1, early onset	NM_007295	-1.7	-1.9	-2.5

TABLE 3-1. Genes predominantly changed by proteasome inhibitor independent of either dexamethasone or 17β-estradiol					
Gene	**Name**	**GenBank**	**MD**	**ME2**	**MG**
SOX4	SRY (sex determining region Y)-box 4	NM_003107	-1.9	-2.1	-2.2
SAP30	Sin3A-associated protein, 30kDa	NM_003864	-1.1	-1.5	-2.2
METTL7A	methyltransferase like 7A	NM_014033	-1.9	-1.6	-1.7
CHD1L	chromodomain helicase DNA binding protein 1-like	NM_004284	1.0	-1.7	-1.7
JMJD2D	jumonji domain containing 2D	NM_018039	-1.1	-1.3	-1.7
JARID2	Jumonji, AT rich interactive domain 2	NM_004973	-1.0	-1.3	-1.4

TABLE 3-2. Genes predominantly changed by proteasome inhibitor, independent or dependent on dexamthasone				
Gene	**Name**	**GenBank**	**DEX**	**MD**
HSPA6	Heat shock protein 70kD 6	NM_002155	-1	45
KRT6C	Keratin 6A	NM_058242	2	25
KLF6/COPEB	Core promoter element binding protein	NM_001300	1	25
NCF2	Neutrophil cytosolic factor 2	NM_000433	1	22
ZNF121	Zinc Finger 121	NM_001008727	1	18
TCIRG1	T-cell immune regulator 1(V-ATPase 116kDa isoform a3)	NM_006019	1	17
TUBB2A	Beta_tubulin 2A	NM_001069	1	16
DDIT3	DNA damage inducible transcript 3	NM_004083	1	15
ATF3	Activating transcription factor 3	NM_004024	1	14
GDF15/PLAB	growth differentiation factor 15	NM_004864	1	13
DNAJB1	DNAJ (Hsp40)	NM_006145	1	13
BAG3	BCL2-associated anthanogene 3	NM_004281	1	12
LCE3E	Late cornified envelope 3E	NM_178435	1	12
GEM	Gene expressed in mitogen stimulated T-cells	NM_005261	1	12
MLLT11/AF1Q	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog) translocated to, 11	NM_006818	1	11
GADD45A	DNA damage inducible transcript 1	NM_001924	1	11
TRIM49/RNF18	tripartite motif-containing 49	NM_020358	1	8
MAFG	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	NM_002359	1	7
ZNF165	zinc finger protein 165	NM_003447	-1	6
NAP1L5	nucleosome assembly protein 1-like 5	NM_153757	-1	6

TABLE 3-2. Genes predominantly changed by proteasome inhibitor, independent or dependent on dexamthasone				
Gene	**Name**	**GenBank**	**DEX**	**MD**
ZNF770	zinc finger protein 770	NM_014106	1	5
CLIC3	Chloride intracellular channel 3	NM_004669	1	-25
SEMA3C	Semaphorin 3C	NM_006379	2	-22
ZNF467	Zinc finger 467–Inhibits components of RNA pol II and III transcription	BC038972	1	-20
COL12A1	Collagen type II alpha 1	NM_004370	1	-18
FAM113B	family with sequence similarity 113, member B11	NM_138371	1	-15
SLC40A1	solute carrier family 40 (iron-regulated transporter), member 1	NM_014585	-1	-13
IFITM2	Interferon induced transmembrane protein 2	NM_006435	1	-11
IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial	NM_002168	1	-11
PGR	Progegesterone receptor	NM_000926	1	-10
LIN28	lin-28 homolog (C. elegans)	NM_024674	-1	-9
BZW2	basic leucine zipper and W2 domains 2	NM_014038	1	-9
NR2F1	Nuclear receptor 2F1	AF087978	3	-9
SREBF1	Sterol regulatory element binding transcription factor 1	NM_001005291	1	-9
NFIB	nuclear factor I/B	NM_005596	-1	-9
TP53I3	tumor protein p53 inducible protein 3	NM_004881	-1	-8
ABCC6	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	BC050733	-1	-8
SOX13	SRY (sex determining region Y)-box 13	NM_005686	-1	-8
S100A4	S100 calcium-binding protein A4	NM_002961	-1	-7
CDH10	Cadherin 10	NM_006727	-1	-7
TCEA2	Transcription elongation factor A (SII) 2	NM_003195	-1	-2
TCEA3	Transcription elongation factor A (SII), 3	NM_003195	-1	-3

TABLE 3-3. Genes predominantly changed by proteasome inhibitor, independent or dependent on 17β-estradiol				
Gene	**Name**	**GenBank**	**E2**	**ME2**
DHRS10	dehydrogenase/reductase (SDR family) member 10	NM_016246	1	37
CRYAB	crystallin, alpha B	NM_001885	-2	34
ZNF121	zinc finger protein 121	NM_001008727	1	26
ATF3	activating transcription factor 3	NM_004024	1	24
DDIT3	DNA-damage-inducible transcript 3	NM_004083	-1	24

