The role of p53.S389 phosphorylation in DNA damage response pathways and tumorigenesis

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Chapter 5

Delayed expression of apoptotic and cell cycle control genes in carcinogen-exposed bladders of mice lacking p53.S389 phosphorylation

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Abstract

Mice with non-phosphorylated Ser389 in p53 are susceptible for bladder tumors induced by 2-AAF. Since p53 is a transcription factor, this might well be preceded by differences in the regulation of gene expression. Microarray analysis was used to determine early transcriptional changes that might underlie this cancer-prone phenotype. Interestingly, lack of Ser389 phosphorylation led to endogenously different gene expression levels. The number of genes affected was, however, rather small. Conversely, after short-term exposure to 2-AAF, wild-type and p53.S389A bladders demonstrated a significant number of differentially-expressed genes. Differences between wild-type and p53.S389A could mainly be attributed to a delayed, rather than complete absence of, transcriptional response of a group of genes, including well-known p53 target genes involved in apoptosis and cell cycle control like *Bax, Perp* and *P21*. An analysis of differentially-expressed genes in non-tumorigenic tissue and bladder tumors of p53.S389A after long-term exposure to 2-AAF revealed 319 genes. Comparison of these with those found after short-term exposure resulted in 23 transcripts. These possible marker genes might be useful for the early prediction of bladder tumor development. In conclusion, our data indicate that, lack of Ser389 phosphorylation results in aberrant expression of genes needed to execute vital responses to DNA damage. Post-translational modifications, like Ser389 phosphorylation, seem crucial for fine-tuning the transcription of a specific set of genes and do not appear to give rise to major changes in transcription patterns. As such, Ser389 phosphorylation is needed for some, but certainly not all, p53 functions.

Introduction

When chemical or physical compounds damage DNA, several defense mechanisms are activated to prevent the DNA from harmful structural alterations that may ultimately lead to cancer. All of these defense mechanisms are aimed at eliminating DNA damage. Examples are cell cycle arrest, DNA repair and apoptotic processes. The latently present p53 protein becomes stabilized and activated in response to DNA damage. Once activated, p53 acts as a tumor suppressor through transcriptional activation of a large variety of target genes [1-3]. Activation of p53 occurs exclusively at the post-translational level through a broad range of modifications; namely (de)phosphorylation, acetylation, ubiquitination, sumoylation, glycosylation, methylation and neddylation. Of these processes, phosphorylation appears to be the most frequent modification of the protein [4-6].

Phosphorylation of the p53 N-terminal region, in particular Ser15 and Ser20, disrupts the interaction with the MDM2 protein, a ubiquitin ligase that inactivates p53 by targeting the protein for proteasomal degradation [7;8]. Ser315 and Ser392 were the first phosphorylation sites identified at the C-terminal domain [9]. Ser315 is phosphorylated in response to both ionizing and UV radiation, whereas Ser392 is only phosphorylated specifically after UV radiation [4;10-12]. Kinases targeting this Ser392 site *in vitro* are Casein Kinase II (CKII) [13;14], the double-stranded, RNA-activated protein kinase (PKR) [15], p38 Map kinase [16;17] and the recently identified cyclin-dependent kinase 9 (cdk9) through direct physical interaction [18;19]. However, it is still not clear how certain types of DNA damage result in phosphorylation of Ser392, and what the ultimate effect on specific cellular defense systems is *in vivo*.

To investigate the function of the Ser392 phosphorylation site (equivalent of mouse Ser389), we generated mice with a single point mutation in the *p53* gene that resulted in a substitution of a serine to an alanine; the p53.S389A mouse model [20]. Phosphorylation of Ser389 is not required

for p53 functioning in either spontaneous or ionizing radiation-induced lymphoma tumor development, since tumor responses were highly comparable in p53.S389A and wild-type mice [20;21]. However, p53.S389A mice appeared to be more susceptible for 2-acetylaminofluorene (2-AAF)-induced urinary bladder tumors and UV-B induced skin tumors [20;21]. This is in line with our *in vitro* findings where cells of these mice demonstrated an impaired DNA-binding capacity and a reduced apoptotic response after UV radiation [20]. We therefore hypothesized that phosphorylation of p53 at codon 389 is substrate dependent, and that lack of Ser389 phosphorylation only has an adverse effect on the functioning of p53 as a tumor suppressor in the case of specific substrates.

In the current study, we further explored which *in vivo* molecular responses are affected when phosphorylation of p53 at codon 389 is impossible. For this, mice were exposed to 2-AAF and gene-expression profiles were analyzed in urinary bladder tissue (further referred to as bladder) of male mice. This was done because the increased incidence of tumors in p53.S389A mice was observed in this specific tissue, and was furthermore restricted to male mice only [21]. Given the well-known function of p53 as a transcription factor, we hypothesized that this increased incidence of bladder tumors is likely to be preceded early in the exposure period by a different gene-expression profile in bladders of p53.S389A mice compared to wild-type mice not susceptible to exposure to 2-AAF. To investigate this, we performed a microarray analysis on wild-type and p53.S389A male bladders exposed for 1 or 2 weeks (short-term) to 2-AAF. Interestingly, a significant number of genes are differentially-expressed in bladders of p53.S389A compared to wild-type mice, including known p53 target genes involved in apoptotic and cell cycle processes. Finally, we also analyzed gene expression patterns in tumorigenic bladders of p53.S389A mice after long-term exposure to 2-AAF, and compared these to the differentially-expressed genes found after short-term exposure to 2-AAF. Several overlapping genes could be identified. Possibly these genes are useful as early markers for bladder cancer development.

Materials and Methods

Wild-type and p53.S389A mice exposed to 2-AAF

Mice were exposed to 300 ppm 2-AAF mixed in feed as used in previous studies [21] for 1 or 2 weeks (short-term) or 39 weeks (long-term) (n=4). The 2-AAF containing feed (Altromin, Lage, Germany), normal feed and water were available ad libitum throughout the course of the studies. All mice used were males of 6-9 weeks of age and at least ten times backcrossed in C57BL/6. Furthermore control mice received normal diet.

RNA isolation

Total RNA was isolated from mouse bladder using the RNeasy Mini kit (Qiagen, Valencia, USA), followed by a DNase treatment with RNase-Free DNase Set (Qiagen Valencia, CA, USA)). RNA quality was assessed with the Bioanalyzer 2100 (Agilent Technologies, Palo Alto, USA) All assays were performed according to the manufacturer's protocols.

Microarray analysis

The mouse oligonucleotide libraries (Cat # MOULIBST & Cat # MOULIB384B) were obtained from Sigma-Compugen Incorporated. Technical support was supplied by LabOnWeb (http://www.labonweb.com/cgi-bin/chips/full_loader.cgi). The libraries represent in total 21,766 LEADS™ clusters plus 231 controls. The oligonucleotide library was printed with a Lucidea

Spotter (Amersham Pharmacia Biosciences, Piscataway, USA) on commercial UltraGAPS slides (amino-silane-coated slides, Corning 40017) and processed according to the manufacturer's instructions. The slides contained 65-mer oligonucleotides and the batch was checked for the quality of spotting by hybridizing with SpotCheck Cy3 labeled nonamers (Genetix,New Milton Hampshire, UK).
1.5 µg total RNA samples were used in randomized batches, according to a common reference design, with a mix of all samples as common reference. The RNA was amplified using the Amino Allyl MessageAmp aRNA kit (Ambion, Austin, USA), and labeled with Cy3 (experimental samples) and Cy5 (common reference) reactive dye according to the manufacturer's instructions. The microarrays were hybridized overnight with 200 µl hybridization mixture, consisting of 50 µl Cy3-and Cy5-labeled and aRNA (in a 1 to 3 molar ratio), 100 µl Formamide and 50 µl 4 x RPK0325 MicroArray Hybridization Buffer (Amersham Pharmacia Biosciences, Piscataway, USA) at 37°C, washed in an Automated Slide Processor (Amersham Pharmacia Biosciences, Piscataway, USA), and subsequently scanned (Agilent DNA MicroArray Scanner, Agilent Technologies, Palo Alto, USA).

Data analysis and statistics
Microarray spot intensities were quantified as artifact removed densities, using Array Vision software (version 6.0). Further processing of the data was performed using R (version 2.2.1) and the Bioconductor MAANOVA package (version 0.98.8). All slides were subjected to a set of quality control checks, i.e., visual inspection of the scans, examining the consistency among the replicated samples by principal components analysis (PCA), testing against criteria for signal to noise ratios, testing for consistent performance of the labeling dyes, pen grid plots to check consistent pen performance, and visual inspection of pre- and post-normalized data with box plots and RI plots.
After log2 transformation, the data were normalized by a spatial lowess smoothing procedure, and analyzed using a two-stage mixed-model ANOVA model [22;23] First, array, dye and array-by-dye effects were modeled globally. Subsequently, the residuals from this first model are fed into the gene-specific model to fit treatment, and spot effects on a gene-by-gene basis using a mixed-model ANOVA. These residuals are normalized expression values and throughout used in the graphs to depict (differential) gene expression. For hypothesis testing, a permutation based F1 test was used which allows relaxing the assumption that the data is normally distributed [24]. To account for multiple testing, p-values from the permutation procedure were adjusted to represent a False Discovery Rate (FDR) of 10% [25]. Four tests were performed to identify differential gene expression. 1) A test to find differences between control, 1 or 2 weeks 2-AAF exposed wild-type bladder. 2) A test to find differences between control, 1 or 2 weeks 2-AAF exposed p53.S389A bladder. 3) A test to find differences between genotypes across control, 1 or 2 weeks 2-AAF exposed bladder. 4) A test to find differences between tumorigenic and non-tumorigenic p53.S389A bladder.
The differences in gene expression between the wild-type and p53.S389A mice in their response to 2-AAF was qualitatively determined as follows. First, the normalized expression values for all genes found to be involved in 2-AAF exposure were calculated (the union of list (1) and (2)). Second, for each time point the difference in gene expression between 2-AAF exposed and control bladder was calculated; this difference represents a rate of change in expression. Third, for each time point this difference in gene expression between 2-AAF exposed and control bladder

of wild-type and p53.S389A were compared in a scatter plot. A deviation from a one-to-one relationship in these plots indicated a difference in gene expression rate between wild-type and p53.S389A in their response to 2-AAF exposure. This enabled the identification of delayed up-regulated and delayed down-regulated genes in p53.S389A bladder.
To relate the differences in gene expression to changes in functional biological processes, lists of differentially-expressed genes were analyzed for overrepresentation of specific Gene Ontology (GO) terms using Onto Express (http://vortex.cs.wayne.edu/projects.htm). The list of all gene products on the microarray was then used as the reference set. Relevant GO-terms were selected according to FDR-corrected p-values and the number of genes involved. The F1-statistics from list (1), (2) and (3) were used for Gene Set Enrichment Analysis (GSEA) [26]. All pathways and gene expression signatures contained in the c2 database of the by Molecular Signature Database (MsigDb 1.0; http://www.broad.mit.edu/gsea/msigdb/msigdb_index.html), and an additional p53 pathway reported by Harris and Levine [27] were tested for significance using the Gene-Set-Test facility provided by the Limma package (version 2.7.3) in Bioconductor. Pathways and gene expression signatures with p-values <0.05 and with at least one gene from either list (1), (2) or (3) were reported.

