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Katherine Heminger
Michael Markey
Meldrick Mpagi
Steven J. Berberich

University of Missouri-St. Louis, sjberberich@umsl.edu

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Alterations in gene expression and sensitivity to genotoxic stress following HdmX or Hdm2 knockdown in human tumor cells harboring wild-type p53

Katherine Heminger, Michael Markey, Meldrick Mpagi, and Steven J. Berberich

Wright State University Boonshoft School of Medicine Biochemistry & Molecular Biology Department, Dayton, OH 45435, USA
1 current address: Procter and Gamble Co., Cincinnati OH 45241, USA

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Correspondence: Steven J. Berberich, PhD, Wright State University Boonshoft School of Medicine Biochemistry & Molecular Biology Department, 3640 Colonel Glenn Hwy., Dayton, OH 45435
Received: 11/18/08; accepted: 01/03/09; published on line: 01/07/09
E-mail: steven.berberich@wright.edu

Abstract: While half of all human tumors possess p53 mutations, inactivation of wild-type p53 can also occur through a variety of mechanisms that do not involve p53 gene mutation or deletion. Our laboratory has been interested in tumor cells possessing wild-type p53 protein and elevated levels of HdmX and/or Hdm2, two critical negative regulators of p53 function. In this study we utilized RNAi to knockdown HdmX or Hdm2 in MCF7 human breast cancer cells, which harbor wild-type p53 and elevated levels of HdmX and Hdm2 then examined gene expression changes and effects on cell growth. Cell cycle and growth assays confirmed that the loss of either HdmX or Hdm2 led to a significant growth inhibition and G1 cell cycle arrest. Although the removal of overexpressed HdmX/2 appears limited to an anti-proliferative effect in MCF7 cells, the loss of HdmX and/or Hdm2 enhanced cytotoxicity in these same cells exposed to DNA damage. Through the use of Affymetrix GeneChips and subsequent RT-qPCR validations, we uncovered a subset of anti-proliferative p53 target genes activated upon HdmX/2 knockdown. Interestingly, a second set of genes, normally transactivated by E2F1 as cells transverse the G1-S phase boundary, were found repressed in a p21-dependent manner following HdmX/2 knockdown. Taken together, these results provide novel insights into the reactivation of p53 in cells overexpressing HdmX and Hdm2.

INTRODUCTION

Only half of all human tumors contain mutations in the p53 tumor suppressor gene [1], with the other half retaining wild-type p53 but possessing defects in the expression of p53 regulatory proteins and pathways. Under non-stress conditions, p53 protein is maintained at a low basal level by constant ubiquitination and proteasomal degradation [2]. Upon DNA damage or various types of cellular stress, p53 is stabilized and functions as a transcription factor to induce genes involved in cell cycle arrest, apoptosis, and DNA repair [3]. The stringent regulation of p53 involves a complex network of proteins, and is critical for maintaining genomic stability and suppressing tumor formation.

Hdm2 and its structural homologue HdmX represent two essential negative regulators of p53 as demonstrated by their embryonic lethality in knockout mice and subsequent rescue by concurrent elimination of p53 [4]. Hdm2 inactivates p53 function through direct association resulting in an inhibition of transactivation [5] and, through its E3 ligase activity targeting p53, by ubiquitin-mediated proteasome degradation [6, 7]. While HdmX shows conservation in the Hdm2 E3 ligase ring finger domain through which it
can heterodimerize with Hdm2 [8, 9]. HdmX lacks the ability to ubiquitinate p53 in vivo [10, 11] and thus can only antagonize p53 transactivation [12]. The heterodimerization of Hdm2 and HdmX also plays a critical role in the response to DNA damage enabling Hdm2 to promote the ubiquitination and rapid proteasomal degradation of HdmX, thereby facilitating the tumor suppressor activity of p53 [13-15]. Thus, the interactions between p53, Hdm2 and HdmX are critical for complete regulation of p53 [4].

The overexpression of either Hdm2 or HdmX can inhibit the activity of p53 and directly contribute to tumor formation. It is not surprising that either one or both proteins are found overexpressed in many human tumors and tumor cell lines which harbor wild-type p53 [16]. Diverse approaches to activate the wild-type p53 in these tumors include the use of small molecule antagonists like Nutlin to inhibit the Hdm2-p53 interaction [17-19], and the use of antisense oligonucleotides, antibodies, and small interfering RNAs directed at Hdm2 or HdmX [20-23]. Recent findings suggest that Hdm2 and HdmX are specific independent therapeutic targets for activating wild-type p53 and that anti-cancer approaches that target both Hdm2 and HdmX should be considered as a means of treatment for tumors [16, 18, 24].

This study undertook an examination of gene expression alterations and the biological effects resulting from RNAi silencing of HdmX and Hdm2 in a breast cancer cell line overexpressing both proteins. Unlike previous studies examining only the biological effect of either HdmX or Hdm2 loss, this study focuses on a cell line where both proteins are overexpressed and further compliments those previous studies with a systematic examination of gene expression changes following loss of HdmX or Hdm2. Interestingly, only p53 target genes primarily associated with cell cycle arrest were induced. More striking was the repression of a large group of E2F-regulated genes upon HdmX/2 knockdown. Using siRNA approaches targeting p21, we were able to show that these E2F2-regulated genes were repressed through p53 activation of p21. Furthermore, cell proliferation and colony formation assays confirmed that loss of HdmX or Hdm2 inhibited tumor cell growth and could sensitize these cells to treatment with doxorubicin. Taken together, these results suggest that in cells where both Hdm2 and HdmX are overexpressed, removal of one leads to an anti-proliferative effect in tumor cells harboring wild-type p53 and induction of p53 cell cycle arrest genes that negatively feedback onto the E2F pathway.

**RESULTS**

**RNAi knockdown of Hdm2 and HdmX in MCF7 cells**

Given that HdmX and Hdm2 are overexpressed in approximately 17% of human tumors [16] the majority of which possess wild-type p53, this study set out to examine how loss of Hdm2/X affected gene expression and tumor cell growth. MCF7, which possess wild-type p53 [25] and elevated levels of both HdmX and Hdm2 (Figure 1A) was the tumor cell line used in these studies. To inactivate HdmX and Hdm2 we employed siRNA targeting each gene as described in the materials and methods.

Before performing the Affymetrix GeneChip experiments we developed a triple transfection protocol that led to over 90% of the MCF7 cells taking up the siRNA (data not shown). Next, the effectiveness of the knockdown was assessed using RT-qPCR (data not shown) and Western blotting. Following the triple transfection protocol HdmX and p53 protein levels were undetectable with Hdm2 showing a greater than 80% reduction in protein expression (Figure 1B). As expected, the loss of either HdmX or Hdm2 led to an increase in the levels of p21. This p21 increase is p53-dependent since no increase in p21 protein levels was detected upon concurrent knockdown of HdmX and p53. While it has been suggested that Hdm2 controls the levels of p53 in non-stressed cells [26, 27], in our hands MCF7 cells showed only a slight increase in p53 protein levels following the combined loss of HdmX and Hdm2. The inability of Hdm2 knockdown to result in an increase in p53 protein could be the result of MCF7 cells harboring an elevated level of HdmX. Consistent with this suggestion, the treatment of MCF7 cells with Nutlin leads to increased p53 protein levels through loss of Hdm2 binding to p53 and concurrent Hdm2 mediated degradation of HdmX [28].

**Loss of Hdm2 and HdmX triggers inhibition of cell growth**

Other groups have reported that in cells where wild-type p53 is kept in check by overexpression of HdmX or Hdm2, their inhibition can trigger alterations in cell growth [29] and in some conditions apoptosis [30]. To assess the growth properties of RNAi knockdown of p53 regulators Hdm2 and HdmX, siRNA-transfected MCF7 cells were plated at low density in 6 well plates and allowed to grow for an additional 10 days. While transfection of siCon or sip53 resulted in only minimal changes in cell growth (Figure 2B), knockdown of either
HdmX or Hdm2, alone or in combination led to significantly fewer colonies (Figure 2A) and suppressed cell growth when compared to siCon (Figure 2B). This decrease in colony formation correlated with an increase in G1 arrest and not apoptosis (i.e. sub-G1) as determined by flow cytometry (data not shown).