TABLE 3-3. Genes predominantly changed by proteasome inhibitor, independent or dependent on 17β-estradiol				
Gene	**Name**	**GenBank**	**E2**	**ME2**
NCF2	neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)	NM_000433	4	22
HSPA6	heat shock 70kDa protein 6 (HSP70B′)	NM_002155	-2	21
ARL14	ADP-ribosylation factor-like 14	NM_025047	-1	21
FTL	ferritin, light polypeptide	NM_000146	-1	18
MLLT11/AF1Q	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog); translocated to, 11	NM_006818	1	16
IL8	interleukin 8	NM_000584	1	15
GDF15/PLAB	growth differentiation factor 15	NM_004864	1	15
DDX43	DEAD (Asp-Glu-Ala-Asp) box polypeptide 43	NM_018665	1	15
KLF6/COPEB	Kruppel-like factor 6	NM_001300	1	13
HMOX1	heme oxygenase (decycling) 1	NM_002133	-1	12
GADD45A	growth arrest and DNA-damage-inducible, alpha	NM_001924	1	12
DDIT3	DNA-damage-inducible transcript 3	NM_004083	1	12
ACOXL	acyl-Coenzyme A oxidase-like	NM_018308	1	11
GABARAPL1	GABA(A) receptor-associated protein like 1	NM_031412	-1	11
SH3BGR	SH3 domain binding glutamic acid-rich protein	NM_007341	-1	11
FBXW10	F-box and WD-40 domain protein 10	NM_031456	-1	11
IFRD1	interferon-related developmental regulator 1	NM_001007245	1	11
RSAD2	radical S-adenosyl methionine domain containing 2	NM_080657	-1	10
GCLM	glutamate-cysteine ligase, modifier subunit	NM_002061	1	10
HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member1	NM_014685	-2	10
BAG3	BCL2-associated athanogene 3	NM_004281	-1	10
PELO	pelota homolog (Drosophila)	NM_015946	1	9
NDRG1	N-myc downstream regulated gene 1	NM_006096	-1	9
CLIC3	chloride intracellular channel 3	NM_004669	-1	-56
KCNMB4	potassium large conductance calcium-activated channel, subfamily M, beta member 4	NM_014505	1	-20
ZNF467	zinc finger protein 467	BC038972	-1	-20
FAM113B	family with sequence similarity 113, member B	NM_138371	-2	-19
SORL1	sortilin-related receptor, L(DLR class) A repeats-containing	NM_003105	1	-15
KCNS3	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	NM_002252	-1	-15

TABLE 3-3. Genes predominantly changed by proteasome inhibitor, independent or dependent on 17β-estradiol

Gene	Name	GenBank	E2	ME2
TMTC4	transmembrane and tetratricopeptide repeat containing 4	NM_032813	1	-14
TRPS1	trichorhinophalangeal syndrome I	NM_014112	-1	-13
BZW2	basic leucine zipper and W2 domains 2	NM_014038	-1	-13
SLC40A1	solute carrier family 40 (iron-regulated transporter), member 1	NM_014585	-2	-12
PIP	prolactin-induced protein	NM_002652	-1	-11
NFIB	nuclear factor I/B	NM_005596	-2	-11
IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial	NM_002168	-1	-10
IFITM2	interferon induced transmembrane protein 2 (1-8D)	NM_006435	-1	-10
NR2F1	Nuclear receptor subfamily 2, group F, member 1	AF087978	-2	-10
KCNK2	potassium channel, subfamily K, member 2	NM_001017424	-1	-10
SREBF1	sterol regulatory element binding transcription factor 1	NM_001005291	-1	-9
MARCKSL1	MARCKS-like 1	NM_023009	1	-9
B3GNT1	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 1	NM_006876	-1	-9
CKMT1B	creatine kinase, mitochondrial 1B	NM_020990	-1	-9
IFITM1	interferon induced transmembrane protein 1 (9-27)	NM_003641	-2	-9
LIN28	lin-28 homolog (C. elegans)	NM_024674	-1	-9
EPN3	epsin 3	NM_017957	-1	-9
PQLC3	PQ loop repeat containing 3	NM_152391	-2	-8
KREMEN2	Homo sapiens kringle containing transmembrane protein 2, transcript variant 3, mRNA	NM_145348	1	-8
NR2F2	nuclear receptor subfamily 2, group F, member 2	NM_021005	-1	-8
DBI	diazepam binding inhibitor (GABA receptor modulator, acyl-Coenzyme A binding protein)	NM_020548	-1	-8
SEMA3C	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C	NM_006379	-1	-8
SERTAD4	SERTA domain containing 4	NM_019605	1	-8
EMP2	epithelial membrane protein 2	NM_001424	1	-8
PLK2/SNK	polo-like kinase 2 (Drosophila)	NM_006622	-2	-8
KRT19	keratin 19	NM_002276	1	-7
INHBB	inhibin, beta B (activin AB beta polypeptide)	NM_002193	-1	-7
S100A4	S100 calcium binding protein A4	NM_002961	-2	-7
TCEA3	transcription elongation factor A (SII), 3	NM_003196	-2	-3

TABLE 3-3. Genes predominantly changed by proteasome inhibitor, independent or dependent on 17β-estradiol					
Gene	**Name**	**GenBank**	**E2**	**ME2**	
TCEA2	transcription elongation factor A (SII), 2	NM_003195	-1	-2	