Real-time PCR
To verify the microarray results, cDNA was generated from RNA by using the high-Capacity cDNA archive kit containing random hexamer primers (Applied Biosystems). mRNA presence was measured with Taqman gene expression assays (Applied Biosystems) on a 7500 Fast Real-Time PCR System, with a two-step PCR procedure according to the manufacturer's protocol. The assays we used were *Ccnb2*; Mm00432351_m1, *Ccng1*; Mm00438084_m1, *Pttg1* Mm00479224_m1 and *Pmaip1*; Mm00451763_m1.

Results

Differentially-expressed genes in unexposed wild-type and p53.S389A bladders

Absence of biologically active p53 protein has a dramatic influence on expression profiles, even in unexposed cells [28;29]. To test comparable effects in cells carrying modified p53 protein, we compared gene-expression profiles between unexposed wild-type and p53.S389A bladders. A total of 44 genes with a clear statistically-significant difference between the genotypes were identified and these are presented in a bar plot in Figure 1. Twenty-three genes had a lower (log2 (expression ratio (p53.S389A/wild-type)) <0) and 21 genes had a higher (log2 (expression ratio (p53.S389A/wild-type)) >0) expression level in p53.S389A bladders compared to wild-type bladders. Interestingly, the two genes with the highest induced ratios were the bladder tumor-related gene *Sfrp1* and the cofactor of transcription *Pparbp* [30]. The two genes with the most reduced ratio were *Igh-6* and the presumed proto-oncogene *Pttg1* [31]. The biological categories, based on GO terms (analyzed using Onto-Express [32]), containing the most affected genes were development, regulation of transcription and transport. The complete lists of differentially-regulated genes found on the basis of genotype differences between wild-type and p53.S389A bladders together with their log2 (expression ratios) is provided in Supplementary Table I. Lack of Ser389 phosphorylation therefore results in clear differences in gene expression levels in unexposed bladders.

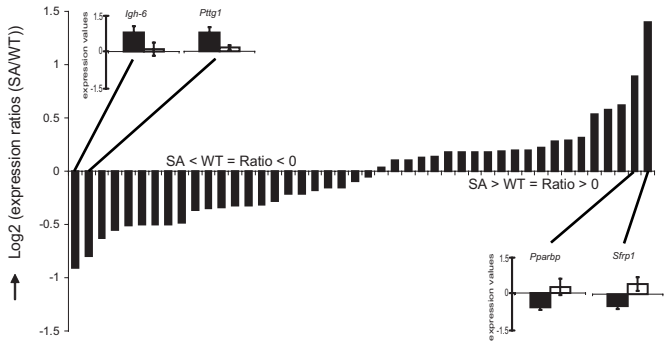

Figure 1 - Bar plot of differentially-expressed genes in wild-type and p53.S389A bladders
Differences in expression of genes across genotypes were tested as described in Materials and Methods. A bar plot representing ratios of the log2 (expression values) of p53.S389A (SA) divided by wild-type (WT) is shown. Each bar represents an individual gene; corresponding values are depicted in Supplementary Table I. The genes with the highest ratios are magnified and represented as a bar plot with the average of log2 (expression values) of both WT (wild-type; black) and SA (p53.S389A ; white) control samples.

Differentially-expressed genes in wild-type and p53.S389A bladders after exposure to 2-AAF

To analyze whether differences in gene expression shortly after exposure to 2-AAF can explain differences in tumor outcome later in life, we exposed wild-type and p53.S389A mice for one and two weeks to 2-AAF and analyzed gene expression by microarrays in RNA isolated from bladder tissues.

A PCA with a two-dimensional representation of the relationship between the wild-type and p53.S389A samples demonstrated the segregation between the control and samples exposed to 2-AAF for 1 or 2 weeks (Figure 2A). Importantly, a clear segregation between the genotypes was observed as well. Apparently, lack of Ser389 phosphorylation of p53 resulted in different gene expression responses *in vivo* after exposure to 2-AAF.

Differential expression of the genes found to have significantly changed over time was analyzed with the help of a one-way ANOVA analysis on the different groups of mice (control, 1 or 2 weeks 2-AAF), and the construction of Self-Organizing Maps (SOMs). Figure 2B shows the expression profiles of both genotypes as well as the number of genes per SOM. The complete lists of differentially-expressed genes found in wild-type and p53.S389A bladders, together with fold-change levels based on their corresponding controls is provided in Supplementary Tables II and III, respectively. Expression levels measured by microarray analysis were highly similar to results obtained with real-time PCR (Supplementary Figure 1).

As a consequence of 2-AAF exposure, there were groups of genes in both genotypes that exhibited continuous changes in gene expression. However, induction and repression levels were rather small (panels A, B, E, and F). Furthermore, there were groups with more pronounced expression profiles (panels C, D, G, and H). With respect to differences between wild-type and p53.S389A, a group of 82 genes (panels C and D) in the wild-type demonstrated a clear increase in gene expression after 1 week and a marked decrease after 2 weeks, which was not observed in p53.S389A (panels G and H). A careful evaluation revealed that those genes peak at 1 week in wild-type, whereas in p53.S389A they only reached this peak after 2 weeks. Interestingly, this group included apoptosis-related genes such as *Pmaip1* (*Noxa*) [33], *Bax* [34] and *Birc5* (*Survivin*) [35], genes involved in damage recovery and growth promotion like *Ccng1* (*Cyclin G1*) and *Ccnb2* (*Cyclin B2*) [36], and the DNA repair gene *Mgmt*.

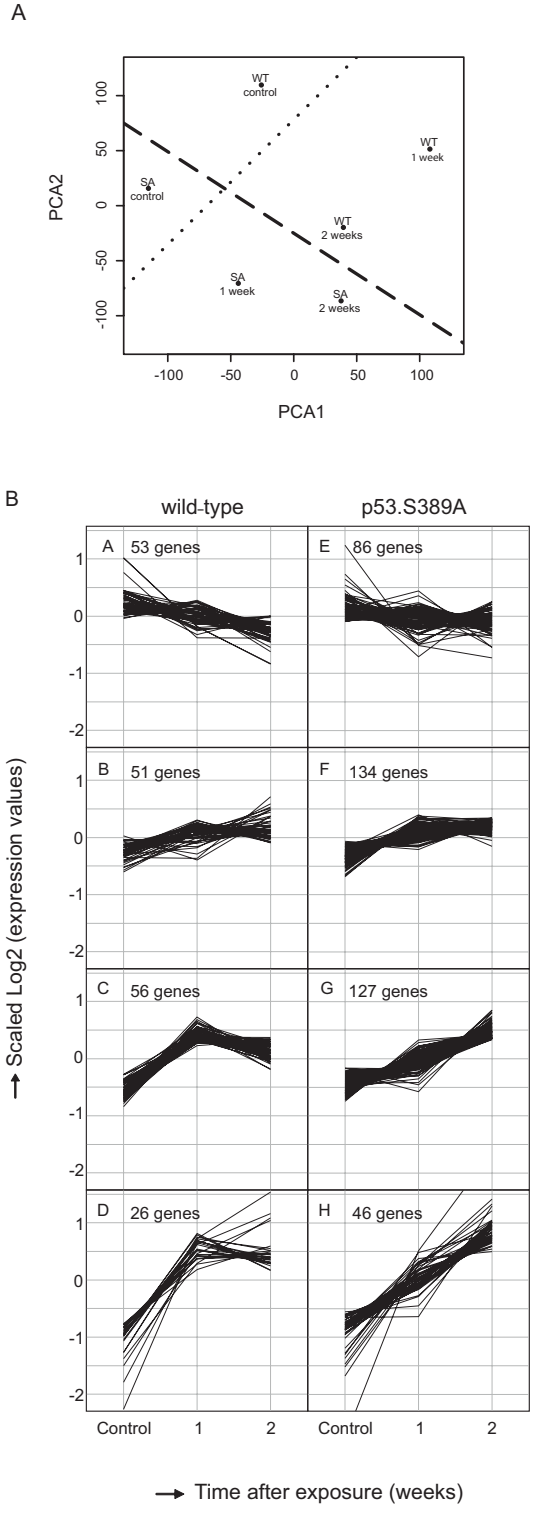

Figure 2 - Presentation of differentially-expressed genes in wild-type and p53.S389A bladders after short-term exposure to 2-AAF

A) PCA of differentially-expressed genes in wild-type (WT) and p53.S389A (SA) bladders, providing a two-dimensional representation of the relationships between wild-type and p53.S389A gene expression of control bladders and bladders after short-term exposure to 2-AAF. Each group represents 4 individual replicates. The dashed line shows segregation between wild-type and p53.S389A, and the dotted line between the control group and groups exposed short-term (1 week and 2 weeks) to 2-AAF.

B) SOM of differentially-expressed genes in bladders wild-type and p53.S389A mice after short-term exposure to 2-AAF. All differentially-expressed genes were grouped into four clusters per genotype (panel A-D for wild-type and panel E-H for p53.S389A). Each SOM cluster represents the gene expression pattern for genes within the specific cluster, with the number of genes in each cluster indicated. Log2 (expression values) scaled relative to the mean are shown on the *y*-axis, with the corresponding time points on the *x*-axis.

1 = one week exposure to 2-AAF, 2 = two weeks exposure to 2-AAF

Exploring the differentially-expressed genes in wild-type and p53.S389A bladders after exposure to 2-AAF

To gain a better understanding of the processes involved in mouse bladders after short-term exposure to 2-AAF, we analyzed all genes that were differentially-expressed in wild-type and/or p53.S389A (i.e., union, see Materials and Methods) using Onto-Express [32]. Supplementary Table IV shows the GO categories with a corrected p-value ≤0.05 and at least 3 genes present. Within the GO categories found to be significant, the most interesting were: cell cycle (arrest), DNA repair, and induction of apoptosis. These are processes known to be affected after exposure to a genotoxic agent [37]. As expected after exposure to 2-AAF, the category 'response to DNA damage stimulus' was also found. Furthermore, mitosis and mitotic-related processes such as DNA replication, cytokinesis and DNA metabolism are significantly present. In addition to the GO analysis, we used GSEA (recently described as a powerful analytical method for interpreting microarray data) to obtain more insight into the biological pathways involved [26]. Table I provides a complete list of pathways that were significantly affected (p ≤0.005). Four processes with known p53 involvement were ranked in the top ten when selecting for highest p-values in wild-type bladders. For example, the p53 signaling pathway was highly significantly altered, as well as a general p53 pathway and the p53 hypoxia pathway. Furthermore, a variety of cell cycle and apoptotic-related processes were found. In conclusion, short-term *in vivo* exposure to 2-AAF of wild-type mice resulted in altered expression of genes in bladders mostly involved in p53-related pathways, such as cell cycle regulation and apoptosis. Given this observation, it might be expected that these p53-related pathways in particular, are aberrantly regulated by exposure to 2-AAF when p53 functioning is inhibited through mutational inactivation of the Ser389 phosphorylation site. Indeed, GSEA of the differentially-regulated genes in bladders from p53.S389A mice exposed to 2-AAF resulted in an almost entirely different order of this list (Table I). Only two of the p53-related processes, p53-UP and p53 signaling, were also found to