Figure 1. (A) RT-PCR analysis of hdmX and hdm2 gene expression in various human cell lines. The endogenous levels of hdmX and hdm2 were determined relative to H1299 cells. All samples were normalized to GAPDH. (B) RNAi knockdown of HdmX or Hdm2 triggers p53-dependent p21 induction. Western blot analysis of indicated proteins from the various siRNA or doxorubicin (Dox) treated MCF7 cells. Knockdowns of the indicated proteins were greater than 80%. Protein extracts were made 24 hours after the last siRNA transfection or treatment with 5 μg/ml doxorubicin.
Loss of HdmX or Hdm2 sensitizes MCF7 cells to DNA damage

Several recent studies using Nutlin and various DNA damaging agents reported that blocking Mdm2:p53 association led to increased chemosensitivity to DNA damaging agents [31, 32]. To examine whether knockdown of HdmX and Hdm2 can also elicit increased cytotoxicity to DNA damage, MCF7 cells were transfected with the indicated siRNA leading to alterations of gene expression (Figure 3B). Cells were then treated with varying doses of doxorubicin and cell viability assessed. siRNAs targeting HdmX or Hdm2 increased doxorubicin cytotoxicity, while removing both HdmX and Hdm2 led to the greatest level of chemosensitivity (Figure 3A). Enhanced chemosensitivity was also observed in cisplatin treatment of siHdmX or siHdm2 MCF7 cells (data not shown).

Gene expression profiles of MCF7 cells lacking HdmX or Hdm2

Having established an effective knockdown approach with effects on cell growth and increased sensitivity to DNA damage, we performed an Affymetrix GeneChip experiment to assess how loss of HdmX or Hdm2 affected global gene expression in MCF7 cells. Each RNAi transfection was performed in three separate biological replicates. The data analysis was carried out using GeneSpring GX software. Given the similarity of biological function uncovered in the previous experiments we focused our informatics on genes commonly altered following RNAi treatment with siHdmX or siHdm2. In summary, .cel files were normalized using GCRMA, genes filtered by ANOVA and fold change, and genes significantly altered by both siHdmX and siHdm2 but not siHdmX + sip53 identified (see materials and methods for detailed approach). From this approach we uncovered 394 gene alterations common to knockdown of both siHdmX and siHdm2 (Table 1).

p53 activation following loss of HdmX or Hdm2

The initial examination of the 394 genes focused on those genes (n=222) that were increased following siHdmX or siHdm2 treatment relative to siCon. Thirteen genes were identified that were known p53-regulated genes (Figure 4). As expected these genes increased with siHdmX or siHdm2 treatment but had expression levels comparable or lower than siCon when treated with siHdmX + sip53 or sip53. Interestingly, with the exception of Fas, this list of p53 target genes consisted predominately of genes encoding proteins involved in cell cycle arrest or DNA repair. Consistent with a model whereby p53 proapoptotic target genes require p53 that is phosphorylated at serine 46 by HIPK2 [33-35], we observed no detectable phosphorylation at serines 6, 15, 20, 46, or 392 following the RNAi transfection protocol employed in these studies (data not shown).

To confirm these results, we performed RT-qPCR using TaqMan primers targeting five known p53 target genes, three of which were identified in our analysis. p21, BTG2 and ACTA2 are p53 target genes that are associated with cell cycle arrest or growth inhibition [36-38], while Hdm2 is a negative regulator of p53 and Noxa a pro-apoptotic factor not observed in our list of.
altered genes [39]. MCF7 cells were either mock transfected (Mock), transfected with siRNA that does not target any human gene (siCon) or transfected with siRNA to HdmX or Hdm2 either alone or in combination. The results in Figure 5 demonstrate that relative to siCon, knockdown of HdmX led to significant increases in hdm2, p21, BTG2 and ACTA2 gene expression. No significant change in gene expression was observed with Noxa, which is consistent with our GeneChip results. With the obvious exception of hdm2, siRNA-targeting Hdm2 led to similar alterations in gene expression (Figure 5). Finally, when both HdmX and Hdm2 were eliminated, the levels of the cell cycle arrest genes p21, BTG2 and ACTA2 increased either synergistically or additively while levels of Noxa remained unchanged. These results validate our GeneChip data that p53-target genes were induced upon HdmX or Hdm2 knockdown and that several of these genes encode proteins involved in the cell cycle arrest.

**p53 upregulation of p21 leads to global repression of E2F regulated genes**

After searching for genes that were directly upregulated by p53 we next evaluated those genes that were repressed (N=172) following HdmX and Hdm2 knockdown (Figure 7). Within the list of downregulated genes were a set of genes that encode proteins involved in G1-S phase transition, the majority of which were known E2F1 regulated genes. It is concomitant decrease in both CCNA2 and E2F1 (Figure 7). In contrast, loss of Hdm2/X and p21 completely abrogated CCNA2 and E2F1 repression consistent with p53 activation inactivating E2F1 transactivation via p21 induction.

**Figure 4. GeneChip expression of 13 known p53-regulated genes that were induced by knockdown of either siHdmX or siHdm2.** Y-axis represents the average fold change (log2) for each of the genes in the indicated siRNA transfections relative to siCon (X-axis, conditions labeled at the top of the chart).
DISCUSSION

As an essential tumor suppressor it is no surprise that human tumors demonstrate a diverse array of genetic mechanisms to inactivate p53 function. Central to this present study are tumors where one or both of the negative regulators of p53, Hdm2 and HdmX, are overexpressed leading to loss of p53 activity. Previous studies have focused on Hdm2 overexpression, where a small molecule inhibitor Nutlin 3 has proven to activate wild-type p53 in cell lines with elevated Hdm2, triggering apoptosis when combined with genotoxic agents that do not function as anti-mitotics [44]. Unfortunately, Nutlins have not proven as effective in tumors where HdmX is overexpressed [18, 45-47], suggesting the need for additional approaches aimed at blocking the HdmX:p53 association particularly given the recent observation of HdmX overexpression in retinoblastoma [48].

Here we have employed RNAi approaches and DNA microarrays to better understand the activation of p53 in cells overexpressing Hdm2 and HdmX. In MCF7 cells a growth arrest with no detectable apoptosis was observed following knockdown of either Hdm2 or HdmX (Figure 2 and data not shown). While loss of either HdmX or Hdm2 was sufficient to trigger an anti-proliferative effect, the combined loss of both HdmX and Hdm2 resulted in a more significant growth inhibition.

Even though this RNAi approach appears to activate p53 without triggering its phosphorylation (data not shown), the loss of either HdmX or Hdm2 did effectively sensitize the cells to doxorubicin with the loss of both Hdm2 and HdmX being most sensitive to DNA damage (Figure 3). Surprisingly our results showed only a modest elevation of endogenous p53 levels following loss of HdmX and Hdm2 (Figure 1). This result maybe unique to MCF7 cells which harbor elevated Hdm2 and HdmX, in contrast to most tumor cell lines with wild-type p53 that possessed only elevated Hdm2 (Figure 1A). Consistent with the need for only one negative regulator to be elevated 65% of retinoblastoma tumors overexpress HdmX and possess wild-type p53 [48]. Based on our previous HdmX overexpression studies [10] we would predict that the overexpression of HdmX might inhibit Hdm2 degradation of p53 in MCF7 cells and thus could explain why modulating Hdm2 levels in MCF7 cells has no dramatic effect on p53 levels.

The DNA microarray experiment directly tested whether HdmX or Hdm2 knockdown triggered an increase in p53-regulated genes. While 394 genes were...
significantly altered by either HdmX or Hdm2 knockdown (Table 1), only a small group was previously identified p53 targets (Figure 4). A few of the remaining genes induced by HdmX or Hdm2 loss are likely novel p53 regulated genes (S. Berberich, personal communication) but most probably represent downstream effects of the cell cycle arrest induced by p53. Within the 13 identified p53 target genes it is noteworthy that only one apoptotic gene (Fas) was found activated by loss of either HdmX or Hdm2. Upon careful examination of 16 known p53 pro-apoptotic genes we found that several of them were repressed following p53 knockdown, suggesting that their failure to be induced by loss of HdmX or Hdm2 was not a cell-type specific phenotype. Rather, we propose that the non-genotoxic release of p53 from Hdm2 of HdmX results in a preferential activation of growth arrest target genes, like p21 (Figure 5). This model is consistent with recent work suggesting that p53 promoter selection is dependent on its phosphorylation [49].