TABLE 3-4. Common genes between proteasome inhibitor and proteasome inhibitor and hormone					
Gene	**Name**	**GenBank**	**MD**	**ME2**	**MG**
CRYAB	crystallin, alpha B	NM_001885	81	34	47
NCF2	neutrophil cytosolic factor 2	NM_000433	22	22	42
HSPA6	heat shock 70kDa protein 6 (HSP70B′)	NM_002155	45	21	36
ATF3	activating transcription factor 3	NM_004024	14	24	29
ZNF121	zinc finger protein 121	NM_001008727	18	26	28
DDIT3	DNA-damage-inducible transcript 3	NM_004083	15	24	26
GEM	GTP binding protein overexpressed in skeletal muscle	NM_005261	12	24	26
MLLT11/AF1Q	myeloid/lymphoid or MLL (trithorax homolog) translocated to, 11	NM_006818	11	16	24
ARL14	ADP-ribosylation factor-like 14	NM_025047	3	21	22
HMOX1	heme oxygenase (decycling) 1	NM_002133	14	12	17
FTL	ferritin, light polypeptide	NM_000146	12	18	17
LCN2	lipocalin 2 (oncogene 24p3)	NM_005564	4	18	16
TUBB2A	tubulin, beta 2A	NM_001069	16	16	16
ACOXL	acyl-Coenzyme A oxidase-like	NM_018308	3	11	15
HERPUD1	homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1	NM_014685	10	10	14
KLF6	Kruppel-like factor 6	NM_001300	25	13	14
GDF15/PLAB	growth differentiation factor 15	NM_004864	13	15	13
GADD45A	growth arrest and DNA-damage-inducible, alpha	NM_001924	11	12	12
IL8	interleukin 8	NM_000584	2	15	12
DDIT3	DNA-damage-inducible transcript 3	NM_004083	7	12	10
PRNP	prion protein (p27-30) (Creutzfeldt-Jakob disease, Gerstmann-Strausler-Scheinker syndrome, fatal familial insomnia)	NM_000311	8	9	10
NDRG1	N-myc downstream regulated gene 1	NM_006096	17	9	9
MAFG	v-maf musculoaponeurotic fibrosarcoma oncogene homolog G (avian)	NM_002359	7	7	9
DUSP1	dual specificity phosphatase 1	NM_004417	27	5	9
BAG3	BCL2-associated athanogene 3	NM_004281	12	10	9
CHD1	chromodomain helicase DNA binding protein 1	NM_001270	2	2	2

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TABLE 3-4. Common genes between proteasome inhibitor and proteasome inhibitor and hormone

Gene	Name	GenBank	MD	ME2	MG
AMIGO2	adhesion molecule with Ig-like domain 2	NM_181847	-57	-45	-52
CLIC3	chloride intracellular channel 3	NM_004669	-25	-56	-47
CXXC4/IDAX	CXXC finger 4	NM_025212	-36	-25	-42
S100A8	S100 calcium binding protein A8	NM_002964	-45	-29	-34
NCAM2	neural cell adhesion molecule 2	U75330	-9	-7	-17
COL12A1	collagen, type XII, alpha 1	NM_004370	-18	-20	-16
ZNF467	zinc finger protein 467	BC038972	-20	-20	-15
TMTC4	transmembrane and tetratricopeptide repeat containing 4	NM_032813	-11	-14	-14
NFIB	nuclear factor I/B	NM_005596	-9	-11	-14
KCNMB4	potassium large conductance calcium-activated channel, subfamily M, beta member 4	NM_014505	-10	-20	-13
SLC40A1	solute carrier family 40 (iron-regulated transporter), member 1	NM_014585	-13	-12	-13
KCNS3	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 3	NM_002252	-11	-15	-13
CDH10	cadherin 10, type 2 (T2-cadherin)	NM_006727	-7	-16	-12
PIP	prolactin-induced protein	NM_002652	-8	-11	-12
BZW2	basic leucine zipper and W2 domains 2	NM_014038	-9	-13	-10
SREBF1	sterol regulatory element binding transcription factor 1	NM_001005291	-9	-9	-10
FAM113B	family with sequence similarity 113, member B	NM_138371	-15	-19	-10
SERTAD4	SERTA domain containing 4	NM_019605	-8	-8	-10
CREB3L4/AIBZIP	cAMP responsive element binding protein 3-like 4	NM_130898	-10	-10	-10
LIN28	lin-28 homolog (C. elegans)	NM_024674	-9	-9	-10
IFITM2	interferon induced transmembrane protein 2 (1-8D)	NM_006435	-11	-10	-9
IDH2	isocitrate dehydrogenase 2 (NADP+), mitochondrial	NM_002168	-11	-10	-9
MARCKSL1	MARCKS-like 1	NM_023009	-8	-9	-9
PGR	progesterone receptor	NM_000926	-10	-6	-8
SOX13	SRY (sex determining region Y)-box 13	NM_005686	-8	-9	-8
LTBP1	latent transforming growth factor beta binding protein 1	NM_206943	-10	-9	-8
ABCC6	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	BC050733	-8	-6	-8
NR2F2	nuclear receptor subfamily 2, group F, member 2	NM_021005	-5	-8	-7
NR2F1	Nuclear receptor subfamily 2, group F, member 1	AF087978	-9	-10	-6

TABLE 3-4. Common genes between proteasome inhibitor and proteasome inhibitor and hormone

Gene	Name	GenBank	MD	ME2	MG
SMYD2	SET and MYND domain containing 2	NM_020197	-3	-4	-4
TARBP1	Tar (HIV-1) RNA binding protein 1	NM_005646	-3	-2	-2

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Table 4

TABLE 4-1 Effect of proteasome inhibition on transcripts encoding RNA Polymerase II regulatory factors