Table I - Biological pathways in mouse bladders after exposure to 2-AAF that were found to be differential using GSEA analysis

Wild type			p53.S389A		
Pathway	**Description**	**p-value***	**Pathway**	**Description**	**p-value***
p53_signalling	BioCarta	<0.0001	PGC	Manually Curated	0,0004
PGC	Manually Curated	<0.0001	MAP00600_Sphingoglycolipid_metabolism	GenMAPP	0,0009
P53_UP	Kannan_et_al_2001	<0.0001	MYC_MUT	BLACK	0.001
GLUT_UP	Peng_at_al_2002	<0.0001	PROLIF_GENES	Manually Curated	0.0013
CBF_LEUKEMIA_DOWNING_AML	Manually Curated	<0.0001	MAP00531_Glycosaminoglycan_degradation	GenMAPP	0.0018
p53Pathway	BioCarta	6,00E-04	MAP00480_Glutathione_metabolism	GenMAPP	0.0019
p53hypoxiaPathway	BioCarta	7,00E-04	GLUT_UP	Peng_at_al_2002	0.0021
PROLIF_GENES	Manually Curated	8,00E-04	CR_CELL_CYCLE	PNAS_2004	0.0022
MAP00512_O_Glycans_biosynthesis	GenMAPP	0,0015	ST_Dictyostelium_discoideum_cAMP_Chemotaxis_Pathway	Signalling Transduction KE	0.0024
radiation_sensitivity	BioCarta	0,0016	**P53_UP**	Kannan_et_al_2001	0.0025
cell_cycle_arrest		0,0018	**p53_signalling**	BioCarta	0.0026
BRCA_UP	Welcsh_et_al_2002	0,002	MAP00052_Galactose_metabolism	GenMAPP	0.0034
cellcyclePathway		0,0021	cell_cycle_arrest		0.0036
drug_resistance_and_metabolism	BioCarta	0,0021	GO_ROS	FO	0.0036
GO_ROS	FO	0,0021	breast_cancer_estrogen_signalling	GEArray	0.0037
MAP00480_Glutathione_metabolism	GenMAPP	0,0024	AR_MOUSE_PLUS_TESTO_FROM_NETAFFX	na	0.0038
SA_PROGRAMMED_CELL_DEATH	SigmaAldrich	0,0024	CBF_LEUKEMIA_DOWNING_AML	Manually Curated	0.0038
CR_CELL_CYCLE	PNAS_2004	0,0027	freePathway		0.0043
breast_cancer_estrogen_signalling	GEArray	0,0032	g1Pathway	BioCarta	0.0049
DNA_DAMAGE_SIGNALLING	GO	0,0034	cell_proliferation		0.005
badPathway		0,0035			
FRASOR_ER_DOWN	Frasor_et_al_2004	0,0036			
g1Pathway	BioCarta	0,0038			
ceramidePathway		0,0042			
MAP00230_Purine_metabolism	GenMAPP	0,0049			

*Pathways with a p-value <0.005 are shown

be significantly present. However for the corresponding processes, the associated p-values were considerably higher in the p53.S389A bladders than those found in wild-type mice, indicating that these p53-related pathways are activated/de-activated to a lesser extent in p53.S389A bladders exposed to 2-AAF.

Delayed gene expression in p53.S389A bladders exposed to 2-AAF

To unravel the exact nature of the observed differences in gene expression between wild-type and p53.S389A mice, the union of the 2-AAF induced differential genes was tested for its distribution of genes along the log2 (expression values) axis. As depicted in Figure 3A, left panel, the line chart with the log2 (expression values) of genes in wild-type and p53.S389A control bladders had a slope of 1.04 and a correlation coefficient of 0.98, indicating equal log2 (expression values) of the genes in unexposed bladders of mice of both genotypes (i.e., lack of significant differences). However after 1 week of exposure to 2-AAF, the distribution along the 1:1 line of these genes was divergent since the slope was only 0.71 and the correlation coefficient 0.64. This means the log2 values (fold change levels) compared to their corresponding control values of genes in p53.S389A bladders were either higher or lower than those of the same genes in wild-type bladders. Intriguingly, after 2 weeks of exposure to 2-AAF, the distribution of log2 (fold change levels) of the selected genes was becoming more comparable again, with a slope of 1.16 and a correlation coefficient of 0.83. These results indicated that although expression levels in unexposed samples were highly comparable, a mutation at Ser389 resulted in different kinetics of gene expression after short-term exposure to 2-AAF compared to wild-type mice. Since this difference in transcriptional response was most evident after 1 week, and seemed to decrease again after 2 weeks of exposure towards endogenous levels, responses in p53.S389A bladders appeared to be delayed compared to their wild-type counterparts.

K-means clustering was subsequently performed on a selection of these genes (n=247), in which induction or repression were delayed in p53.S389A compared to wild-type bladders after 1 week of exposure to 2-AAF (Figure 3B). Although the resulting groups of genes did not have similar response patterns, responses in p53.S389A bladders were consistently delayed compared to wild-type responses, both in down-regulation as well as in up-regulation of genes. In some groups (I, IV and V) the expression value in p53.S389A bladders after 2 weeks of exposure to 2-AAF reached more or less the same value as observed in wild-type bladders after 1 week of exposure to 2-AAF. In the remaining groups the minimum expression values in p53.S389A bladders were either higher (II), or reached a lower (III) or higher (VI) maximum than those in the wild-type. This indicated that although all 247 genes demonstrated delayed responses, the exact nature of this delay was highly dependent on the individual gene concerned. A representative gene was selected for each group (I to VI) and the expression levels of these are depicted in Figure 3C. Use of the GO categories revealed that these delayed genes were involved in transport and mitotic-related processes (cytokinesis, DNA replication, electron transport, metabolism, mitosis). In addition, categories involved in the p53-dependent processes like apoptosis and cell cycle regulation were also present (see Supplementary Table V).

Based on the above-mentioned results, we investigated the behavior of the known p53-related pathways of cell cycle arrest and apoptosis, and the p53 target genes involved in these processes. Harris and Levine recently reviewed the downstream events of the p53 pathway and this led to a model of important p53 target genes and their function [27]. Figure 4 shows an adapted version of this model, provides an overview of the genes present on our microarray and lists

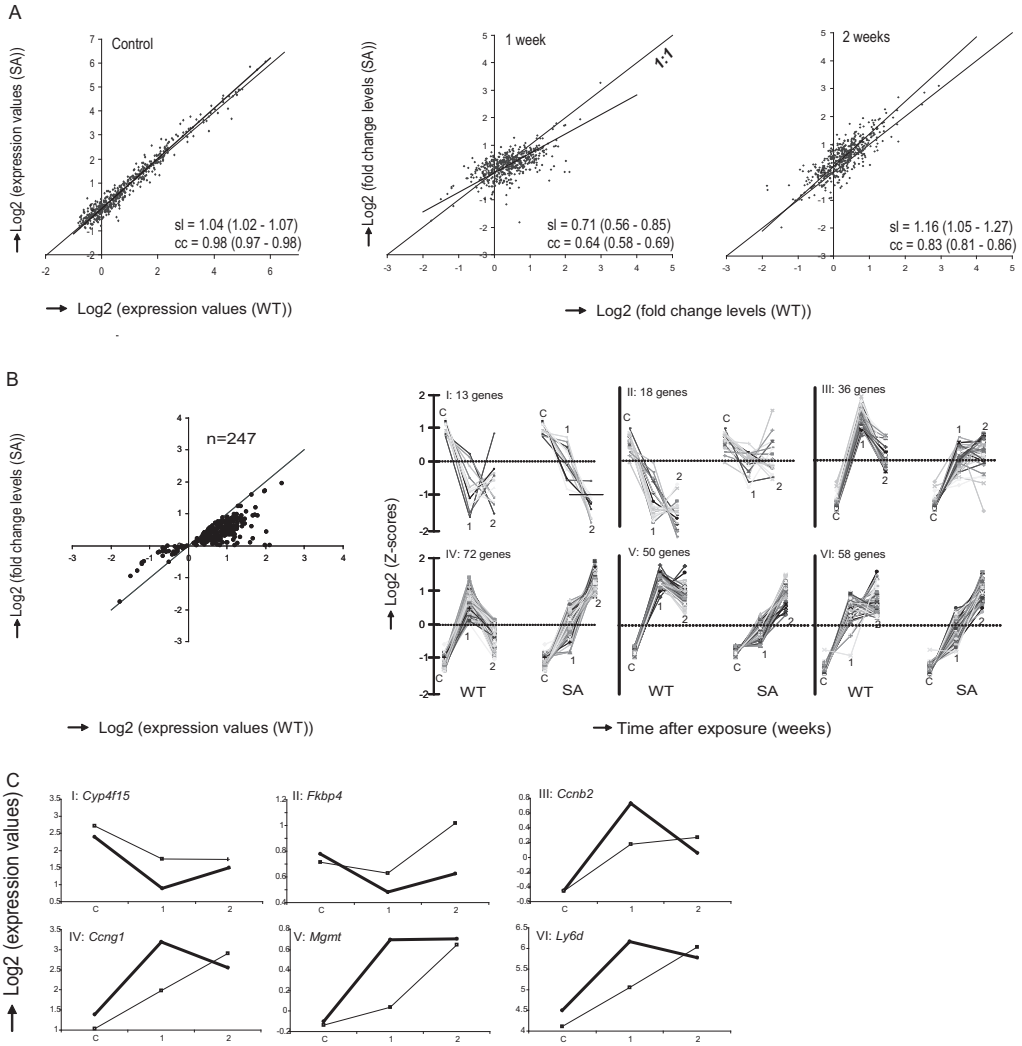

Figure 3 - Graphical configuration of differentially-expressed genes in wild-type and p53.S389A bladders after short-term exposure to 2-AAF

A) All differentially-expressed genes are displayed in a scatter plot to compare the expression values of the differentially-expressed genes found in bladders of wild-type (WT) and p53.S389A (SA) mice after short-term exposure to 2-AAF. Each spot represents an individual gene, where the axis shows the log2 (expression values) for the 1st plot and log2 (fold change levels) based on their corresponding controls for the 2nd and 3rd plot of wild-type (*x*-axis) and p53.S389A (*y*-axis). An orthogonal regression line is added to show the slope of the data points. Equal expression values of a gene in both wild-type and p53.S389A give a slope of 1. The slope (sl) of this line is displayed in the chart, as well as the corresponding correlation coefficient (cc).

B) A selection of genes (n=247) with a delayed response in p53.S389A (SA) compared to wild-type (WT) mice after 1 week of exposure (represented in A) is shown. Six pairs of line graphs have been made for these genes. In each pair the left plot are wild-type (WT) and the right plot are p53.S389A (SA) values of the selected genes. The number of genes in each plot is indicated above the corresponding pair of graphs.

C) Log2 (expression values) of 6 individual genes corresponding with the line graphs depicted under B. Bold line: wild-type, Thin line: p53.S389A, C = control, 1 = one week exposure to 2-AAF exposure, 2 = two weeks exposure to 2-AAF.