While this report focused on genes commonly regulated by HdmX and Hdm2, it is worth mentioning that within genes uniquely regulated by either HdmX or Hdm2 we did not observe any additional p53 regulated genes (M. Markey, personal communication). The common biological effects of HdmX or Hdm2-loss and significant overlap of gene expression patterns are in contrast to recent in vivo studies where the knockout of Mdm2 or MdmX in adult mouse tissues lead to non-overlapping roles in regards to regulating p53 activity [51]. We believe these findings point to either differences in cell culture verses tissue studies or more likely represent a significant departure in the roles that Hdm2 and HdmX play when expressed at physiological levels compared to the elevated levels in tumor cells.

Finally these studies demonstrate that non-genotoxic activation of p53 by knockdown of its inhibitors Hdm2 and HdmX leads to the induction of genes involved in cell-cycle arrest, as well as repression of genes along the E2F/Rb pathway that promote cell cycle entry. These alterations in gene expression resulted in a decreased population of proliferative cells without necessarily increasing apoptosis. A non-genotoxic activation of p53 is one possible mechanism for the reduction in cellular proliferation observed during aging. This further underscores the critical importance of tumor suppressor activation in senescence and organismal aging.

**MATERIALS AND METHODS**

Cell lines, antibodies, siRNA and chemotherapeutic agents. The human breast tumor cell line MCF7 was grown in Dulbecco’s modified Eagle medium (DMEM) supplemented with 10% bovine growth serum (BGS), and 10 µg/ml gentamicin unless otherwise indicated. HdmX polyclonal antibody (Bethyl Laboratories, Inc.), p21 polyclonal antibody C-19 (Santa Cruz Biotechnology, Inc.), p53 monoclonal antibody Ab-6 (Oncogene), Hdm2 monoclonal antibody SMP-14 (Santa Cruz Biotechnology, Inc.) and beta-actin monoclonal antibody (Sigma, Inc.) were used as indicated. A phosphorylation-specific p53 polyclonal antibody kit (Cell Signaling Technology, Inc.) was utilized per manufacturer’s protocol. Horseradish peroxidase (HRP)-conjugated anti-mouse or anti-rabbit secondary antibodies (Promega) were used with Super Signal substrate (Pierce) for chemiluminescence detection of proteins. siGENOME duplex RNA targeting mRNA from hdmX, hdm2, or p53 was purchased from Dharmacon Research, Inc. and siRNA transfection was performed using Oligofectamine or Lipofectamine 2000 (Invitrogen) as described below. Doxorubicin hydro-

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Figure 7. Repression of E2F1-regulated genes by Hdm2 or HdmX knockdown is blocked by concurrent knockdown of p21. MCF7 cells were transfected with the indicated siRNA combinations. Twenty-four hours later, RNA was isolated and subjected to RT-qPCR to quantify expression of CCNA2, p21 and E2F1 after normalization to GAPDH. Expression levels (Y-axis) were relative to siCon and reported as RQ values. Error bars represent the 95% confidence interval of the relative expression.

Another interesting finding within the microarray data was a subgroup of genes that were repressed upon HdmX and Hdm2 knockdown and could be classified as known E2F-regulated genes. Other groups have noted that p53 activation of p21 could lead to the repression of TERT [42] or Chk2 [41], known E2F-target genes, and another group recently reported similar findings using microarray assays [50].
chloride (Tocris Bioscience) was prepared as a 5 mg/ml stock solution in water.

siRNA transfection. Cells were seeded at 200,000 cells per well in 6-well plates (for RNA isolation), or at 700,000 cells per 6-cm dish (for protein extraction) in antibiotic free DMEM containing 1% BGS in a small volume. Cells were reverse transfected with 100 nM siRNA (Dharmacon Research, Inc.) at time of seeding using Lipofectamine 2000 (Invitrogen). After a five hour incubation, the media was removed and cells were refed with DMEM containing 10% BGS. Twenty hours later, the cells were transfected again with 100 nM siRNA in a small volume of serum free media using Oligofectamine (Invitrogen). After a four-hour incubation, an equal volume of DMEM containing 20% BGS was added to each well or dish without removing the transfection mixture. Total RNA was isolated 24 hours post siRNA transfection and protein was extracted at 48 hours post siRNA unless otherwise indicated.

Analysis of Affymetrix GeneChips. The Affymetrix HG-U133 plus 2.0 GeneChips containing probe sets detecting over 54,000 transcripts were used in this study and each transfection condition was performed in triplicate. GeneChip cel files were imported into GeneSpring GX and preprocessed by GCRMA. Measurements less than 0.01 were then set to 0.01, and a p-value < 0.05 were then made for siHdm2 versus siCon treatments by 1.5 fold with a p-value < 0.05. Initially all genes were filtered in GeneSpring GX first by Welch ANOVA to find expression changes based on siRNA treatment, using a p-value cut off of 0.05 and the Benjamini and Hochberg False Discovery Rate as a multiple testing correction. The cross-gene error model was active and based on replicates. From this list, genes were removed which varied between the mock and siCon treatments by 1.5 fold with a p-value < 0.05. Next, lists of genes with expression changes of 1.5 fold and a p-value < 0.05 were then made for siHdm2 versus siCon and siHdmX versus siCon. We then eliminated all but the union between these two lists. One gene that was repressed in the siHdm2 condition but upregulated in the siHdmX condition (encoding hypothetical protein MGC5370) was manually removed. Finally, genes that were not changed 1.5 fold with a p-value of <0.05 between the siHdmX and siHdmX + sip53 conditions were removed leaving a total of 394 selected genes.

Quantitative RT-pPCR. Cells were lysed directly in the culture dish and total RNA was isolated using the RNeasy kit (Qiagen) according to manufacturer’s protocol. The RNA was quantified by spectrophotometer reading at 260 nm, and 1 µg RNA was reverse transcribed with random hexamers to create cDNA using the TaqMan Reverse transcription kit (Applied Biosystems). Quantitative PCR was performed in a 96-well micro titer plate format on an ABI Prism 7900HT sequence detection system using 1 µl cDNA, TaqMan Universal PCR master mix and Assay-on-Demand Gene Expression products (Applied Biosystems) specific for genes of interest. Each cDNA sample was analyzed in triplicate and fold change relative to control was calculated based on a PCR efficiency of two and normalized to GAPDH (endogenous control) RNA levels. Average fold change and standard deviation were obtained from 2-3 biological replicate samples per treatment assayed in triplicate.

Western blot analysis. Frozen cells were lysed in an aqueous extraction buffer composed of 120 mM NaCl, 50 mM Tris-HCl (pH 8.0), 5 mM EGTA, 1 mM EDTA, 5 mM NaP pi, 10 mM NaF, 30 mM para-nitrophenylphosphate, 1 mM Benznidamide, 0.1% NP-40 (Ipegal Ca-630), 0.2 mM PMSF, and 1% protease inhibitor cocktail (Sigma), and soluble protein was recovered by centrifugation. Protein concentration was determined using Bradford reagent (Bio-Rad), and proteins were resolved on a sodium dodecyl sulfate-10% polyacrylamide gel followed by transfer of proteins to a polyvinylidene difluoride membrane (Millipore) using a Transblot system (Bio-Rad). Immunoblotting was performed as previously described [52] using appropriate primary antibodies at 1:1000-1:10,000 dilution and secondary antibodies (goat anti-mouse or goat anti-rabbit HRP-conjugated, Promega) at 1:5000-1:10,000 dilution. Blots were exposed to chemiluminescent reagent (Pierce) and protein was visualized on a FUJIFILM LAS-3000 image reader.

Colony formation and cell viability assays. Twenty-four hours after the second siRNA transfection, the cells were trypsinized, counted and seeded at 500 cells per well in 6-well plates for the colony formation assay. The cells were allowed to grow for ten days, and then the colonies were fixed and stained in 1% crystal violet in 70% methanol. The cell viability assays were performed in 96-well plates using either CellQuanti-Blue™ Reagent (BioAssay Systems) according to manufacturer’s protocol or by staining the cells with crystal violet, extracting the stain in 10% acetic acid, and then reading absorbance at 590 nm. Again, cells were trypsinized after the second siRNA transfection, counted and seeded at 20,000 cells per well. Cell
viability was determined at various time points post-seeding or following treatment with chemotherapeutic agents for the times indicated.