Gene	Name	GenBank	DEX	MD	E2	ME2	MG
ELL2	elongation factor, RNA polymerase II, 2	NM_012081	2.8	11.9	-1.3	6.2	5.7
TAF13	TAF13 RNA polymerase II, TATA box binding protein-associated factor, 18kDa	NM_005645	1.1	4.8	1.2	3.3	3.1
MED10	mediator of RNA polymerase II transcription, subunit 10 homolog	NM_032286	1.2	3.7	1.3	2.6	3.5
TAF1A	TATA box binding protein (TBP)-associated factor, RNA polymerase I, A, 48kDa	NM_005681	1.1	2.2	1.4	2.9	3.2
TAF9	TAF9 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 32kDa	NM_001015891	1.1	2.0	1.1	2.7	2.6
ELL2	elongation factor, RNA polymerase II, 2	BX538289	1.2	1.7			1.3
SSU72	SSU72 RNA polymerase II CTD phosphatase homolog (S. cerevisiae)	NM_014188	-1.1	-2.3		-1.5	-1.8
TAF10	TAF10 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 30kDa	NM_006284	1.0	-2.4		-1.9	-2.6
CTDSP1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1	NM_021198	-1.3	-2.7	-1.4	-2.6	-2.9
CTDSPL	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase-like	NM_001008392	-1.0	-2.7		-2.4	-3.8
TAF1B	TATA box binding protein associated factor, RNA polymerase I, B, 63kDa	NM_005680	-1.1	-3.3	-1.2	-1.9	-2.3
ELL3	elongation factor RNA polymerase II-like 3	NM_025165	-1.0	-3.4	-1.1	-3.3	-3.2
TAF2	TAF2 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 150kDa	NM_003184			-1.0	2.4	2.6
MED6	mediator of RNA polymerase II transcription, subunit 6 homolog (S. cerevisiae)	NM_005466			-1.0	1.8	1.1
MED28	Mediator of RNA polymerase II transcription, subunit 28 homolog (S. cerevisiae)	AF317680			1.3	1.7	1.8
CTDP1	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) phosphatase, subunit 1	NM_004715			-1.6	1.7	1.9
ELL	elongation factor RNA polymerase II	NM_006532			1.1	1.4	1.5
CDC73/Paf1	cell division cycle 73, Paf1/RNA polymerase II complex component, homolog (S. cerevisiae)	NM_024529			-1.1	1.4	1.5
ELL	elongation factor RNA polymerase II	NM_006532			-1.0	-1.4	-1.1
BTAF1	BTAF1 RNA polymerase II, B-TFIID transcription factor-associated, 170kDa (Mot1 homolog, S. cerevisiae)	NM_003972	-1.1	1.7	1.1	1.7	1.8
BRF2	BRF2, subunit of RNA polymerase III transcription initiation factor, BRF1-like	NM_018310	1.0	3.2	-1.1	4.4	3.9
BRF1	BRF1 homolog, subunit of RNA polymerase III transcription initiation factor IIIB (S. cerevisiae)	NM_145685			-1.8	-1.0	
TAF7	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 55kDa	NM_005642		1.7		1.7	2.3
CCNK	cyclin K	NM_003858	1.1	1.6	-1.2	2.2	
CDK9	cyclin-dependent kinase 9 (CDC2-related kinase)	NM_001261			-1.5	2.0	

TABLE 4-2. Effect of proteasome inhibition on transcription elongation and translation initiation factors							
Gene	**Name**	**GenBank**	**DEX**	**MD**	**E2**	**ME2**	**MG**
TCEAL1	transcription elongation factor A (SII)-like 1	NM_001006640	2.5	-2.1			
TCEAL4	transcription elongation factor A (SII)-like 4	NM_024863	1.8	-1.7			
TCEA1	transcription elongation factor A (SII), 1	NM_006756	1.5	1.4			
TCEA2	transcription elongation factor A (SII), 2	NM_003195	-1.2	-1.8	-1.2	-1.9	-1.8
TCEA3	transcription elongation factor A (SII), 3	NM_003196	-1.3	-2.8	-1.5	-3.1	-4.1
TCEAL8	transcription elongation factor A (SII)-like 8	NM_153333	-1.2	-2.9	1.1	-1.9	-2.4
TCEAL5	transcription elongation factor A (SII)-like 5	NM_001012979	1.4	-3.4	1.2	-3.3	-3.1
EEF1B2	eukaryotic translation elongation factor 1 beta 2	NM_001959			1.5	1.1	
TCEB3	transcription elongation factor B (SIII), polypeptide 3 (elongin A)	NM_003198			-1.4	1.8	1.9
EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	NM_004280			1.6	1.7	1.8
EEFSEC	eukaryotic elongation factor, selenocysteine-tRNA-specific	NM_021937			1.3	-2.7	-2.1
TCERG1	transcription elongation regulator 1	NM_0067061		1.3		1.6	1.5
EIF5	eukaryotic translation initiation factor 5	NM_001969	-1.1	2.8	1.2	3.0	3.1
EIF1B	eukaryotic translation initiation factor 1B	NM_005875	1.2	2.7	-1.0	4.0	4.4
EIF2AK3	eukaryotic translation initiation factor 2-alpha kinase 3	NM_004836	1.2	2.6	1.3	3.2	3.0
EIF1	eukaryotic translation initiation factor 1	NM_005801	1.3	2.5	1.3	3.2	3.1
EIF2A	eukaryotic translation initiation factor 2A, 65kDa	NM_032025	1.3	2.3	1.3	2.6	
EIF2B2	eukaryotic translation initiation factor 2B, subunit 2 beta, 39kDa	NM_014239	1.2	1.8	-1.1	1.6	
EIF2AK2	eukaryotic translation initiation factor 2-alpha kinase 2	NM_002759	-1.4	-1.6			-1.0
EIF4G2	eukaryotic translation initiation factor 4 gamma, 2	NM_001418	-1.2	-1.9			-1.1
EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2	NM_004096	1.4	-3.0			
EIF3S1	eukaryotic translation initiation factor 3, subunit 1 alpha, 35kDa	NM_003758			1.7	2.2	2.1
EEF1B2	eukaryotic translation elongation factor 1 beta 2	NM_001959			1.5	1.1	
EIF2AK3	eukaryotic translation initiation factor 2-alpha kinase 3	NM_004836			1.3	3.2	
EIF1	eukaryotic translation initiation factor 1	NM_005801	1.2	2.5	1.3	3.1	3.1
EIF3S6	eukaryotic translation initiation factor 3, subunit 6 48kDa	NM_001568			1.7	2.1	1.8
EIF2S2	eukaryotic translation initiation factor 2, subunit 2 beta, 38kDa	NM_003908			1.5	1.9	1.7
EIF2S1	eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa	NM_004094			1.5	1.9	1.7
EEF1E1	eukaryotic translation elongation factor 1 epsilon 1	NM_004280			1.5	1.7	1.8