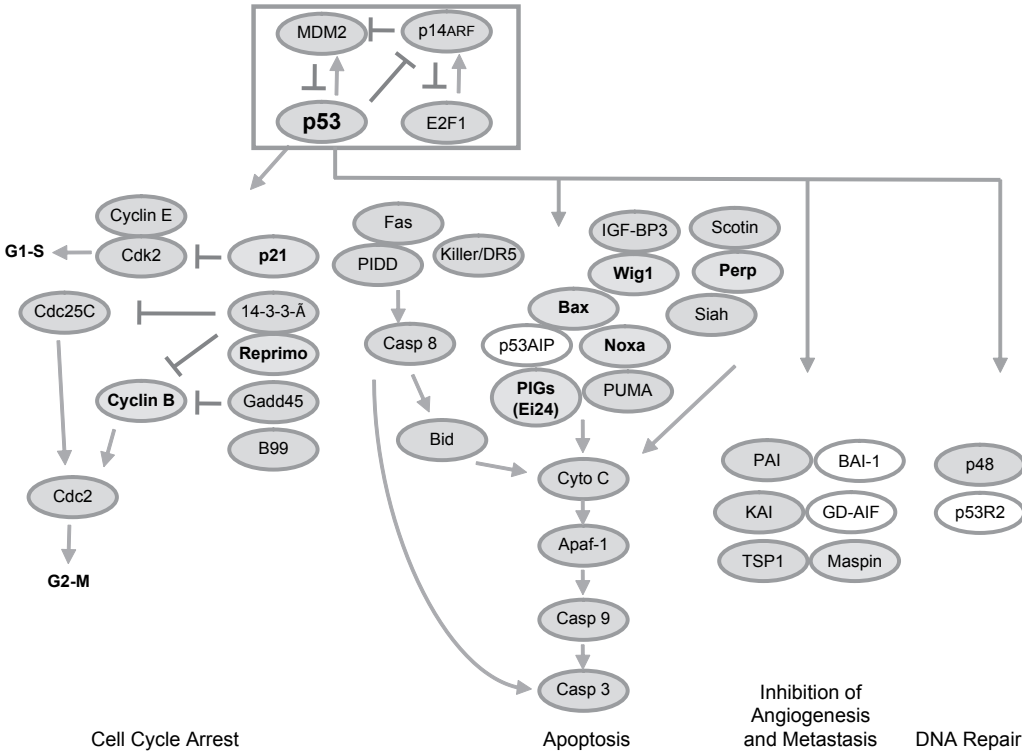

Figure 4 - Adversely affected p53 target genes in bladders of p53.S389A mice after short-term exposure to 2-AAF

Known p53 target genes involved in the apoptotic, cell cycle arrest, inhibition of angiogenesis and metastasis and DNA repair processes are presented (model adapted from [27]). Genes represented in yellow show a delayed response in bladders of p53.S389A mice compared to wild-type after exposure to 2-AAF. Genes represented in blue are present on our microarray but not differential between p53.S389A and wild-type; genes in white are absent on our microarray. For color figure, see page 183.

the genes showing a delayed response in our mutant mouse model. Several genes involved in these p53-dependent pathways were severely altered by the mutation at Ser389. In the cell cycle arrest pathway, three of the ten p53 target genes, *Cdkn1a* (*P21*), *Reprimo* and *Ccnb2* (*Cyclin B*), demonstrated a delayed response. Also, five of the nine analyzed genes involved in the intrinsic apoptosis pathway were affected by the p53.S389A mutation; *Wig1* (*PAG608*), *Perp*, *Bax*, *Pmaip1* (*Noxa*) and *Ei24* (*Pig8*). Interestingly, the extrinsic apoptotic pathway seemed not to be affected, at least not those genes described in the Harris and Levine model. Even in the pathway involving angiogenesis and metastasis expression, one of the four genes analyzed was affected; *Maspin*. In summary, differences in gene expression between p53.S389A and wild-type bladders after exposure to 2-AAF, as detected by microarray analysis, could be mapped to specific p53-dependent pathways.

Analysis of gene expression in p53.S389A bladder tumors

The detected differences in gene-expression profiles of bladders of wild-type and p53.S389A mice after short-term exposure to 2-AAF, might explain the increased susceptibility of our mutant model for development of bladder tumors. To analyze whether these differentially-expressed

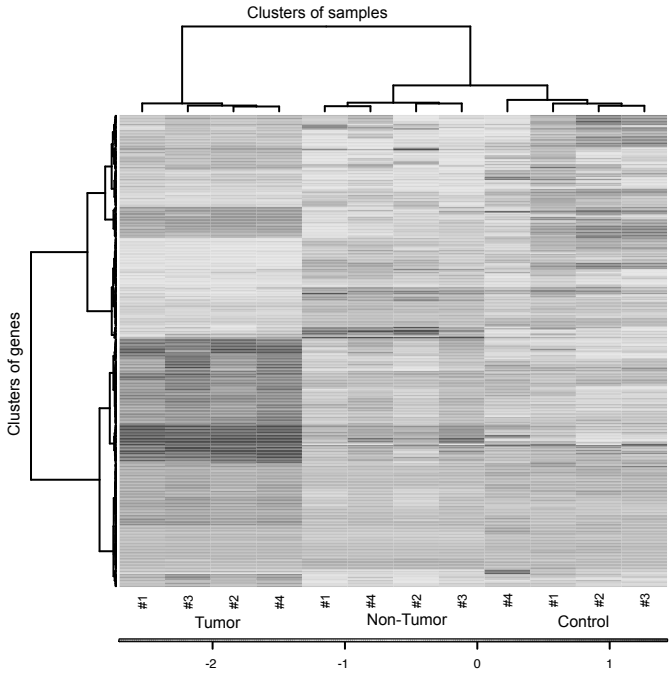

Figure 5 - Hierarchical clustering of genes found to be differentially-expressed in tumorigenic versus non-tumorigenic p53.S389A bladders after long-term exposure to 2-AAF

A total of 319 differentially-expressed transcripts were found by analysis of tumorigenic versus non-tumorigenic bladders of p53.S389A mice after long-term exposure to 2-AAF. Clusters were created using hierarchical clustering of the gene-expression profiles, where each row represents an individual gene. The degree of redness and greenness represent induction and repression respectively.
non-tumor = bladders after 39 weeks exposure to 2-AAF without tumorigenic bladder, tumor = bladders after 39 weeks of exposure to 2-AAF with tumorigenic bladder. For color figure, see page 183.

genes could be directly linked to bladder tumor development, we compared gene-expression profiles in both non-tumorigenic bladders and bladder tumor tissues. Since wild-type mice did not develop bladder tumors using the current exposure protocol, these analyses were restricted to p53.S389A mice only. A total of 319 significant genes were found between non-tumorigenic and bladder tumors of p53.S389A mice (Supplementary Table VI). Hierarchical clustering of the individual samples of the control, tumorigenic and non-tumorigenic bladders separated the groups, as a strong correlation across samples was found between the 319 differentially-expressed genes (Figure 5). Non-tumorigenic tissue exposed long-term to 2-AAF, clustered more closely to the controls than tumor-bearing bladders. In addition, absolute expression levels of the majority of genes appeared to be higher in the tumorigenic bladders compared to the non-tumorigenic bladders or controls. This seemed plausible given the high levels of cell proliferation or suppression of survival genes known to be present in tumors in general. Examples of significant biological processes found after GO analysis in this group of differential genes were metabolism, cell adhesion, muscle development, apoptosis and cytoskeleton organization. All of these are processes known to be involved in or affected by tumor formation (Supplementary Table VII). Some processes demonstrated a reduced expression level of genes in tumorigenic compared to non-tumorigenic bladder (e.g., cytoskeleton organization and muscle development), whereas others show an increased expression of most genes (e.g., cell adhesion).

To find out whether altered gene expression after short-term exposure coincided with genes affected in tumors, a comparison was made of genes found to be differentially-expressed in tumorigenic bladders (long-term exposure to 2-AAF) and genes found in p53.S389A bladders exposed short-term to 2-AAF. Interestingly, this comparison revealed 23 genes found both in short-term exposed bladders as well as in bladder tumors. Ratios of short-term exposed (i.e., 1 week) p53.S389A/wild-type and long-term exposed tumorigenic/non-tumorigenic

Table II - Differentially-expressed genes detected in p53.S389A bladders after both long-term and short-term exposure to 2-AAF

			Ratios	
Accno	**GO category**	**symbol**	**tumor/non-tumor***	**SA/WT 1 week****
NM_013834	Signal Transduction	**Sfrp1**	2,0	2,2
NM_019645	Signal Transduction	**Pkp1**	1,9	-0,1
NM_009704	Cell cycle	**Areg**	1,6	-0,2
NM_019736	Lipid Metabolism	**Acate2**	1,3	0,0
NM_009448	Nuclear Congression	**Tuba6**	1,2	0,3
NM_008385	Cell Growth and/or Maintenance	**Inpp5b**	1,0	-0,4
AF036898	DNA Replication	**Pole2**	0,8	-0,1
NM_010282	Lipid Metabolism	**Ggps1**	0,6	-0,1
NM_026662	Neurogenesis	**Prps2**	0,5	0,0
AK016670	Germ-Cell Development	**Bcl2l14**	-0,4	-0,1
AF109905	Chromatin Silencing at Ribosomal DNA	**Ly6g6c**	-0,5	-0,2
AK017677		**Cds2**	-0,7	-0,2
AK008108		**Sulf2**	-0,7	-0,7
NM_011663	Developmental Processes	**U2af1-rs1**	-0,7	-0,5
NM_007554	Cell Growth and/or Maintenance	**Bmp4**	-0,7	-0,5
NM_011390	Transport	**Slc12a7**	-0,7	0,0
NM_011812	Cell Matrix Adhesion	**Fbln5**	-1,1	-0,8
AK004418		**Synpo2**	-1,2	0,3
NM_009994	Electron Transport	**Cyp1b1**	-1,5	-0,7
NM_010145	Nitrogen Metabolism	**Ephx1**	-1,5	-1,4
U12961	Heat Shock Response	**Nqo1**	-1,6	-0,2
NM_007436	Aldehyde Metabolism	**Aldh3a1**	-1,9	-0,6
NM_008182	Stress Response	**Gsta2**	-1,9	-1,3

* Ratio > 0 = tumor > non-tumor, ** Ratio > 0 = SA > WT
SA = p53.S389A, WT = wild-type

were subsequently calculated, and are presented in Table II. Of the genes overexpressed in tumorigenic bladders after long-term exposure to 2-AAF, only two genes (*Sfrp1* and *Tuba6*) were also overexpressed in p53.S389A bladders exposed short-term, compared to wild-type. However, most of the genes repressed in the tumors were also decreased in p53.S389A bladders exposed short-term, compared to wild-type.

Discussion

Previously we demonstrated that 39 weeks of *in vivo* 2-AAF exposure results in an increased incidence of bladder tumors in p53.S389A compared to wild-type mice [21]. Although the underlying molecular and cellular mechanisms remained unclear, it was thought that changes in gene expression might, at least in part, provide some insight in these processes.
Microarray experiments were performed with bladders from wild-type and p53.S389A mice that were exposed to the genotoxic carcinogen 2-AAF for either 1 or 2 weeks. These time points were chosen because a clear and significant induction of cell proliferation and apoptotic cells were previously observed after exposure to 2-AAF in wild-type and p53 knock-out mice [38]. Short-term 2-AAF exposure resulted in a large group of differentially-expressed genes, confirming the involvement of gene expression in response to a genotoxic carcinogen at early time points.