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CONFLICT OF INTERESTS STATEMENT

The authors of this manuscript have no conflict of interests to declare.

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Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID | Fold Change vs. siCon | Gene Symbol | Description |
|--------|-----------------------|-------------|-------------|
| 212354_s_at | 5.873 | SULF1 | sulfatase 1 |
| 205916_s_at | 5.6 | S100A7 | S100 calcium binding protein A7 (psoriasin 1) |
| 211893_s_at | 5.167 | TNFRSF10C | tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain |
| 206222_s_at | 4.986 | TNFRSF10C | tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain |
| 208180_s_at | 4.603 | HIST1H4H | histone 1, H4h |
| 206488_s_at | 4.424 | CD36 | CD36 antigen (collagen type 1 receptor, thrombospondin receptor) |
| 237737_at | 4.4 | LOC375010 | hypothetical LOC375010; hypothetical LOC40131 |
| 232035_s_at | 4.209 | HIST1H4H | histone 1, H4h |
| 218252_s_at | 3.97 | FAS | Fas (TNF receptor superfamily, member 6) |
| 213110_s_at | 3.929 | COL4A5 | collagen, type IV, alpha 5 (Alport syndrome) |
| 209566_s_at | 3.927 | CD36 | CD36 antigen (collagen type 1 receptor, thrombospondin receptor) |
| 228331_at | 3.756 | SPATA18 | spermatogenesis associated 18 homolog (rat) |
| 228786_at | 3.703 | CD36 | CD36 antigen (collagen type 1 receptor, thrombospondin receptor) |
| 208083_s_at | 3.691 | ITGB6 | integrin, beta 6 |
| 212097_s_at | 3.831 | CAV1 | caveolin 1, caveolae protein, 22kDa |
| 204781_s_at | 3.627 | FAS | Fas (TNF receptor superfamily, member 6) |
| 202917_s_at | 3.61 | S100A8 | S100 calcium binding protein A8 (calgranulin A) |
| 225912_at | 3.59 | TP53INP1 | tumor protein p53 inducible nuclear protein 1 |
| 215856_at | 3.493 | CD3D | CD3D antigen-like 3 |
| 215719_s_at | 3.479 | FAS | Fas (TNF receptor superfamily, member 6) |
| 226535_at | 3.47 | ITGB6 | integrin, beta 6 |
| 212344_at | 3.331 | SULF1 | sulfatase 1 |
| 202939_s_at | 3.198 | SERPINA1 | serpin peptidase inhibitor, clade A (alpha-1 antitrypsin, anti-trypsin), member 1 |
| 209504_s_at | 3.136 | PLEKHB1 | pleckstrin homology domain containing, family B (eukaryote) member 1 |
| 218985_at | 3.104 | FLJ20366 | hypothetical protein FLJ20366 |
| 200996_s_at | 3.103 | COL2A1 | collagen, type XXI, alpha 1; collagen, type XXI, alpha 1 |
| 204780_s_at | 3.049 | FAS | Fas (TNF receptor superfamily, member 6) |
| 209503_s_at | 3.027 | CAPN2 | calpain 2, (mfo) large subunit |
| 219628_at | 2.982 | WIG1 | p53 target zinc finger protein |
| 211423_s_at | 2.976 | SERPINA1 | serpin peptidase inhibitor, clade A (alpha-1 antitrypsin, anti-trypsin), member 1 |
| 1554062_at | 2.871 | XG | XG blood group (pseudoautosomal boundary-divided on the X chromosome) |
| 207899_s_at | 2.847 | IGSF1 | immunoglobulin superfamily, member 1 |
| 212288_at | 2.819 | NRP1 | neurophin 1 |
| 201236_s_at | 2.8 | BTG2 | BTG family, member 2 |
| 207392_x_at | 2.795 | UGT2B15 | UDP glucuronosyltransferase 2 family, polypeptide B15 |
| 215125_s_at | 2.784 | UGT1A10 | UDP glucuronosyltransferase 1 family, polypeptide A10 |
| 210387_at | 2.776 | HIST1H2BG | histone 1, H2bg |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID   | Fold Change vs. siCon | Gene Symbol | Description                                                                 |
|----------|-----------------------|-------------|-----------------------------------------------------------------------------|
| 209596   | 2.739                 | UDP glucuronosyltransferase 1 family, polypeptide A10 | C1q and tumor necrosis factor related protein 6                              |
| 208984   | 2.587                 | ITG96       | major histocompatibility complex, class II, DQ beta 1; major histocompatibility complex, class II, DQ beta 1 |
| 242444   | 2.685                 | C1QTNF6     | phosphoinositol-3-kinase, regulatory subunit 3 (p55, gamma)                |
| 212998   | 2.650                 | HLA-DQB1     | tumor necrosis factor (ligand) superfamily, member 10                       |
| 202743   | 2.468                 | PIK3R3      | tumor necrosis factor (ligand) superfamily, member 10                       |
| 202688   | 2.635                 | TNFSP10     | kynurenine 3-monoxygenase (kynurenine 3-hydroxylase)                        |
| 205306   | 2.633                 | KMO         | MAX dimerization protein 4                                                  |
| 212347   | 2.622                 | COL3A1      | collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant) |
| 227853   | 2.577                 | CTSD        | cytoplasmic FMR1 interacting protein 2; cytoplasmic FMR1 interacting protein 2 |
| 220959   | 2.573                 | CYFIP2      | cathepsin D (lysosomal aspartyl peptidase)                                  |
| 155997   | 2.559                 | AD-020      | Chromosome 1 open reading frame 119                                        |
| 221150   | 2.555                 | LOC64103    | hypothetical protein LOC64103                                              |
| 206260   | 2.533                 | CDH18       | cathepin 18, type 2                                                        |
| 223316   | 2.526                 | CDNA FLJ31683 fis, clone NT2R1/2005353 | Homo sapiens, clone IMAGE:4400004, mRNA                                     |
| 1557779  | 2.523                 | ACTA2       | actin, alpha 2, smooth muscle, aorta                                        |
| 200974   | 2.521                 | MGC17330    | HGF; gene; HGF, gene                                                        |
| 202180   | 2.503                 | MVP         | major vault protein                                                         |
| 221218   | 2.484                 | TPK1        | thiamin pyrophosphokinase 1                                                 |
| 219049   | 2.479                 | ChGn        | chondroitin beta1,4-N-acetylgalactosaminytransferase                       |
| 227020   | 2.448                 | YPEL2       | pyrophosphate- and aspartic acid 2 (Drosophila)                            |
| 225207   | 2.441                 | PKC4        | pyruvate dehydrogenase kinase, isoenzyme 4                                 |
| 215779   | 2.439                 | HIST1H2B2G  | histone 1, H2b                                                               |
| 210778   | 2.432                 | MBD4        | MAX dimerization protein 4                                                  |
| 202284   | 2.428                 | CDKN1A      | cyclin-dependent kinase inhibitor 1A (p21, Cip1)                            |
| 211598   | 2.405                 | PIK3R3      | phosphoinositol-3-kinase, regulatory subunit 3 (p55, gamma)                |
| 213216   | 2.388                 | LBA1        | lupus brain antigen 1                                                       |
| 215785   | 2.388                 | CYFIP2      | cytoplasmic FMR1 interacting protein 2                                       |
| 210218   | 2.381                 | SP100       | nuclear antigen Sp100                                                       |
| 215465   | 2.375                 | ABCA12      | ATP-binding cassette, sub-family A (ABC1), member 12                        |
| 203055   | 2.368                 | PAPS2       | 3'-phosphoadenosine 5'-phosphosulfate synthase 2                           |
| 200984   | 2.356                 | CD59        | CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16.3A5, E J16, EJ30, EL32 and G344) |
| 225613   | 2.346                 | MAST4       | microtubule associated serine/threonine kinase family member 4             |
| AffyID | Fold Change vs. siCon | Gene Symbol | Description |
|--------|----------------------|-------------|-------------|
| 212463 at | 2.34 | CD59 | CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16,3A6, E16, EJ30, EL32 and G344) |
| 204648 at | 2.338 | GIP | erythroblastin (ferrooxidase) |
| 236835 at | 2.336 | FUT8 | fucosyltransferase 8 (alpha (1,6) fucosyltransferase) |
| 236278 at | 2.333 | H2b | histone 1, H2b |
| 214616 at | 2.322 | HIST1H3E | histone 1, H3e |
| 202737 at | 2.31 | MAGI2 | membrane associated guanylate kinase, WW and PDZ domain containing 2 |
| 203060 s at | 2.304 | PAPSS2 | 3-phosphoadenosine 5'-phosphosulfate synthase 2 |
| 1552632 a at | 2.303 | KIAA1001 | Arylsulfatase G |
| 205480 at | 2.302 | ABAT | 4-amino butyrate amino transferase |
| 207664 at | 2.264 | ADAM2 | ADAM metalloprotease domain 2 (fertilin beta) |
| 200696 s | 2.249 | GSN | gelsolin (amyloidosis, Finnish type) |
| 238439 at | 2.24 | ANKRD22 | ankyrin repeat domain 22 |
| 223385 at | 2.237 | NTN4 | netrin 4 |
| 224847 at | 2.237 | CDK6 | cyclin-dependent kinase 6 |
| 242039 s at | 2.234 | THAP1 | thiamin pyrophosphokinase 1 |
| 223686 at | 2.208 | TPK1 | thiamin pyrophosphokinase 1 |
| 201484 s at | 2.204 | TNFRSF11C | tumor necrosis factor receptor superfamily, member 10c, decoy without an intracellular domain; hypothetical protein MGC31957 |
| 201652 x at | 2.203 | COL3A1 | collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant) |
| 1564573 at | 2.193 | LOC402778 | similar to RIKEN cDNA 6330512M04 gene (mouse) |
| 213744 at | 2.192 | ATRNL1 | attractin-like 1 |
| 226553 at | 2.192 | PGAM2L1 | phosphoglucomutase 2-like 1 |
| 233600 e at | 2.191 | KIAA1683 | KIAA1683 |
| 209160 at | 2.185 | AKR1C3 | aldo-keto reductase family 1, member C3 (3-alpha hydroxy steroid dehydrogenase, type II) |
| 211138 s at | 2.18 | KMO | kynurenine 3-monooxygenase (kynurenine 3-hydroxylase) |
| 225890 at | 2.18 | CENDR | CONA clone IMAGE:5259272 |
| 206483 s at | 2.172 | OHR2 | 4-amino butyrate amino transferase (SDF1 family) member 2 |
| 223346 s at | 2.169 | MDX4 | MAX dimerization protein 4 |
| 1555756 a at | 2.164 | CLEC7A | C-type lectin domain family 7, member A |
| 214455 at | 2.15 | HIST1H2B8C | histone 1, H2b |
| 228151 at | 2.148 | H2b | histone 1, H2b |