TABLE 4-2. Effect of proteasome inhibition on transcription elongation and translation initiation factors							
Gene	**Name**	**GenBank**	**DEX**	**MD**	**E2**	**ME2**	**MG**
EIF3S2	eukaryotic translation initiation factor 3, subunit 2 beta, 36kDa	NM_003757			-1.0	1.6	1.7
EIF4G3	eukaryotic translation initiation factor 4 gamma, 3	NM_003760			1.0	1.5	1.2
EIF4EBP2	eukaryotic translation initiation factor 4E binding protein 2	NM_004096			1.3	-3.9	
EIF2C3	eukaryotic translation initiation factor 2C, 3	NM_024852		1.4		1.5	1.8

Table 4-3. Effect of proteasome inhibition on nuclear receptor co-regulators							
Gene	**Name**	**GenBank**	**DEX**	**MG**	**E2**	**ME2**	**MG**
NAT5	N-acetyltransferase 5	NM_016100			1.2	2.9	2.7
NAT13	N-acetyltransferase 13	NM_025146		1.95	1.3	2.3	2.2
NAT1	N-acetyltransferase 1 (arylamine N-acetyltransferase)	NM_000662			-1.2	-1.8	-1.8
NAT11	N-acetyltransferase 11	NM_024771		-1.4		-1.1	-1.2
HDAC3	histone deacetylase 3	NM_003883	-1.8	1.3			
SAP30	Sin3A-associated protein, 30kDa	NM_003864	2.6	-1.1			
HDAC8	histone deacetylase 8	NM_018486	1.3	-1.6			-1.4
HDAC1	histone deacetylase 1	NM_004964	-1.1	-2.5			-1.5
NCOR2	nuclear receptor co-repressor 2	NM_006312	-1.2	-1.8	-1.0	-2.1	-2.2
NRIP3	nuclear receptor interacting protein 3	NM_020645	1.1	2.0	1.1	2.7	3.0
NCOA6	nuclear receptor coactivator 6	NM_014071			-1.0	1.5	1.3
NCOA1	nuclear receptor coactivator 1	NM_147223	1.9	-1.4			
NCOA7	nuclear receptor coactivator 7	NM_181782	-1.0	1.5			1.6
NCOA5	nuclear receptor coactivator 5	NM_020967	-1.2	-1.5			-1.2
PNRC2	proline-rich nuclear receptor coactivator 2	NM_017761	-1.3	-1.6			-1.4
TRIP4	thyroid hormone receptor interactor 4	NM_016213	-1.0	1.7	-1.4	1.6	1.9
TRIP12	thyroid hormone receptor interactor 12	NM_004238	1.1	1.6			1.6
TRIP13	thyroid hormone receptor interactor 13	NM_004237			2.1	-1.5	

TABLE 4-4. Effect of proteasome inhibition on histone and DNA modifying enzymes.							
Gene	**Name**	**GenBank**	**DEX**	**MD**	**E2**	**ME2**	**MG**
EHMT1	euchromatic histone-lysine N-methyltransferase 1	NM_024757	1.8	-2.3			-1.5
METTL1	methyltransferase like 1	NM_005371	1.2	1.8	2.5	1.7	1.7

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TABLE 4-4. Effect of proteasome inhibition on histone and DNA modifying enzymes.

Gene	Name	GenBank	DEX	MD	E2	ME2	MG
SETD7	SET domain containing (lysine methyltransferase) 7	NM_030648	-1.0	1.6			1.6
PRMT8	protein arginine methyltransferase 8	NM_019854	1.0	-1.4	-1.1	-1.5	-1.6
EHMT2	euchromatic histone-lysine N-methyltransferase 2	NM_006709	-1.2	-1.6	-1.1	-1.7	-1.5
METTL9	methyltransferase like 9	NM_016025	-1.0	-1.7			-1.5
DNMT1	DNA (cytosine-5-)-methyltransferase 1	NM_001379	-1.3	-2.0	1.8	-1.9	-2.3
PRMT6	protein arginine methyltransferase 6	NM_018137	-1.0	-5.1	-1.1	-4.0	-5.3
DNMT3B	DNA (cytosine-5-)-methyltransferase 3 beta	NM_175850	-1.1	-5.6	-1.2	-3.9	-4.3
JMJD1A	jumonji domain containing 1A	NM_018433	1.2	2.9		2.0	2.8
MLLT2/AFF1	AF4/FMR2 family, member 1	NM_005935	1.6	2.8	1.0	5.3	4.4
PRMT3	protein arginine methyltransferase 3	NM_005788			1.1	-1.4	-1.1
DNMT3L	DNA (cytosine-5-)-methyltransferase 3-like	NM_013369			-1.1	-1.6	-1.1
METTL7A	methyltransferase like 7A	NM_014033		-1.9		-1.6	-1.7
SETD1A	SET domain containing 1A	NM_014712			-1.1	1.8	1.8
SETDB1	SET domain, bifurcated 1	NM_012432			-1.2	1.7	1.8
SUV39H2	suppressor of variegation 3-9 homolog 2 (Drosophila)	NM_024670	-1.2	-2.1		-1.8	-1.8
MLLT11/AF1Q	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 11	NM_006818	1.1	11	1.2	16.3	23.6
MLLT3/AF9	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 3	NM_004529	1.1	-1.7			-1.7
MLLT1/ENL	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila); translocated to, 1	NM_005934			1.5	-2.6	-2.1
RBBP2	Jumonji, AT rich interactive domain 1A (RBBP2-like)	AF090884			1.0	-2.2	
JARID2	Jumonji, AT rich interactive domain 2	NM_004973		-1.0		-1.3	-1.4
JMJD2D	jumonji domain containing 2D	NM_018039		-1.1		-1.3	-1.8
WHSC1/NSD2/MMSET	Wolf-Hirschhorn syndrome candidate 1	NM_133334	-1.0	-2.3	1.4	-2.4	-1.9
WHSC1L1/NSD3	Wolf-Hirschhorn syndrome candidate 1-like 1	BG680979			-2.0	-3.3	-4.6
HIP2	huntingtin interacting protein 2	NM_005339	1.3	1.7			2.1
SMYD1	SET and MYND domain containing 1	NM_198274		1.1		1.2	1.5
SMYD2	SET and MYND domain containing 2	NM_020197	1.6	-2.6	-1.1	-4.3	-4.4