Differentially-expressed genes between unexposed wild-type and p53.S389A bladders

Merely the lack of Ser389 phosphorylation resulted in a different expression pattern without the introduction of damage by 2-AAF, even though only 44 genes were found. An explanation

for the difference in genotypes under unexposed conditions could lie in different responses to spontaneously formed DNA damages, like reactive oxygen species (ROS) or depurination [reviewed in [39]]. Apparently, the basal p53 tumor suppressor system is readjusted to cope with spontaneous DNA damages, since p53.S389A mice are not tumor prone under unexposed conditions [20]. However, these readjusted responses together with an altered response to genotoxic compounds, might contribute to an increased susceptibility for bladder tumor development. A good example is the gene with the highest fold endogenous change level; *Sfrp1*. This gene is frequently affected in bladder tumors, and is used as a biomarker for bladder cancer detection in humans [40]. Another interesting example with one of the lowest fold endogenous change levels is *Pttg1 (Securin*), which plays a role in p53-mediated cellular response to DNA damage. Rustgi *et al.* have already demonstrated that the C-terminus of p53 can interact with the amino-terminus of *Pttg1*, regulating apoptosis and transcription activity [41]. The S389A alteration at the C-terminus of p53 might therefore prohibit binding of *Pttg1*. In our experiment *Pttg1* was not specifically induced after exposure to 2-AAF but the expression level was already found to be reduced in p53.S389A unexposed bladders compared to wild-type. A constitutive expression level of *Pttg1* probably gives an overall steady level of some target genes involved in DNA damage response, so that wild-type cells can immediately react to 2-AAF exposure. Alternatively, *Pttg1* might be necessary to respond to endogenous DNA damage, and this response is constitutively activated through Ser389 phosphorylation. *Pttg1* is also known to be involved in the induction of apoptosis through the p53-dependent, pro-apoptotic gene *Bax* [42], and mice lacking *Pttg1* have aberrant cell cycle progression, premature centromere division and problems with chromosomal stability [43]. In our experiment, levels of *Bax* induction are indeed reduced in p53.S389A compared to wild-type bladders after exposure to 2-AAF, and this might finally lead to a less effective defense against carcinogens. Altogether, it seems likely that readjustments of basal gene expression level in response to the p53.S389A mutation plays a role in the bladder tumorigenesis upon exposure to 2-AAF.

Processes involved in the responses to 2-AAF

Short-term 2-AAF exposure induced rather large effects on gene expression levels in bladders of mice, with most of the differentially-expressed genes functioning in apoptotic and cell cycle control processes. These findings are in line with a previous study using 2-AAF as the damaging agent, where it was found that apoptotic and cell proliferation related genes were up-regulated, whereas immune system related genes were down-regulated (note that these assays were examined in rat livers and B-cells respectively [44]). In our present study, however, we did not find differences in expression of immune response related genes in the bladders of mice after short-term exposure to 2-AAF.

Delayed transcriptional response to 2-AAF in p53.S389A bladders

For the first time we demonstrated *in vivo* that the mutation at Ser389, a site involved in the post-translational modification of p53, caused altered gene expression patterns in bladders after exposure to a genotoxic carcinogen (Figure 4). In particular, we identified a set of genes whose change of expression after 1 week of exposure was clearly delayed in the p53.S389A mutant bladders. This group of 'delayed genes' included several p53 target genes, such as *Mgmt* (O-6-methylguanine-DNA methyltransferase), which is involved in DNA repair [45]. Recently, a microarray study demonstrated the largest induction for *Mgmt* after 28 days of 2-AAF administration [46]. Even

though this was specifically described for rat livers, our experiment also clearly revealed an induction of *Mgmt* in wild-type bladders after short-term exposure to 2-AAF, with a reduced activation of *Mgmt* after 1 week in p53.S389A mutant bladders. Apparently, phosphorylation of Ser389 is, at least in part, necessary for a rapid induction of *Mgmt* by p53.
The group of delayed genes also included the well-known p53 target genes involved in the p53-dependent apoptotic or cell cycle arrest responses to DNA damage (see also model by Harris and Levine, Figure 4 and [27]) like *Pmaip1* (*Noxa*), [47], *Bax* [48], *Wig1* [49], *Perp* [50], *Ei24* (*Pig8*) [51], *P21* [52], *Reprimo* [53], *Ccnb2* (*Cyclin B2*) [54] and *Ccng1* (*Cyclin G1*) [55]. Inactivation of these targets commonly results in an oncogenic phenotype. Therefore, the reduced expression levels in p53.S389A mutant bladders after exposure to 2-AAF, as observed in this study, can be clearly linked to increased tumor susceptibility. Preliminary results using immunohistochemical analysis of apoptosis in epithelial tissue of bladders after short-term exposure to 2-AAF, indicate a slight reduction of apoptotic levels *in vivo* in the p53.S389A mice compared to wild-type.

Genes found differentially-expressed in tumors and after short-term exposure to 2-AAF
To identify possible tumor markers, i.e., genes that are associated with tumor formation, differentially-expressed genes found with the analysis of tumorigenic versus non-tumorigenic bladders after long-term exposure to 2-AAF were compared with genes found differentially-expressed in bladders after short-term exposure. A fraction of these genes, 23, indeed overlapped, indicating that these tumor-related genes are already affected by 2-AAF exposure at early time points. The list of genes as presented in Table II, contains some genes related to drug metabolism and tumor development, like *Fbln5* and *Sfrp1.* Expression of *Fbln5* has previously been associated with the suppression of tumor formation through its control of cell proliferation [56], and reduced expression of this gene has also been associated with the progression of malignant lymphoma [57]. In line with this, expression levels in tumorigenic tissue were decreased compared to non-tumorigenic p53.S389A bladders in our study, indicating phosphorylation of Ser389 is needed for optimal functioning of *Fbln5* in tumor suppression in the case of bladders after long-term exposure to 2-AAF. Other examples of overlapping genes are the cytochrome P450 family member *Cyp1b1* and the key enzyme involved in defense against reactive forms of oxygen *Nqo1*, which was also found to be required for the stabilization of p53 protein in response to DNA-damaging stimuli. Both are repressed after short-term 2-AAF exposure in p53.S389A bladders and in the bladder tumor. A final example is the Wnt-antagonist *Sfrp1* with induced expression levels both in unexposed p53.S389A bladders and in the bladder tumor. However, for the above-mentioned genes in our experiment, the ratio of expression levels of genes found in tumorigenic compared to non-tumorigenic tissue was the opposite of that in studies performed by others [40;58-62]. This might be because our experiment was performed in bladders of mutant mice, while these other studies mainly used bladder cancer samples or related cell lines from human patients that probably contain other genetic alterations that affect gene expression. In summary, our work has identified some genes differentially-expressed after short-term exposure to 2-AAF, which are associated with tumor formation in bladders of mice lacking the Ser389 phosphorylation event.

Concluding remarks
As tumor formation is a process of several sequential stages like initiation, promotion and progression, the differences in response to bladder tumors between wild-type and p53.S389A mice

are expected to be present at all of these stages. This was particularly the case in our experimental set up, since it was previously reported that arylamines (such as 2-AAF) are involved in both initiating as well as promoting activities in carcinogenesis [63]. In this study we were interested in the gene-expression profiles after 1 and 2 weeks of exposure to 2-AAF in relation to a p53.S389A mutation, as this can give an impression of the initiation step of tumor development. In general we measured many subtle differences in gene expression. Mapping these findings to a recently published model of the p53 activation pathway demonstrated that a considerable number of these model genes were differentially-expressed in bladders of p53.S389A compared to wild-type mice after exposure to 2-AAF. These subtle changes in gene expression can be important for tumor development, as bladder cancer develops due to the accumulation of various molecular changes: i) chromosomal alterations, ii) loss of cell cycle regulation, iii) growth control events such as angiogenesis, resulting in metastasis and iiii) decrease in cellular apoptosis [64]. Our results demonstrated a possible effect on chromosomal stability, as *Pttg1* expression is differential between the genotypes. Further, a clear effect on cell cycle regulation and apoptotic responses could be detected. Although angiogenesis-related genes and pathways were not found, the regulation of these processes might arise at a later stage in tumor development, i.e., far beyond the 2 weeks time point. In conclusion, a mutation at the phosphorylation site Ser389 results, *in vivo*, in specific altered gene-expression profiles in bladders exposed short term to carcinogens, which finally leads to increased susceptibility for tumor development in the bladder. Since responses of several (p53) target genes are not completely absent but only partially disturbed, and since only 14% of the p53.S389A mice developed tumors after 39 weeks, p53 still seems to be partially active in response to DNA damage induced by 2-AAF. Activation of p53 in the p53.S389A mice might presumably go through other post-translational modifications [21]. Mouse models with mutations at other phosphorylation sites also displayed intermediate cellular responses, underlining the idea of p53 functioning being fine tuned through post-translational modifications [65-68]. These results do, however, indicate the importance of post-translational modifications for proper p53 functioning. Phosphorylation of Ser389 appears to play a key role in fine-tuning the expression of a variety of important genes, as opposed to being the result of major changes in a few genes, because a reduction or delay in expression is measurable as opposed to a complete elimination. Finally, we demonstrated that phosphorylation of Ser389 is involved in some, but not all, functions of p53.

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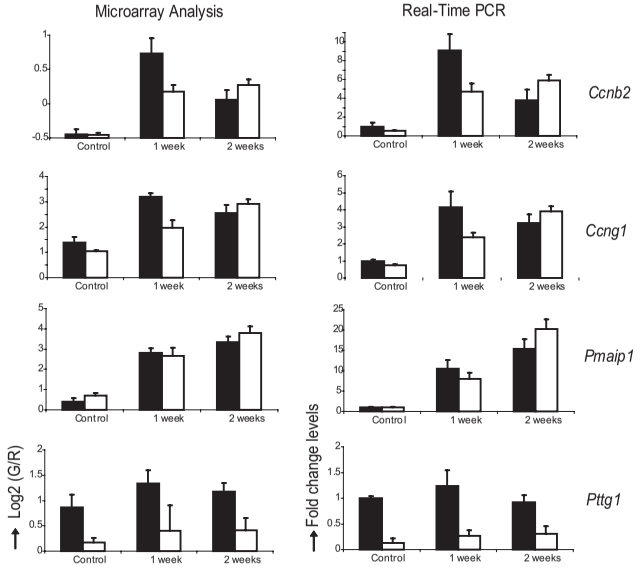
Supplementary tables and figures

Sup. Figure 1 - Validation of gene-expression profiles obtained from microarray analysis by real time PCR



A real-time quantitative gene-expression analysis using a Taqman-based detection method was used to verify differentially-expressed genes found by means of microarray analysis. Four genes, *Ccng1* (*Cyclin G1*), *Pmaip1* (*Noxa*), *Ccnb2* (*Cyclin B2*) and *Ptgg1* were randomly selected for this verification. Left plots show average expression level (log2 (G/R)) values calculated with microarray analysis; right plots represent the fold changes found with real-time PCR analysis (unexposed wild-type samples were taken as basal level with value 1).