| 1559322 at | 2.145 | PTP4A1 | protein tyrosine phosphatase type IVA, member 1 |
| 203543 s at | 2.146 | KLF9 | Kruppel-like factor 9 |
| 2061776 at | 2.124 | FMID | flavin containing monooxygenase 5 |
| 206110 at | 2.122 | HIST1H3 | histone 1, H3h |
| 40016 g at | 2.119 | MAST4 | microtubule associated serine/threonine kinase family member 4 |
| 205053 s at | 2.114 | IGA | iduronidase, alpha-L- |
| 205983 at | 2.113 | RXF5 | regulatory factor X, 5 (influences HLA class II expression) |
| 213664 at | 2.105 | SLC1A1 | solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), member 1 |
| 218280 x at | 2.101 | HIST2H2AA | histone 2, H2aa |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID | Fold Change vs. siCon | Gene Symbol | Description |
|--------|-----------------------|-------------|-------------|
| 214686 | 5.24                  | MGC14376    | hypothetical protein MGC14376 |
| 225725 | 5.09                  | CDNA FLJ31689 fis, clone NT2R12053535 | cyclin-dependent kinase 6 |
| 224848 | 5.25                  | CDK6        | regulatory factor X, 5 (influences HLA class II expression) |
| 202364 | 5.24                  | RFX5        | Ribosomal protein S27-like |
| 236395 | 5.24                  | PK2R2       | phosphoinositide-3-kinase, regulatory subunit 2 (p65 beta) |
| 223201 | 5.09                  | PTIP3-88   | hypothetical protein FLJ22679 |
| 218346 | 5.09                  | SEsN1       | septin 1 |
| 202329 | 5.09                  | MGP         | matrix Gla protein |
| 223087 | 5.25                  | TB6D        | thrombomodulin |
| 235903 | 5.25                  | TSGA2       | tests specific A2 homolog (mouse) |
| 219399 | 5.09                  | C12orf5     | chromosome 12 open reading frame 5 |
| 223441 | 5.25                  | PRS23       | Protease, serine, 23 |
| 203076 | 5.09                  | COL3A1      | collagen, type III, alpha 1 (Ohiens-Danlos syndrome type IV, autosomal dominant) |
| 202073 | 5.09                  | OPTN        | optineurin |
| 223057 | 5.09                  | BF          | B-factor, properdin |
| 227221 | 5.09                  | CDNA FLJ31689 fis, clone NT2R12053535 | fibroblast growth factor 13 |
| 205110 | 5.09                  | FGF-13      | fibroblast growth factor 13 |
| 203988 | 5.09                  | THBD        | thrombomodulin |
| 235911 | 5.09                  | C12orf116   | chromosome 12 open reading frame 116 |
| 223888 | 5.09                  | INPP4B      | inositol polyphosphate-4-phosphatase, type II, 105kDa |
| 223179 | 5.09                  | YPEL3       | yippee-like 3 (Drosophila) |
| 220574 | 5.09                  | OPCT        | glutamimy-peptide cyclotransferase (glutamimyl cyclase) |
| 221513 | 5.09                  | TMEM2       | transmembrane protein 2 |
| 235934 | 5.09                  | Homo sapiens, clone IMAGE 5723825 | mRNA |
| 200963 | 5.09                  | CD59        | CD59 antigen p18-20 (antigen identified by monoclonal antibodies 16, 3AS, EJ16, EL30, EL32 and G344) |
| 211884 | 5.09                  | FER1L3      | fer-1-like 3, myoferlin (C. elegans) |
| 206482 | 5.09                  | PTK6        | PTK6 protein tyrosine kinase 6 |
| 223434 | 5.09                  | GBP3        | guanylate binding protein 3 |
| 221316 | 5.09                  | SEsN2       | septin 2 |
| 155303 | 5.09                  | SYT5L       | synaptotagmin-like 5 |
| 225771 | 5.09                  | ATP8B2      | ATPase, Class I, type 8B, member 2 |
| 201798 | 5.09                  | FER1L3      | fer-1-like 3, myoferlin (C. elegans) |
| 227134 | 5.09                  | SYT1L       | synaptotagmin-like 1 |
| 202708 | 5.09                  | HIST2H2BE   | histone 2, H2be |
| 225666 | 5.09                  | LOC440444   | hypothetical gene supported by AF086204 |
| 225326 | 5.09                  | RAMP3       | receptor (calmodulin) activity modifying protein 3 |
| 235673 | 5.09                  | TMEM7       | Transcribed locus |
| 222450 | 5.09                  | SMP3A1      | transmembrane, prostate androgen induced RNA |
| 225927 | 5.09                  | MAP2K1      | mitogen-activated protein kinase kinase kinase 1 |
| 213142 | 5.09                  | LOC54103    | hypothetical protein LOC54103 |
| 155630 | 5.09                  | FLJ33674    | hypothetical protein FLJ33674 |
| 225922 | 5.09                  | MGC17299    | hypothetical protein MGC17299 |
| 208796 | 5.09                  | CCNG1       | cyclin G1 |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID   | Fold Change vs. siHdmX | Gene Symbol | Description |
|----------|-------------------------|-------------|-------------|
| 226403_at| 1.735                   | TMC4        | transmembrane channel-like 4 |
| 209333_at| 1.794                   | ULK1        | unc-51-like kinase 1 (C. elegans) |
| 228664_at| 1.794                   | PKA         | Protein kinase (AMP-dependent, catalytic) inhibitor alpha |
| 203059_s_at| 1.787                | PAPSS2      | 3-phosphoadenosine 5'-phosphosulfate synthase 2 |
| 214290_s_at| 1.773                | HIST2H2AA   | Histone H2aa |
| 205726_at| 1.766                   | DiAPH2      | diaphansus homolog 2 (Drosophila) |
| 219410_at| 1.753                   | TMEM45A     | transmembrane protein 45A |
| 37996_s_at| 1.752                   | DMPK        | dystrophia myotonica-protein kinase |
| 200766_at| 1.744                   | CTSD        | cathepsin D (lysosomal aspartic peptidase) |
| 202306_at| 1.742                   | CDH26       | cadherin-like 26 |
| 217419_x_at| 1.732                | AGRN        | agrin |
| 219561_at| 1.723                   | COPZ2       | coatomer protein complex, subunit zeta 2 |
| 216264_s_at| 1.718                | LAMB2       | laminin, beta 2 (laminin S) |
| 212120_at| 1.712                   | RHOQ        | Rho homolog gene family, member Q |
| 212285_s_at| 1.711                | AGRN        | agrin |
| 218007_s_at| 1.707                | RPS27L      | ribosomal protein S27-like |
| 230750_at| 1.707                   | CDNA FLJ31839 fis, clone NT2RP7000060 |
| 207855_s_at| 1.7                    | BLNK        | B-cell linker |
| 231406_at| 1.7                    | LOC401394   | hypothetical LOC401394, hypothetrical LOC402578 |
| 204462_s_at| 1.699                | SLC16A2     | solute carrier family 16 (monocarboxylic acid transporters), member 2 |
| 214481_at| 1.699                   | HIST1H2AM   | Histone H2am |
| 231766_s_at| 1.693                | COL12A1     | collagen, type XII, alpha 1 |
| 219687_at| 1.692                   | H-HAT       | hedgehog acetyltransferase |
| 202376_at| 1.69                    | SERPINA3    | serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3 |
| 204954_s_at| 1.689                | Dyrk1B      | dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B |
| 208792_s_at| 1.677                | CLU         | clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J) |
| 217529_at| 1.676                   | LOC401394   | hypothetical LOC401394, hypothetical LOC402578 |
| 218471_s_at| 1.673                | BBS1        | Bardet-Biedl syndrome 1 |
| 203767_s_at| 1.664                | STS         | steroid sulfatase (microsomal), arylsulfatase C, isozyme S |
| 208919_at| 1.663                   | CLU         | clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J) |
| 201646_at| 1.654                   | JAK1        | Janus kinase 1 (a protein tyrosine kinase) |
| 202917_s_at| 1.546                | TP53AP1     | TP53 activated protein 1 |
| 212456_at| 1.546                   | KIAA0256    | KIAA0256 gene product |
| 222243_at| 1.536                   | CLU         | clusterin (complement lysis inhibitor, SP-40,40, sulfated glycoprotein 2, testosterone-repressed prostate message 2, apolipoprotein J) |
| 204546_at| 1.523                   | KIAA0513    | KIAA0513 |
| 236068_at| 1.521                   | CDNA clone IMAGE 5312086 |
| 209523_at| 1.518                   | MCCC2       | methylcrotonoyl-Coenzyme A carboxylase 2 (beta) |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID  | Fold Change vs. siCon | Gene Symbol                | Description                                                                 |
|---------|-----------------------|----------------------------|----------------------------------------------------------------------------|
| 209380  | s_at                  | 1.616                      | RUNX1                        | runt-related transcription factor 1 (acute myeloid leukemia 1, amy1 oncogene) |
| 220513  | s_at                  | 1.614                      | SYTL2                        | synaptotagmin-like 2               |
| 217767  | s_at                  | 1.613                      | C3                           | complement component 3             |
| 209166  | s_at                  | 1.611                      | MAN2B1                       | mannosidase, alpha, class 2B, member 1 |
| 207913  | s_at                  | 1.611                      | FDXR                         | ferredoxin reductase               |
| 217783  | s_at                  | 1.609                      | YPEL5                        | yipee-like 5 (Drosophila)          |
| 201118  | s_at                  | 1.608                      | CPE                          | carboxypeptidase E                 |
| 209379  | s_at                  | 1.607                      | PNPLA4                       | patatin-like phospholipase domain containing 4 |
| 219529  | s_at                  | 1.602                      | CLIC3                        | chloride intracellular channel 3   |
| 233195  | s_at                  | 1.597                      | SESN2                        | sestrin 2                          |
| 203725  | s_at                  | 1.589                      | GADD45A                      | growth arrest and DNA-damage-inducible, alpha |
| 209216  | s_at                  | 1.583                      | WDR45                        | WD repeat domain 45                |