TABLE 4-5. Proteasome inhibition alters transcription of histone genes

Gene	Name	GenBank	DEX	MD	E2	ME2	MG
H2BFC	H2B histone family member C	NM_003519	-1.3	-1.8	-1.4	-1.3	-1.7

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TABLE 4-5. Proteasome inhibition alters transcription of histone genes

Gene	Name	GenBank	DEX	MD	E2	ME2	MG
H3FI	H3 histone family member I	NM_003530	-1.0	-1.9	-1.3	-1.5	-1.6
H2A_S	H2A histone family member,	NM_080596	-1.2	-2.8	-2.0	-1.6	-2.1
H2BFF	H2B histone family member F	NM_021062	-1.4	-2.8	-1.5	-1.6	-2.0
H2AFL	Testis specific H2A histone family	NM_003512	-1.0	-3.5	-2.3	-1.8	-2.3
H3FT	H3 histone family member	NM_003493	-1.1	-1.9	-1.2	-1.5	-1.6
H2BFD	H2B histone family member D	NM_080593	-1.3	-2.3	-1.2	-1.6	-1.9
H2AFY2	A subtype of histone H2A that contains a unique non-histone domain	NM_018649	1.1	-2.8	1.4	-2.3	-2.6
H1F4	H1 histone family member 4, may maintain a low methylation state in CpG-rich DNA and linker DNA, a role in DNA accessibility during apoptotic DNA fragmentation	NM_005321	-1.4	-4.7	-1.1	-2.3	-3.8
H2BFH	H2B histone family member H	NM_003523	-1.3	-2.7	-1.3	-1.4	-1.8
H2AFA	H2A histone family member A	NM_021052	-1.2	-2.8	-2.3	-1.6	-2.8
H2BFQ	H2B histone family member Q	NM-003528			-2.3	-1.0	
H2AFE	H2A histone family member E	NM_021066	-1.2	-1.9			-1.9
H2BFE	H2B histone family member E	NM_003521	-1.4	-2.1			
H2BFB	H2B histone family member B	NM_021063	-1.3	-2.6			-1.9
H2BFK	H2B histone family member K Expression is likely replication-dependent	NM_003525	-1.4	-2.7			-1.9
H2AFO	H2A histone family member O	NM_003516	1.3	-3.0			-2.5
H2AFJ	Protein with strong similarity to human H2AFX	NM_177925	-1.2	-2.0			
H1F0	H1(0)-type member of the H1 histone family	NM_005318	1.1	-1.6			-1.5
H2BFS	Protein with high similarity to histone H2B	NM_017445	-1.4	-1.7			

Table 5

Table 5-1. Proteasome inhibition affects transcription of genes involved in development							
Gene	**Name**	**GenBank**	**DEX**	**MD**	**E2**	**ME2**	**MG**
HOXA1	homeobox A1	NM_153620			-1.8	2.9	-1.7
HOXA5	homeobox A5	NM_019102			1.1	-2.0	-1.8
HOXA10	homeobox A10	NM_018951	-1.3	-2.4			-1.8
HOXB2	homeobox B2	NM_002145	-1.1	-4.9	-1.0	-4.5	-3.5
HOXC6	homeobox C6	NM_153693			1.1	-1.9	-1.3
HOXC8	homeobox C8	NM_022658	1.0	-3.1			-5.6
HOXC9	homeobox C9	NM_006897	-1.1	-2.1	-1.3	-2.1	-2.7
HOXC10	homeobox C10	NM_017409	-1.1	-1.7	-1.1	-2.0	-1.5
HOXC11	homeobox C11	NM_014212	1.0	1.4			1.1
HOXC13	homeobox C13	NM_017410	-1.2	-4.4	-1.2	-4.3	-4.9
HOXD3	homeobox D3	NM_006898			1.4	1.6	1.3
HOXD8	homeobox D8	NM_019558	1.0	-1.8			-1.3
HOXD9	homeobox D9	NM_014213	-1.7	-1.3			
HOXD13	homeobox D13	NM_000523	1.1	1.5			1.5
LIN28	lin-28 homolog (C. elegans)	NM_024674	-1.2	-9.4	-1.0	-9.2	-9.7
LIN7A	lin-7 homolog A (C. elegans)	NM_004664		1.2		1.3	1.7
LIN7C	lin-7 homolog C (C. elegans)	NM_018362		1.6		2.2	1.7
LIN7B	lin-7 homolog B (C. elegans)	NM_022165		-1.6		-2.7	-2.1
SEL1L	sel-1 suppressor of lin-12-like (C. elegans)	NM_0050615	-1.1	2.4	-1.4	3.4	2.8