Sup. Table I - Log2 (expression ratios (p53.S389A/wild-type) of differentially expressed genes in unexposed bladders, based on genotype differences

Symbol	Accession Number	Log2 (expression ratios)
Igh-6	AF052834	-0,90
Pttg1	NM_013917	-0,80
Cd209a	AF373408	-0,63
Trf	J03299	-0,55
Dap	BC010828	-0,51
Siat7b	NM_009180	-0,50
4432412D15Rik	AK014490	-0,50
Fbln5	NM_011812	-0,50
Mmp2	NM_008610	-0,48
Ly6c	NM_010741	-0,36
Atp6ap1	NM_018794	-0,34
Trim16	AF220134	-0,34
Pja1	NM_008853	-0,33
Bmp4	NM_007554	-0,32
-	U55701	-0,32
Ccl11	NM_011330	-0,28
D11Ertd99e	AK019025	-0,21
Pecam1	NM_008816	-0,21
Trim11	AF220124	-0,18
Alox15b	U93277	-0,15
Rbbp9	NM_015754	-0,15
Rpl21	NM_019647	-0,09
Abcg2	NM_011920	-0,05
2700008N14Rik	AK012224	0,03
2310015A16Rik	AK009349	0,10
Tle4	AK017548	0,10
4930544O15Rik	AK016041	0,12
5730409G07Rik	AK016076	0,14
1300015D01Rik	AK005014	0,18
Ap3d1	NM_007460	0,18
Efcbp1	AK005641	0,18
4930484H19Rik	AK015621	0,18
4933434L15Rik	AK013967	0,19
1700047E16Rik	AK006705	0,20
3830403N18Rik	AK014417	0,20
2900053O20Rik	AK013680	0,22
Pctp	NM_008796	0,28
Meis1	NM_010789	0,29
-	M30432	0,31
Prkwnk4	AK008047	0,53
5730405I09Rik	AK017493	0,57
1700052K11Rik	AK006771	0,62
Pparbp	NM_013634	0,89
Sfrp1	NM_013834	1,40

Sup. Table II - Differentially-expressed genes in wild-type bladders after short-term exposure to 2-AAF

Symbol	Acc. Nr.	FC 1 wk	FC 2 wks
Krt20	AK018567	-2,3	-3,6
9230106L14Rik	AF276974	-2,3	-3,6
D12Bwg1266e	Y13832	-2,2	-2,2
2310020J12Rik	AK009423	-1,6	-1,2
4930426D05Rik	AK015205	-1,6	-1,5
Chrm4	NM_007699	-1,5	-1,4
Rnut1	AK002717	-1,5	-1,4
Fabp9	NM_011598	-1,5	-1,6
Bcas1	AK008957	-1,4	-1,4
4930524O07Rik	AK019696	-1,4	-1,6
D6Bwg1452e	AK004185	-1,4	-1,8
-	M94308	-1,4	-1,5
1300013J15Rik	NM_026183	-1,4	-1,9
Ltb4dh	NM_025968	-1,3	1,3
Adssl1	NM_007421	-1,3	-1,5
Smoc1	AF070470	-1,3	-1,4
Lmod1	AF237627	-1,3	-1,8
-	K02109	-1,3	-1,8
Col19a1	NM_007733	-1,3	-1,3
Kcnmb4	NM_021452	-1,2	-1,3
Fkbp4	NM_010219	-1,2	-1,1
Synpo2	AK004418	-1,2	-2,1
Nmb	NM_026523	-1,2	-1,3
Atp2a2	NM_009722	-1,2	-1,9
Grik5	NM_008168	-1,2	-1,2
Chn1	AK019340	-1,2	-1,2
-	AF121981	-1,2	-1,3
Fhit	NM_010210	-1,2	1,4
Sntg2	AF367760	-1,2	-1,6
-	AJ409496	-1,1	-1,5
Alox12	NM_007440	-1,1	-1,2
Slc12a7	NM_011390	-1,1	-1,6
C78915	NM_019704	-1,1	-1,1
1110057K04Rik	AK004285	-1,1	-1,4
Ly6g6c	AF109905	-1,1	-1,2
-	BC012698	-1,1	-1,1
Fancl	NM_025923	-1,1	-1,3
Sod1	M35725	-1,1	-1,2
-	AF207705	-1,0	1,1
GIG18	AF374476	-1,0	-1,2
Trim25	D63902	-1,0	1,3
Pkd1	NM_013630	-1,0	-1,3
3110001N18Rik	NM_026517	-1,0	-1,5
4632432E15Rik	AK014604	-1,0	1,1
4930542G03Rik	BC005547	-1,0	2,1
Ptprz1	BC002298	1,0	-1,2
Stac	NM_016853	1,0	-1,4
Cds2	AK017677	1,0	-1,6
Cdkn1a	NM_007669	1,0	1,1
Ptpn11	BC003980	1,0	-1,2
1110021P09Rik	AK003907	1,0	-1,1
4921504P20Rik	AK014816	1,0	1,2
-	X04211	1,0	1,1
3010001K23Rik	BC005542	1,1	-1,1
5033428C03Rik	AK017200	1,1	1,1
Lgals3	X16834	1,1	1,7
2900073G15Rik	NM_026064	1,1	1,2
Itpkb	AK004067	1,1	-1,2
Aox1	NM_009676	1,1	-1,4
Grb14	NM_016719	1,1	-1,2
D10Ertd610e	AK010452	1,1	-1,3
Trim35	AK003000	1,1	-1,1
Sspn	NM_010656	1,1	-1,3
3110050N22Rik	AK012273	1,1	1,2
Sgol2	AK003555	1,2	1,1
Cap1	NM_007598	1,2	1,2
Helz	AK020667	1,2	1,1
Nudt5	NM_016918	1,2	1,3
Dp1l1	AB039933	1,2	-1,2
Acyp2	AK009134	1,2	-1,1
1600029D21Rik	AK005558	1,2	1,8
4833427G06Rik	AK014780	1,3	1,4
Slc25a4	U27315	1,3	1,0
Hn1	NM_008258	1,3	1,3
Abhd9	AK010021	1,3	1,3
Def6	AK010356	1,3	1,4
Pole2	AF036898	1,3	1,1
1300006M19Rik	BC015459	1,3	1,1
Arrdc4	AK010352	1,3	1,3
Rrm2	NM_009104	1,3	1,3
Fabp2	NM_007980	1,3	1,5
Ddx28	AK010396	1,3	1,3
Gna13	NM_010303	1,3	1,1
Ppp1r14b	NM_008889	1,3	1,3
U2af1-rs1	NM_011663	1,4	1,1
Srf	NM_020493	1,4	1,2
Mre11a	NM_018736	1,4	1,3
Lzic	AK007657	1,4	1,2
Sprr2j	NM_011476	1,4	2,2
Tuft1	NM_011656	1,4	1,5
Bbs2	AF342737	1,4	1,1
BC004728	BC004728	1,4	1,4
Dgka	NM_016811	1,4	1,2
Elovl4	AF277093	1,4	1,4
Dpagt1	NM_007875	1,4	1,1
Reprimo	AB043586	1,4	1,8
1810037I17Rik	BC002135	1,4	2,1
Glb1	NM_009752	1,5	1,3
Cgref1	AK003420	1,5	1,6
Col17a1	NM_007732	1,5	1,2
C1galt1	AF157962	1,5	1,2
2010005A06Rik	AK008111	1,5	1,8
Pold3	AF294329	1,5	1,5
Sra1	AF092039	1,5	1,2
Galnt3	NM_015736	1,6	1,3
Dpp8	AK016546	1,6	1,4
Rtkn	NM_009106	1,6	2,2
1200015M12Rik	AK004796	1,6	2,0
Rfc5	AK011489	1,6	1,3
Lxn	NM_016753	1,7	1,3
9130019I15Rik	AK018636	1,7	1,1
1200015A22Rik	AK004778	1,7	1,4
D11Ertd603e	NM_026023	1,7	1,3
Il18	NM_008360	1,7	1,5
Dutp	AF091101	1,7	1,3
Wnt4	NM_009523	1,7	1,8
Mgmt	NM_008598	1,7	1,7
-	NM_007629	1,7	1,4
Mrpl53	AK003549	1,8	1,3
1700007K13Rik	AK005731	1,8	1,8
Hebp2	NM_019487	1,8	1,8
Birc5	NM_009689	1,8	1,4
2310061G07Rik	BC003749	1,8	1,9
4933424G05Rik	AK016892	1,8	1,5
Msh6	NM_010830	1,9	1,1
Pgam1	AF283667	1,9	1,4
Nqo1	U12961	1,9	1,2
Gfer	AF148688	1,9	1,9
1700020I14Rik	AK006166	1,9	1,5
Gpr115	AK019508	1,9	1,8
Trim16	AF220134	1,9	2,6
Ak1	NM_021515	2,0	1,8
0610012G03Rik	NM_025320	2,0	1,6
Itgb4	L04678	2,0	1,5
Serpinb5	NM_009257	2,0	1,4
Epha1	AF131197	2,0	1,8
Bzw1	NM_025824	2,0	1,4
Gss	NM_008180	2,0	2,1
2410001H17Rik	NM_025889	2,1	1,7
Sprr2g	NM_011473	2,1	2,4
9230117N10Rik	BC003847	2,1	1,6
Rrm1	NM_009103	2,1	1,6
Ripk3	NM_019955	2,2	1,9
Anxa8	NM_013473	2,2	2,2
Rbx1	NM_019712	2,2	1,7
Scarb2	NM_007644	2,2	1,7
Gaa	NM_008064	2,2	1,5
Tusc4	NM_018879	2,2	1,7
Pkp1	NM_019645	2,2	2,3
Bax	NM_007527	2,2	2,0
Txnl2	AF118650	2,2	1,7
Gsta1	NM_008181	2,2	1,8
Rps27l	NM_026467	2,3	1,6
Wig1	NM_009517	2,3	1,9
Ccnb2	NM_007630	2,3	1,4
1810027I20Rik	AK007617	2,3	2,1
-	NM_019669	2,3	2,2
S100a14	NM_025393	2,3	2,4
-	NM_018806	2,3	1,8
2210023G05Rik	AK008775	2,4	2,3
Ube2c	AK003722	2,4	1,5
1190003J15Rik	AK004470	2,4	2,6
Sptlc2	NM_011479	2,5	2,0
Cdkn2b	NM_007670	2,5	2,6
Krt2-4	NM_008475	2,6	2,2
Ptp4a3	NM_008975	2,6	2,1
Akr1b3	NM_009658	2,6	1,8
Mki67	X82786	2,6	1,5
Dmp1	U65020	2,6	2,3
Gsta2	NM_008182	2,6	1,7
S100a11	U41341	2,8	2,1
Sprr1a	NM_009264	2,8	2,7
Commd3	AK007318	2,9	2,3
Ephx1	NM_010145	3,0	2,3
Mal	NM_010762	3,1	2,3
Nos3	AK010032	3,2	5,5
Ly6d	NM_010742	3,2	2,4
Anxa3	NM_013470	3,3	2,7
Cyp1b1	NM_009994	3,3	2,2
Krt1-15	NM_008469	3,3	2,8
Ccng1	NM_009831	3,5	2,2
Areg	NM_009704	3,9	5,8
Krt1-23	NM_033373	4,0	3,5
Ckmt1	NM_009897	4,0	3,4
Pmaip1	NM_021451	5,3	7,7
Sprr2f	NM_011472	7,9	13,9