| 234644  | x_at                  | 1.570                      | CDNA: FLJ22426 fis, clone HRC08780 |
| 214524  | x_at                  | 1.571                      | HIST1H2Ai                    | histone 1, H2ai                    |
| 210886  | x_at                  | 1.571                      | TP53AP1                       | TP53 activated protein 1           |
| 201939  | x_at                  | 1.571                      | PLK2                         | polo-like kinase 2 (Drosophila)    |
| 208950  | x_at                  | 1.563                      | PLXNB2                       | plexin B2                         |
| 211979  | x_at                  | 1.559                      | GPR107                        | G protein-coupled receptor 107     |
| 210241  | x_at                  | 1.558                      | TP53AP1                       | TP53 activated protein 1           |
| 210930  | s_at                  | 1.557                      | ERBB2                        | v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuroglioblastoma derived oncogene homolog (avian) |
| 218706  | s_at                  | 1.557                      | NSSTP2                       | HCV NS3-transactivated protein 2   |
| 202387  | s_at                  | 1.545                      | BAG1                         | BCL2-associated athanogene ; BCL2-associated athanogene |
| 225968  | s_at                  | 1.545                      | PRICKLE2                     | pricklike-2 (Drosophila)          |
| 209920  | s_at                  | 1.544                      | BTG1                         | B-cell translocation gene 1, anti-proliferative |
| 218080  | s_at                  | 1.539                      | FADS3                        | fatty acid desaturase 3            |
| 32428   | s_at                  | 1.537                      | AQP3                         | aquaporin 3                        |
| 217270  | s_at                  | 1.535                      | DYRK1B                        | dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B |
| 214433  | s_at                  | 1.534                      | SELENBP1                     | selenium binding protein 1 ; selenium binding protein 1 |
| 210224  | at                    | 1.524                      | MR1                          | major histocompatibility complex, class i-related |
| 224836  | at                    | 1.512                      | TP53IP2                      | tumor protein p53 inducible nuclear protein 2 |
| 212890  | at                    | 1.511                      | MGC15523                      | hypothetical protein MGC15523     |
| 214066  | s_at                  | 0.666                      | PARP2                        | poly (ADP-ribose) polymerase family member 2 |
| 213346  | s_at                  | 0.663                      | LCC903811                     | hypothetical protein LCC903811    |
| 228559  | at                    | 0.663                      | CDNA clone IMAGE043059       | |
| 227337  | at                    | 0.663                      | ANKR37                       | ankyrin repeat domain 3            |
| 230425  | at                    | 0.662                      | SGOL2                        | shugoshin-like 2 (S. pombe)        |
| 204435  | at                    | 0.661                      | NPL1                         | nucleoporin like 1                 |
| 201860  | at                    | 0.658                      | RRM2                         | ribonucleotide reductase M2 polypeptide |
| 222840  | s_at                  | 0.658                      | C1orf112                     | chromosome 1 open reading frame 112 |
| 222834  | at                    | 0.658                      | FIGNL1                       | fidgetin-like 1                    |
| 204240  | s_at                  | 0.657                      | SMC2L1                       | SMC2 structural maintenance of chromosomes 2-like 1 (yeast) |
| 228273  | at                    | 0.657                      | FLJ11029                     | Hypothetical protein FLJ11029     |
| 203625  | s_at                  | 0.656                      | SKP2                         | S-phase kinase-associated protein 2 (p45) |
| 218350  | s_at                  | 0.656                      | GMNN                         | geminin, DNA replication inhibitor |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID   | Fold Change vs. siHdmX | Gene Symbol | Gene Description                                                                 |
|----------|------------------------|-------------|----------------------------------------------------------------------------------|
| 219502   | 0.856                  | NEIL3       | Nucleotide excision repair endonuclease VIII-like 3 (E. coli)                     |
| 209906   | 0.655                  | ACAT2       | Acetyl-Coenzyme A acetyltransferase 2 (Acetoacetyl Coenzyme A thiolaase)          |
| 203213   | 0.543                  | CDC2        | Cell division cycle 2, G1 to S and G2 to M                                       |
| 227787   | 0.639                  | THRAP6      | Thyroid hormone receptor associated protein 6                                    |
| 219555   | 0.65                   | BM039       | Uncharacterized bone marrow protein BM039                                          |
| 203302   | 0.569                  | DCK         | Deoxycofactor kinase                                                              |
| 222508   | 0.54                   | ANLN        | Anillin, actin binding protein (scraps homolog, Drosophila)                       |
| 222249   | 0.473                  | ATAD2       | ATPase family, AAA domain containing 2                                            |
| 219522   | 0.541                  | WDHD1       | WD repeat and HMG-box DNA binding protein 1                                       |
| 222466   | 0.464                  | PKSG14      | Leucine zipper protein PKSG14                                                     |
| 220896   | 0.541                  | TPRT        | Trans-prenyltransferase                                                           |
| 203334   | 0.541                  | CHEK1       | CHK1 checkpoint homolog (S. pombe)                                                |
| 232556   | 0.568                  | KIAA1333    | KIAA1333                                                                          |
| 229442   | 0.618                  | C18orf54    | Chromosome 18 open reading frame 54                                               |
| 204531   | 0.61                   | BRCA1       | Breast cancer 1, early onset                                                       |
| 209754   | 0.59                   | TMPO        | Thymopoietin                                                                      |
| 211767   | 0.613                  | SL5        | SL5 homolog, SL5 homolog                                                          |
| 223265   | 0.564                  | KIAA1333    | KIAA1333                                                                          |
| 225300   | 0.539                  | C15orf23    | Chromosome 15 open reading frame 23                                               |
| 228865   | 0.627                  | FLJ32363    | FLJ32363 protein                                                                  |
| 209709   | 0.639                  | HMR         | Hyaluronan-mediated motility receptor (RHAMM)                                     |
| 218750   | 0.568                  | KIF20A2     | Kinesin family member 20A                                                         |
| 156850   | 0.657                  | TRAP        | Trophin associated protein (fastin)                                               |
| 129531   | 0.653                  | Cep72       | Centrosomal protein 72 kDa                                                        |
| 227545   | 0.626                  | BARD1       | BRCA1 associated RING domain 1                                                     |
| 234944   | 0.597                  | FAM54A      | Family with sequence similarity 54, member A                                      |
| 230705   | 0.601                  | CHEK1       | CHK1 checkpoint homolog (S. pombe)                                                |
| 204962   | 0.666                  | CENPA       | Centromere protein A, 17kDa                                                        |
| 222339   | 0.582                  | LOCI46909   | Hypothetical protein LOCI46909                                                    |
| 207030   | 0.651                  | CCNB2       | Cyclin B2                                                                         |
| 223610   | 0.613                  | FLJ40629    | Hypothetical protein FLJ40629                                                     |
| 218550   | 0.605                  | FLJ20105    | FLJ20105 protein                                                                  |
| 201663   | 0.618                  | SMC4L1      | SMC4 structural maintenance of chromosomes 4-like 1 (yeast)                       |
| 218883   | 0.641                  | MFL1IP      | MFL1 interacting protein                                                           |
| 209715   | 0.621                  | CBX5        | Chromobox homolog 5 (HP1 alpha homolog, Drosophila)                               |
| 220239   | 0.626                  | KLHL7       | Kelch-like 7 (Drosophila)                                                         |
| 203690   | 0.531                  | KIFC1       | Kinesin family member C1                                                           |
| 218768   | 0.61                    | NUP107      | Nucleoporin 107 kDa                                                               |
| 38158    | 0.661                  | EPS1L       | Extra spindle poles like 1 (S. cerevisiae)                                        |