TABLE 5-2. Proteasome inhibition affects transcription of proteasome subunits								
Gene	**Name**	**GenBank**	**Other names**	**DEX**	**MD**	**E2**	**ME2**	**MG**
PSMC1	Proteasome 26S subunit ATPase 1, interacts with the papilloma virus oncoprotein E7	NM_002802	S4, Rpt2	-1	1.9	-1.0	3.0	2.5
PSMC2	26S protease regulatory subunit 7, ATPase subunit of the 26S proteasome, interacts with Tat protein, may be involved in cell cycle control and has a role in the activation of human immunodeficiency virus-1 (HIV-1) gene transcription	NM_002803	S7, MSS1		-1.5			-1.6
PSMC4	Proteasome (prosome, macropain) 26S subunit ATPase 4, interacts with an orphan nuclear hormone receptor and with HIV tat protein	NM_006503	S6,Rpt3,TBP7	1	3.2	-1	4.5	4.0

TABLE 5-2. Proteasome inhibition affects transcription of proteasome subunits

Gene	Name	GenBank	Other names	DEX	MD	E2	ME2	MG
PSMC5	ATPase subunit 5 of the 26S proteasome, which is a multicatalytic proteinase complex involved in cellular protein degradation; may also function as a transcriptional modulator	NM_002805	S8,Rpt6,Sug1, TBP10,Trip1	-1	1.7	-1	2.3	2.0
PSMC6	Proteasome (prosome, macropain 26S subunit ATPase 6, may be involved in spermatogenesis	NM_002806	S10b,Sug2,Rpt4	1	2.8	-1	3.7	3.7
PSMD1	Proteasome (prosome, macropain) 26S subunit (non-ATPase,1)	NM_002807	Sen3,S1, p112, Rpn1	1	2.0	1	2.4	2.3
PSMD2	Proteasome 26S non-ATPase subunit 2	NM_002808	S2,Rpn2, TRAP2	-1	2	-1	2.9	3.1
PSMD8	Non-ATPase subunit 8 of the 26S proteasome (prosome macropain), may play a role in regulating the cell cycle	NM_002812	S14,HIP6,HYPF,p31	1	1.4			1.3
PSMD9	Non-ATPase subunit 9 of the 26S	NM_002813	p27-L			-1	1.7	1.4
PSMD11	Subunit 9 of the 26S proteasome (proteasome 26S subunit non-ATPase 11), a non-ATPase subunit of the 19S	NM_002815	S9,Rpn6,Trip15			-1	3.4	2.5
PSMD12	Proteasome (prosome, macropain) 26S subunit (non-ATPase, 12), a regulatory subunit of the 26S proteasome				2.1			2.5
PSMD14	26S proteasome-associated pad1 homolog, a subunit of the 26S proteasome, confers multidrug resistance and resistance to ultraviolet light when overexpressed	NM_005805	Rpn11, POH1, PAD1	1	2.3	-1	4.5	3.9
PSMA1	Alpha type 1 proteasome (prosome, macropain) subunit	NM_148976	NU,HC2,Pros30		1.9	-1	2.3	2.5
PSMA3	Proteasome subunit alpha type 3, the C8 subunit of the 20S core proteasome, which is a multicatalytic proteinase complex involved in cellular protein degradation; expression is increased in skeletal muscle of slim AIDS patients	NM_002788	HC8,PSC3, pre10	-1	1.8			2.2
PSMA4	Proteasome subunit alpha type 4, expressed at abnormally high levels in renal carcinomas	NM_002789	Pre9, Prs5,HC9			-1	2.0	2.3
PSMA5	Proteasome subunit alpha type 5	NM_002790	PSC5, ZETA	1	1.7	-1	2.6	2.3
PSMA7	Proteasome (prosome, macropain) subunit (alpha type) 7, a subunit of the 20S core proteasome, a target of hepatitis B virus X protein; may be involved in pathogenesis of pancreatic cancer	NM_152255	Pre6, XAPC7, RC6-1, HSPC	-1	1.5	1	1.9	1.8
PSMB1	Proteasome subunit beta type 1, the C5 subunit of the proteasome, which is a multicatalytic proteinase complex involved in cellular protein degradation, interacts with Alzheimers disease associated protein, presenilin 1 (PSEN1)	NM_002793	HC5,PSC5		-1			1.4
PSMB2	Proteasome subunit beta type 2, putative beta type subunit of the 20S proteolytic core of proteasomes, which are multicatalytic proteinase complexes involved in cellular protein degradation	NM_002794	Pre1, HC7-1		1.5	1	2.1	1.9
PSMB3	Beta subunit 3 of the proteasome core, which is a multicatalytic protease complex involved in cellular protein degradation	NM_002795	Theta,Pup3, HC10-II			-1	2.3	2.0
PSMB4	Proteasome subunit, beta type, 4, binds human immunodeficiency virus type 1 Nef protein	NM_002796	Pre4, PROS26		1.9	1	2.1	2.0
PSMB5	Proteasome (prosome, macropain) subunit beta type 5, may be inhibited by the HIV1 protease inhibitor Ritonavir	NM_002797	pPre2, Lmp17X, MB1, LMPX			-1.0	1.8	1.5
PSMB6	Proteasome (prosome, macropain) subunit beta 6	NM_002798	Pre3, DELTA, LMPY			-1	2.6	2.1
PSMB7	Proteasome (prosome, macropain) subunit beta 7, a subunit of the 26S proteasome, replaced by PSMB10 upon interferon gamma (IFNG) stimulation	NM_002799	LMP19, Pup1,Z	-1	1.7	-1	2.6	2.1