Acc.Nr. = Accession Number
FC = Fold Change
wk = week

Sup. Table III - Differentially-expressed genes in p53.S389A bladders after short-term exposure to 2-AAF

Symbol	Acc.Nr.	FC 1 wk	FC 2 wks
Ttr	NM_013697	-3,4	-3,9
D12Bwg1266e	Y13832	-2,4	-2,0
Slc25a4	U27315	-2,2	-1,1
Cyp4f15	AF233645	-2,0	-2,0
-	BC006792	-1,8	-1,3
-	BC004015	-1,7	-1,1
Upk2	NM_009476	-1,7	-1,0
Map1lc3b	NM_026160	-1,6	-1,9
-	M94308	-1,5	-1,4
Anubl1	AK012639	-1,5	-1,1
9230106L14Rik	AF276974	-1,4	-1,5
-	L43818	-1,4	-1,3
2310032D16Rik	BC006887	-1,4	-1,4
Elf5	NM_010125	-1,4	-1,4
Depdc6	AK014624	-1,3	-1,4
5730511K23Rik	NM_019458	-1,3	1,1
Cyp3a41	NM_017396	-1,3	-1,1
Dgka	NM_016811	-1,3	1,1
Krtap8-2	NM_010676	-1,3	-1,6
Rarg	NM_011244	-1,3	1,1
Plce1	AF233885	-1,3	-1,1
Ctse	NM_007799	-1,3	2,0
ICRFP703B1614Q5.6	NM_020616	-1,3	1,0
Kcnmb4	NM_021452	-1,3	-1,2
2310044G17Rik	AK009800	-1,3	-1,0
Stm	NM_011501	-1,2	-1,1
Commd3	AK007318	-1,2	1,7
B230118H07Rik	NM_026592	-1,2	-1,0
Usmg4	AJ290946	-1,2	-1,5
Tbcc	AK012950	-1,2	1,0
Rps14	NM_020600	-1,2	1,0
Fabp2	NM_007980	-1,2	1,1
Brd3	AF269193	-1,2	1,1
Adh4	AC079832	-1,2	-1,1
Psme1	NM_011189	-1,2	1,1
-	NM_025561	-1,2	-1,3
Exo1	NM_012012	-1,2	-1,1
Trpm7	NM_021450	-1,2	-1,1
Gdap8	Y17857	-1,2	-1,3
Cds2	AK017677	-1,2	-1,3
Susd2	AK004703	-1,2	-1,4
Npy6r	NM_010935	-1,2	-1,2
Pthlh	NM_008970	-1,2	-1,9
Cklfsf7	AF188504	-1,1	-1,4
Trim7	AF220033	-1,1	-1,2
5430430B14Rik	AK017364	-1,1	1,1
Prdm5	AK014501	-1,1	-1,4
1700026J14Rik	AK006390	-1,1	-1,0
Ndrl	NM_008681	-1,1	1,0
Smox	BC004831	-1,1	1,1
4933406C10Rik	AK016682	-1,1	-1,3
1110004E09Rik	AK018510	-1,1	1,3
Bspry	AJ276690	-1,1	1,1
-	AL358892	-1,1	1,2
-	U01314	-1,1	-1,1
Yap1	NM_009534	-1,0	1,5
5430425C04Rik	AK017334	-1,0	1,3
1110054N06Rik	AK004254	-1,0	-1,0
Opn3	NM_010098	-1,0	1,1
Irebf1	NM_013714	-1,0	1,0
Mapk14	NM_011951	-1,0	1,2
Tep1	NM_009351	-1,0	1,3
Ptges	AB041997	-1,0	-1,4
1700016G05Rik	NM_026317	-1,0	-1,4
2310003F16Rik	NM_026318	-1,0	1,6
Ppp1r14b	NM_008889	-1,0	1,1
Klf3	NM_008453	-1,0	1,4
Bcas1	AK008957	-1,0	-1,1
Slc12a7	NM_011390	-1,0	1,0
Kif20a	NM_009004	-1,0	-1,0
Sema7a	AK019344	-1,0	1,6
Mrpl27	AB049648	1,0	1,3
Fbxl12	NM_013911	1,0	1,2
Cstb	NM_007793	1,0	2,4
1110003O08Rik	AK003388	1,0	-1,1
Ivl	NM_008412	1,0	3,9
-	NM_018806	1,0	1,9
4930430E12Rik	AK015249	1,0	-1,0
Foxd3	NM_010425	1,0	-1,1
Procr	NM_011171	1,0	1,2
Nit1	NM_012049	1,0	1,6
Sorl1	AB015790	1,0	1,3
5730509C05Rik	AK003700	1,1	1,5
Bace2	AK004306	1,1	1,1
Eya4	NM_010167	1,1	1,5
Kl	NM_013823	1,1	-1,1
Calu	NM_007594	1,1	1,2
Agtrl1	NM_011784	1,1	-1,1
Car14	NM_011797	1,1	-1,2
Abhd5	NM_026179	1,1	1,6
Adamdec1	NM_021475	1,1	1,5
1700124P09Rik	AK007274	1,1	-1,1
Gsdm1	NM_021347	1,1	1,5
Fut2	NM_018876	1,1	1,5
Ephx1	NM_010145	1,1	3,0
Adam28	AF153350	1,1	2,2
Gstm1	NM_010358	1,1	1,0
Efhc1	AK006489	1,1	1,1
4933405A16Rik	AK016659	1,1	1,1
Ptk9	NM_008971	1,1	1,3
Krt2-1	NM_008473	1,1	1,3
2400003N08Rik	AK010274	1,1	1,6
Rap2b	NM_028712	1,1	1,5
Prim1	NM_008921	1,1	1,5
Ltb4dh	NM_025968	1,1	2,5
Mrpl28	NM_024227	1,1	1,1
Pzp	NM_007376	1,1	1,6
Bloc1s2	AK010745	1,1	1,7
2010002A20Rik	NM_025655	1,1	1,1
Plek2	NM_013738	1,1	1,3
Katna1	NM_011835	1,1	1,8
Arpc1a	NM_019767	1,1	1,4
Pde9a	NM_008804	1,1	-1,0
Bcl2l14	AK016670	1,1	1,6
-	Z12192	1,1	-1,1
Il1rap	NM_008364	1,1	1,2
Mrpl13	AB049641	1,1	1,3
Mgmt	NM_008598	1,1	1,7
Sca2	NM_009125	1,1	1,1
Txnl2	AF118650	1,1	2,1
9530027K23Rik	AK020578	1,1	2,1
1810011O16Rik	NM_025456	1,1	1,6
5730547N13Rik	AK017825	1,1	1,4
Lgals8	NM_018886	1,1	1,4
Ppib	M60456	1,1	-1,1
Pold3	AF294329	1,1	1,5
2310004I24Rik	NM_025510	1,1	1,3
Lincr	AK008501	1,1	1,3
9430072K23Rik	AK020483	1,1	1,8
Psmd10	NM_016883	1,1	1,5
Slc2a9	BC006076	1,1	1,4
2210037E17Rik	AK008779	1,1	1,4
0610030E20Rik	AK002701	1,2	1,4
Pigf	NM_008838	1,2	1,5
Vamp8	NM_016794	1,2	1,7
Pycs	NM_019698	1,2	-1,1
Slc22a8	AF078869	1,2	-1,0
Iapp	NM_010491	1,2	-1,1
-	AF143541	1,2	1,3
Arhgap24	AK002660	1,2	1,1
Lrrfip2	BC014761	1,2	1,7
Ntn4	NM_021320	1,2	1,4
-	NM_008252	1,2	2,0
Tjp3	NM_013769	1,2	1,8
Map3k7	D76446	1,2	1,8
2900024C23Rik	AK013580	1,2	-1,1
-	U19782	1,2	1,1
Rog	NM_021397	1,2	1,3
Pak1	NM_011035	1,2	1,2
Btnl6	AF050157	1,2	-1,2
2010004M13Rik	AK008102	1,2	1,4
Zfp617	AB020542	1,2	1,2
Cyp27a1	NM_024264	1,2	2,0
Ubxd4	BC004632	1,2	1,3
Rrh	NM_009102	1,2	-1,0
Pdlim1	NM_016861	1,2	1,6
Rage	NM_011973	1,2	1,2
Padi3	NM_011060	1,2	1,2
-	NM_020294	1,2	1,2
Spint1	NM_016907	1,2	2,4
Sptlc2	NM_011479	1,2	2,5
Sulf2	AK008108	1,2	1,9
Nudt14	AK003991	1,2	1,3
1810009O10Rik	AK007413	1,2	1,5
Prkcq	NM_008859	1,2	1,5
Dnaja3	NM_023646	1,2	1,1
Praf2	AF229636	1,2	1,4
Atp8a2	NM_015803	1,2	1,6
Ptprd	D13905	1,2	1,4
Tuft1	NM_011656	1,2	2,0
DDA3	NM_019976	1,2	1,6
1810034K20Rik	NM_023397	1,2	1,6
Capn10	NM_011796	1,2	1,2
Nudt4	NM_027722	1,2	1,8
1300002C08Rik	AF354708	1,2	-1,0
Tyms-ps	M30774	1,2	1,6
1700025B16Rik	BC013487	1,2	1,7
4631405K08Rik	AK014513	1,2	1,5
Gzmm	NM_008504	1,2	1,1
Mad2l2	BC011282	1,3	1,4
Ugdh	NM_009466	1,3	1,7
5730409N24Rik	AK017539	1,3	-1,6
Petl12l	BC005709	1,3	1,5
Capza1	U16740	1,3	2,1
0610012C01Rik	AK002581	1,3	1,5
1810009A15Rik	NM_025463	1,3	1,7
Grpel2	AF041060	1,3	1,6
5830417C01Rik	BC002200	1,3	1,5
Hcngp	NM_020483	1,3	1,4
2510001I10Rik	AK010881	1,3	1,6
A530065E19Rik	BC005625	1,3	1,3
Sprr2j	NM_011476	1,3	2,9
Scarb2	NM_007644	1,3	2,3
0610012G03Rik	NM_025320	1,3	2,0
Ei24	NM_007915	1,3	2,0
3110004L20Rik	AK013991	1,3	1,7
Perp	AF249870	1,3	1,8
Ggps1	NM_010282	1,3	1,6
Stx16	AK017291	1,3	1,7
Inpp5b	NM_008385	1,3	1,8
Scap2	NM_018773	1,3	2,0
4931431L11Rik	AK016498	1,3	1,3
Zfp185	NM_009549	1,3	1,2
Fxyd4	AF362729	1,3	1,4
Car2	NM_009801	1,3	1,7
1200015A22Rik	AK004778	1,3	2,0
Wnt10a	NM_009518	1,3	1,5
1700009P17Rik	AK005804	1,3	1,7
2310033P09Rik	NM_024210	1,3	1,5
D15Wsu75e	AK003172	1,3	1,6
8430408E17Rik	AK018395	1,3	-1,2
Bin3	NM_021328	1,3	1,6
2410030K01Rik	AK011728	1,3	1,2
Rnpep	BC010520	1,3	1,4
Brp17	NM_019999	1,3	1,3
Cib1	NM_011870	1,3	2,2
Asb4	AF155355	1,3	1,3