| 204127   | 0.626                  | RFC3        | Replication factor C (activator 1) 3, 34kDa                                       |
| 209714   | 0.629                  | CDKN3       | Cyclin-dependent kinase inhibitor 3 (CDK2-associated dual specificity phosphatase) |
| 235545   | 0.558                  | DEPDC1      | DEP domain containing 1                                                            |
| 209856   | 0.604                  | DUT         | DUTP pyrophosphatase                                                               |
| 212211   | 0.629                  | PSRC1       | Proline/serine-rich coiled-coil 1                                                 |
| 213647   | 0.465                  | DNA2L       | DNA2 DNA replication helicase 2-like (yeast)                                      |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyId | Fold Change vs. siCon | Gene Symbol | Description |
|--------|-----------------------|-------------|-------------|
| 204822 | 0.62                  | TTK         | TTK protein kinase |
| 204825 | 0.62                  | MELK        | maternal embryonic leucine zipper kinase |
| 215773 | 0.62                  | PARP2       | poly (ADP-ribose) polymerase family, member 2 |
| 204162 | 0.619                 | KNTC2       | kinetochore associated 2 |
| 205363 | 0.619                 | CHEK1       | CHK1 checkpoint homolog (S. pombe) |
| 221665 | 0.619                 | PLJ20364    | hypothetical protein FLJ20364 |
| 227298 | 0.619                 | PLJ20641    | hypothetical protein FLJ20641 |
| 226069 | 0.619                 | FAM54A      | family with sequence similarity 54, member A |
| 230165 | 0.619                 | SGOL2       | shugoshin-like 2 (S. pombe) |
| 218169 | 0.618                 | DTL         | dentateless homolog (Drosophila) |
| 218365 | 0.616                 | KIF4A       | kinesin family member 4A |
| 223307 | 0.616                 | CDC3A       | cell division cycle associated 3 |
| 218039 | 0.615                 | NUSAP1      | nucleolar and spindle associated protein 1 |
| 204033 | 0.614                 | TRIP13      | thyroid hormone receptor interactor 13 |
| 225867 | 0.613                 | C20orf129   | chromosome 20 open reading frame 129 |
| 226308 | 0.613                 | NY-SAR-48   | sarcoma antigen NY-SAR-48 |
| 204752 | 0.608                 | PARP2       | poly (ADP-ribose) polymerase family, member 2 |
| 205853 | 0.608                 | POLR3G      | POLR3G (RNA III (DNA directed) polypeptide G (32kD)) |
| 210931 | 0.608                 | MCM7        | MCM7 minichromosome maintenance deficient 7 (S. cerevisiae) |
| 218727 | 0.608                 | ATAD2       | ATPase family, AAA domain containing 2 |
| 219258 | 0.608                 | PLJ20461    | timeless-interacting protein |
| 208795 | 0.607                 | MCM7        | MCM7 minichromosome maintenance deficient 7 (S. cerevisiae) |
| 220060 | 0.607                 | PLJ20641    | hypothetical protein FLJ20641 |
| 221436 | 0.607                 | CDC3A       | cell division cycle associated 3 ; cell division cycle associated 3 |
| 223542 | 0.607                 | AKR3D2      | ankyrin repeat domain 32 |
| 155544 | 0.604                 | FANCA       | Fanconi anemia, complementation group B |
| 219041 | 0.604                 | C20orf45    | chromosome 20 open reading frame 45 |
| 221591 | 0.603                 | FAM54A      | family with sequence similarity 54, member A |
| 203836 | 0.602                 | FANCA       | Fanconi anemia, complementation group A ; Fanconi anemia, complementation group A |
| 219978 | 0.601                 | NUSAP1      | nucleolar and spindle associated protein 1 |
| 221879 | 0.601                 | CALML4      | calmodulin-like 4 |
| 203755 | 0.601                 | BUB1B       | BUB1 budding uninhibited by benzimidazoles 1 homolog beta (yeast) |
| 203764 | 0.601                 | DGK7        | discs, large homolog 7 (Drosophila) |
| 204888 | 0.596                 | FLK4        | polo-like kinase 4 (Drosophila) |
| 206659 | 0.596                 | NUP185      | nucleoporin 156kDa |
| 227211 | 0.597                 | PPH19       | PHD finger protein 19 |
| 205363 | 0.598                 | PRIM1       | primase, polypeptide 1, 48kDa |
| 64108  | 0.598                 | CALML4      | calmodulin-like 4 |
| 221521 | 0.597                 | Pfs2        | DNA replication complex GINS protein PSF2 |
| 222962 | 0.595                 | MCM10       | MCM10 minichromosome maintenance deficient 10 (S. cerevisiae) |
| 205159 | 0.594                 | WDR76       | WD repeat domain 76 |
| 219990 | 0.594                 | E2F8        | E2F transcription factor 8 |
| 213226 | 0.592                 | CCNA2       | Cyclin A2 |
| 219703 | 0.592                 | MNS1        | meiosis-specific nuclear structural 1 |
Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID  | Fold Change vs. siCon          | Gene Symbol | Description                                      |
|---------|--------------------------------|-------------|--------------------------------------------------|
| 242564_at| 0.589                          | FLJ13305    | hypothetical protein FLJ13305                    |
| 1552619_at| 0.587                          | ANLN        | alpha II actin binding protein (scraps homolog, Drosophila) |
| 204603_at| 0.583                          | EXO1        | exonuclease 1                                    |
| 223570_at| 0.583                          | MCM10       | MCM10 minichromosome maintenance deficient 10 (S. cerevisiae) |
| 204492_at| 0.582                          | ARHGAP11A   | Rho GTPase activating protein 11A                 |
| 214240_at| 0.582                          | GAL         | galactin                                         |
| 219305_at| 0.582                          | KIF15       | kinesin family member 15                         |
| 203145_at| 0.581                          | SPAG5       | sperm associated antigen 5                       |
| 203969_at| 0.581                          | CDC5        | CDC5 cell division cycle 6 homolog (S. cerevisiae) |
| 230847_at| 0.58                           | WRNIP1      | Werner hereditary interstitial protein 1          |
| 215210_at| 0.57                           | CDC8         | cell division cycle associated 8                 |
| 215294_at| 0.57                           | C6orf139    | chromosome 6 open reading frame 139             |
| 1552927_at| 0.57                           | FIGN1       | fidgetin-like 1                                  |
| 224428_at| 0.57                           | CDC9A       | cell division cycle associated 7; cell division cycle associated 7 |
| 218563_at| 0.56                           | HCAP-G      | chromosome condensation protein G                |
| 1553684_at| 0.56                           | DTYMK       | deoxythymidylate kinase (thymidylate kinase)     |
| 220851_at| 0.57                           | MCM10       | MCM10 minichromosome maintenance deficient 10 (S. cerevisiae) |
| 236641_at| 0.57                           | KIF14       | kinesin family member 14                         |
| 204023_at| 0.57                           | RFC4        | replication factor C (activator) 1, 4, 37kDa     |
| 205024_at| 0.56                           | RAD51       | RAD51 homolog (RecA homolog, E. coli) (S. cerevisiae) |
| 218562_at| 0.56                           | HCAP-G      | chromosome condensation protein G                |
| 220858_at| 0.56                           | DEPDC1      | DEP domain containing 1                          |
| 224787_at| 0.56                           |             |                                                  |
| 1554708_at| 0.56                           | MAD2L1      | MAD2 mitotic arrest deficient-like 1 (yeast) 2   |
| 204541_at| 0.56                           | NEK2        | NIMA (never in mitosis gene a)-related kinase 2 |
| 209773_at| 0.56                           | RRM2        | ribonucleotide reductase M2 polypeptide          |
| 223229_at| 0.56                           | UB22T       | ubiquitin-conjugating enzyme E2T (putative)     |
| 201067_at| 0.56                           | CKS1B       | CDC28 protein kinase regulatory subunit 1B      |
| 214804_at| 0.56                           | FSHPR1      | FSH primary response (LRP1 homolog, rat 1)      |
| 225834_at| 0.56                           | FANCM       | family with sequence similarity 72, member A    |
| 209594_at| 0.56                           | UBE2C       | ubiquitin-conjugating enzyme E2C                |
| 205909_at| 0.56                           | POLE2       | polymerase (DNA directed), epsilon 2 (p59 subunit) |
| 205906_at| 0.55                           | HIST1H4C    | histone 1, H4c                                  |
| 212938_at| 0.55                           | BRRN1       | barre homolog (Drosophila)                       |
| 1553528_at| 0.54                           | TAF5        | TAF5 RNA polymerase II, TATA box binding protein (TBP)-associated factor, 100kDa |
| 207891_at| 0.54                           | TREX2, UTP5 | three prime repair exonuclease 2, 28S proteasome-associated UCH interacting protein 1 |
| 218484_at| 0.54                           | RAD54B      | RAD54 homolog B (S. cerevisiae)                 |
| 209891_at| 0.54                           | SPBC25      | spindle pole body component 25 homolog (S. cerevisiae) |
| 205733_at| 0.54                           | BLM         | Bloom syndrome                                  |
| 227165_at| 0.54                           | C13orf3     | chromosome 13 open reading frame 3              |
| 210416_at| 0.54                           | CHK2        | CHK2 checkpoint homolog (S. pombe)              |
| 215509_at| 0.54                           | BUB1        | BUB1 budding inhibited by benzimidazoles 1 homolog (yeast) |
### Table 1: Genes deregulated by HdmX and Hdm2 in MCF7 cells.