TABLE 5-2. Proteasome inhibition affects transcription of proteasome subunits								
Gene	**Name**	**GenBank**	**Other names**	**DEX**	**MD**	**E2**	**ME2**	**MG**
PSMB10	Proteasome (prosome, macropain) subunit beta type 10, involved in protein degradation and the generation of peptides presented by MHC class I molecules	NM_002801	LMP10,MECL-1	-1	-2.7	-2	-3.2	-4.3
PSME1	Proteasome activator alpha subunit (P28 alpha), activates the 20S proteasome and plays a role in antigen presentation by enhancing the generation of MHC class I binding peptides, expression is induced by IFN-gamma (IFNG)	NM_006263	PA28Alpha, REG alpha	-1	-4.2	-1	-3.3	-2.8
PSME2	Beta subunit of the PA28 proteasome activator, binds to the proteasome complex and enhances the generation of MHC class I binding peptides	NM_002818	REGbeta,PA28beta	-1	-4.8	-1	-2.7	-3.0

Table 5-3. Proteasome inhibition affects transcription of heat shock protein genes							
Gene	**Name**	**GenBank**	**DEX**	**MD**	**E2**	**ME2**	**MG**
HSPD1	heat shock 60kDa protein 1 (chaperonin)	NM_002156	1.8	4.7	2.8	3.6	4.8
HSPA6	heat shock 70kDa protein 6 (HSP70B′)	NM_002155	-1.3	45.2	-1.5	21.4	41.8
HSP90AA1	heat shock protein 90kDa alpha (cytosolic), class A member 1	NM_005348	1.1	7.6	1.5	7.6	6.1
HSPH1	heat shock 105kDa/110kDa protein 1	NM_006644	1.3	7.2	-1.3	4.5	4.0
HSPA5	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	NM_005347	-1.0	6.2	-1.0	7.9	5.6
HSPB8	heat shock 22kDa protein 8	NM_014365	1.1	5.7	1.3	5.1	4.1
HSPA1L	heat shock 70kDa protein 1-like	NM_005527	-1.0	4.4	-1.1	3.7	4.6
HSPE1	heat shock 10kDa protein 1 (chaperonin 10)	NM_002157	1.4	3.6	2.2	3.2	3.3
HSPA9B	heat shock 70kDa protein 9B (mortalin-2)	NM_004134	1.1	2.4	1.2	3.2	3.1
HSP90AB1	heat shock protein 90kDa alpha (cytosolic), class B member 1	NM_007355	1.1	2.4	1.2	5.6	
HSP90B1	heat shock protein 90kDa beta (Grp94), member 1	NM_003299	1.0	4.4	1.2	5.6	5.4
HSP90AB1	heat shock protein 90kDa alpha (cytosolic), class B member 1	NM_007355	1.1	2.4	1.5	3.4	3.0
HSPA1A	heat shock 70kDa protein 1A	NM_005345	-1.0	5.3	-1.1	3.8	4.0
HSPA8	heat shock 70kDa protein 8	NM_006597	1.1	6.4			2.8
HSPA14	heat shock 70kDa protein 14	NM_016299			1.2	1.7	1.8
DNAJB1	DnaJ (Hsp40) homolog, subfamily B, member 1	NM_006145	1.1	13.4	1.1	7.2	8.1
DNAJB9	DnaJ (Hsp40) homolog, subfamily B, member 9	NM_012328	1.5	5.9	1.1	4.9	5.3
DNAJB4	DnaJ (Hsp40) homolog, subfamily B, member 4	NM_007034	-1.2	5.7	1.1	8.7	7.9
DNAJA4	DnaJ (Hsp40) homolog, subfamily A, member 4	NM_018602	1.1	5.0	1.1	4.5	5.2
DNAJC3	DnaJ (Hsp40) homolog, subfamily C, member 3	NM_006260	-1.1	2.7	-1.1	3.3	3.2
DNAJB11	DnaJ (Hsp40) homolog, subfamily B, member 11	NM_016306	-1.1	1.9	-1.1	2.3	2.2

Table 5-3. Proteasome inhibition affects transcription of heat shock protein genes							
Gene	**Name**	**GenBank**	**DEX**	**MD**	**E2**	**ME2**	**MG**
DNAJC19	DnaJ (Hsp40) homolog, subfamily C, member 19	NM_145261	1.1	-2.9	-1.0	-2.2	-2.6
DNAJB2	DnaJ (Hsp40) homolog, subfamily B, member 2	NM_006736	-1.0	3.7			1.8
DNAJB6	DnaJ (Hsp40) homolog, subfamily B, member 6	NM_005494	1.1	2.2			1.9
DNAJC13	Homo sapiens DnaJ (Hsp40) homolog, subfamily C, member 13	NM_173823	1.2	1.7			1.3
DNAJC6	DnaJ (Hsp40) homolog, subfamily C, member 6	NM_014787			-1.1	2.3	1.5
DNAJA1	DnaJ (Hsp40) homolog, subfamily A, member 1	NM_001539			1.3	1.7	1.7