| AffyID  | Fold Change vs. siHdmX | Genes | Symbol | Description |
|---------|------------------------|-------|--------|-------------|
| 37577   | 0.544                  | ARHGAP19 | KIAA0286 | Rho GTPase activating protein 19 |
| 212619  | 0.542                  | KIAA0286 | KIAA0286 | Rho GTPase activating protein 19 |
| 211090  | 0.614                  | NEK2   | KIAA0286 | NIMA (never in mitosis gene a)-related kinase 2 |
| 204128  | 0.539                  | CDC45L | CDC45L | CDC45 cell division cycle 45-like (S. cerevisiae) |
| 223381  | 0.538                  | CDC6   | CDC6   | CDC6 cell division cycle 6 homolog (S. cerevisiae) |
| 203967  | 0.536                  | DEPDC1 | DEPDC1 | DEP domain containing 1 |
| 220295  | 0.535                  | TFDP1  | TFDP1  | transcription factor Dp-1 |
| 222680  | 0.533                  | OTL    | OTL    | doteless homolog (Drosophila) |
| 232278  | 0.533                  | WDHD1  | WDHD1  | WD repeat and HMG-box DNA binding protein 1 |
| 210053  | 0.529                  | TAF5   | TAF5   | TAF5 RNA polymerase II, TATA box binding protein (TBX)-associated factor, 100kDa |
| 206632  | 0.522                  | APOBEC3B | APOBEC3B | apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B |
| 202779  | 0.517                  | UBE2S  | UBE2S  | ubiquitin-conjugating enzyme E2S |
| 209464  | 0.517                  | AURKB  | AURKB  | aurora kinase B |
| 203418  | 0.514                  | CCNA2  | CCNA2  | cyclin A2 |
| 223709  | 0.514                  | GADD46 | GADD46 | GADD46 |
| 203214  | 0.507                  | CDC2   | CDC2   | cell division cycle 2, G1 to S and G2 to M |
| 218726  | 0.507                  | DOK5   | DOK5   | hypothetical protein DOK5 |
| 230022  | 0.507                  | DOK5   | DOK5   | hypothetical protein DOK5 |
| 209408  | 0.506                  | KIF2C  | KIF2C  | kinesin family member 2C |
| 211613  | 0.506                  | KIF2C  | KIF2C  | kinesin family member 2C |
| 205558  | 0.497                  | CDC2   | CDC2   | cell division cycle 2, G1 to S and G2 to M |
| 219000  | 0.497                  | DCC1   | DCC1   | defective in sister chromatid cohesion homolog 1 (S. cerevisiae) |
| 239002  | 0.488                  | ASPM   | ASPM   | asp (abnormal spindle)-like, microcephaly associated (Drosophila) |
| 210334  | 0.483                  | BIRC5  | BIRC5  | baculoviral IAP repeat-containing 5 (survivin) |