Symbol	Acc.Nr.	FC 1 wk	FC 2 wks
Msi2h	AB056103	1,3	1,8
Tm7sf1	AF154337	1,3	1,3
Cdc42ep5	NM_021454	1,3	1,9
D11Ertd603e	NM_026023	1,3	1,6
Hn1	NM_008258	1,3	1,7
Nxt1	NM_019761	1,3	1,5
Rbx1	NM_019712	1,3	2,2
2310058J06Rik	AJ299430	1,3	1,5
4930542G03Rik	BC005547	1,3	2,4
Ddx28	AK010396	1,3	1,7
Prps2	NM_026662	1,3	1,5
Dpp8	AK016546	1,3	1,5
Ang-ps1	U22517	1,3	1,7
Sprrl10	AK004318	1,3	1,4
Ckmt1	NM_009897	1,3	3,3
Tmprss2	NM_015775	1,3	1,7
Gba	NM_008094	1,3	2,0
1110059M19Rik	AK004321	1,3	1,4
Notch3	NM_008716	1,4	1,7
-	NM_018752	1,4	1,7
Indo	NM_008324	1,4	2,6
Stat4	NM_011487	1,4	1,8
Cd44	M30655	1,4	1,5
Panx1	NM_019482	1,4	1,7
Hdh	U24233	1,4	1,4
2610034E01Rik	AK011660	1,4	1,4
Elovl4	AF277093	1,4	1,5
Glb1	NM_009752	1,4	1,4
Adss2	NM_007422	1,4	1,5
Tnfrsf18	NM_009400	1,4	1,5
Plxnb3	NM_019587	1,4	1,4
0710001D07Rik	AK002941	1,4	1,4
Nudt5	NM_016918	1,4	2,2
8030444G23Rik	AK020201	1,4	1,6
Anxa8	NM_013473	1,4	2,5
6330414G21Rik	AK018173	1,4	2,0
Pgam1	AF283667	1,4	1,6
Epha1	AF131197	1,4	2,1
Ddx19	NM_007916	1,4	1,6
Ywhab	NM_018753	1,4	1,8
1700026D08Rik	AK006375	1,4	1,2
Wig1	NM_009517	1,4	1,9
Gfer	AF148688	1,4	2,3
Sat1	NM_009121	1,4	2,5
2700038L12Rik	AF309554	1,4	2,7
5730596K20Rik	BC002149	1,4	1,4
Fmo5	NM_010232	1,4	1,7
Irf5	NM_012057	1,4	1,9
Clic1	BC004658	1,4	1,4
1200003E16Rik	AK004560	1,4	1,9
Dazap1	AF225910	1,4	1,8
Timm8b	NM_013897	1,4	1,9
Fbp2	NM_007994	1,4	1,8
Lzic	AK007657	1,4	1,2
2310020F24Rik	AK009417	1,4	1,5
A830059I20Rik	NM_021427	1,5	1,5
Cst6	AK003744	1,5	1,7
Hmox1	NM_010442	1,5	1,7
1700020I14Rik	AK006166	1,5	2,0
Vamp5	NM_016872	1,5	2,6
1300006M19Rik	BC015459	1,5	1,7
Cast	AB026997	1,5	2,2
Ak1	NM_021515	1,5	1,7
2010005A06Rik	AK008111	1,5	1,7
Muc2	AF121215	1,5	1,5
Tacc3	NM_011524	1,5	1,5
Hgfac	NM_019447	1,5	1,4
E030027H19Rik	BC011340	1,5	2,5
Dnajc9	BC014686	1,5	2,2
Prkar1a	BC003461	1,5	2,4
Brca1	U36475	1,5	1,6
1700007K13Rik	AK005731	1,5	2,4
Scamp5	NM_020270	1,5	2,3
-	NM_007629	1,5	1,5
4632411J06Rik	AK019489	1,5	1,4
Pole3	NM_021498	1,5	1,6
Hexa	NM_010421	1,5	1,7
Trim16	AF220134	1,5	2,2
2610034P21Rik	AK011679	1,5	1,4
Mal	NM_010762	1,5	1,9
Rohn	NM_009708	1,5	2,0
1190003J15Rik	AK004470	1,5	2,8
Gzme	NM_010373	1,5	1,6
5730507H05Rik	AJ237585	1,5	1,4
Cebpg	BC011319	1,5	1,8
Bax	NM_007527	1,5	1,9
Gsta2	NM_008182	1,5	3,3
4632432E15Rik	AK014604	1,5	1,6
Acate2	NM_019736	1,5	1,7
1600029D21Rik	AK005558	1,5	1,5
Ccnb2	NM_007630	1,5	1,7
Psat1	BC004827	1,6	1,1
Gss	NM_008180	1,6	2,4
Rrm1	NM_009103	1,6	1,9
Dpagt1	NM_007875	1,6	1,4
Il18	NM_008360	1,6	1,8
1700013H19Rik	AK005954	1,6	1,7
Homer3	AF093261	1,6	1,5
Sprr1a	NM_009264	1,6	3,5
Dutp	AF091101	1,6	1,5
1190002A23Rik	AK004440	1,6	1,8
Fgfr1op2	AB041650	1,6	2,0
Serpinb5	NM_009257	1,6	2,1
Cd14	NM_009841	1,6	2,0
Hsd17b12	NM_019657	1,6	2,2
Gap43	NM_008083	1,6	1,9
Capg	NM_007599	1,6	2,2
4833427G06Rik	AK014780	1,6	2,0
Dnaja2	NM_019794	1,6	1,7
Cenpa	NM_007681	1,6	1,5
Mcm5	NM_008566	1,6	1,5
3830408G10Rik	AK014427	1,6	2,1
Inhba	NM_008380	1,6	2,1
-	BC006059	1,7	1,3
6820402O18Rik	AK018359	1,7	2,0
Sprr2g	NM_011473	1,7	3,7
Dmp1	U65020	1,7	2,4
Gria3	AK013750	1,7	2,1
Dpysl4	NM_011993	1,7	1,7
-	NM_019389	1,7	1,7
Daf2	NM_007827	1,7	2,3
Gsta1	NM_008181	1,7	2,1
Nasp	NM_016777	1,7	1,8
Sprr1b	NM_009265	1,7	2,1
Cks2	NM_025415	1,7	1,9
Insl6	NM_013754	1,7	2,2
Msx2	NM_013601	1,7	1,7
Ripk3	NM_019955	1,7	2,2
Akr1b3	NM_009658	1,7	3,1
Slc40a1	NM_016917	1,7	3,4
Tusc4	NM_018879	1,7	2,4
Gsta4	NM_010357	1,7	3,3
Phlda3	NM_013750	1,7	2,1
Gaa	NM_008064	1,8	2,7
Sdc1	NM_011519	1,8	2,1
2210023G05Rik	AK008775	1,8	2,9
Slk	NM_009289	1,8	1,9
Clu	NM_013492	1,8	3,8
Tmem27	NM_020626	1,8	1,6
Rtkn	NM_009106	1,8	2,7
S100a11	U41341	1,8	2,6
Wfdc2	AK005519	1,8	3,2
Bzw1	NM_025824	1,8	2,2
1810037I17Rik	BC002135	1,8	3,1
Incenp	NM_016692	1,8	1,6
Cdkn2b	NM_007670	1,8	3,4
Pkp1	NM_019645	1,8	2,2
Ptp4a3	NM_008975	1,9	2,9
S100a14	NM_025393	1,9	2,7
Tacstd2	NM_020047	1,9	2,3
9130019I15Rik	AK018636	1,9	2,6
Ly6a	NM_010738	1,9	3,0
Birc5	NM_009689	1,9	1,7
Ccng1	NM_009831	1,9	3,7
Mki67	X82786	1,9	2,3
Fscn1	NM_007984	1,9	1,8
Ly6d	NM_010742	1,9	3,8
Anxa3	NM_013470	2,0	3,1
Slc2a8	NM_019488	2,0	2,6
Hebp2	NM_019487	2,0	1,9
Ube2c	AK003722	2,0	2,0
Col17a1	NM_007732	2,0	2,2
Aldh3a1	NM_007436	2,1	2,0
2310061G07Rik	BC003749	2,1	2,2
Cyp1b1	NM_009994	2,3	2,7
Tshb	NM_009432	2,4	3,1
Krt2-4	NM_008475	2,4	2,6
Padi2	NM_008812	2,4	3,5
Tffl	NM_009362	2,5	4,7
Tuba6	NM_009448	3,0	4,8
Nos3	AK010032	3,2	5,3
Areg	NM_009704	3,3	7,2
Krt1-23	NM_033373	3,3	6,4
Krt1-15	NM_008469	3,5	4,4
Pmaip1	NM_021451	3,8	8,6
Sprr2f	NM_011472	9,7	32,5

Acc.Nr. = Accession Number
FC = Fold Change
wk = week

Sup. Table IV - Biological processes (i.e., GO categories) containing genes involved in 2-AAF responses in mouse bladders

GO category	Total*	Corrected p-value*
Cell cycle	14	0,0084
Electron transport	13	0,0224
Cytokinesis	10	0,0035
DNA replication	9	<0.001
Response to DNA damage stimulus	9	0,0051
Carbohydrate metabolism	8	0,0298
Protein ubiquitination	9	0,0309
DNA repair	7	0,0406
Lipid metabolism	7	0,0291
Mitosis	7	0,0027
Induction of apoptosis	6	0,0033
Angiogenesis	4	0,0466
Cell cycle arrest	4	0,0043
Negative regulation of cell proliferation	4	0,0249
Neuropeptide signaling pathway	4	0,0464
Nucleobase, nucleoside, nucleotide and nucleic acid metabolism	4	0,0028
Rho protein signal transduction	4	0,0033
DNA metabolism	3	0,0291
DNA replication and chromosome cycle	3	<0.001
Induction of apoptosis by intracellular signals	3	<0.001
One-carbon compound metabolism	3	0,0323
Protein catabolism	3	0,0115
Protein localization	3	0,0079
Response to UV	3	<0.001
Thyroid hormone generation	3	<0.001

*Processes with at least 3 genes present and a corrected p-value <0.05 are shown

Sup. Table V - Biological processes (i.e., GO categories) containing genes with a delayed response in p53.S389A bladders compared to wild-type bladders

GO category	Total
Transport	15
Cell cycle	9
Proteolysis and peptidolysis	9
Cytokinesis	7
Development	7
DNA replication	7
Electron transport	6
Metabolism	6
Regulation of transcription, DNA dependent	6
Signal transduction	6
Induction of apoptosis	5
Mitosis	5
Protein amino acid phosphorylation	5
Response to DNA damage stimulus	5
Apoptosis	4
Carbohydrate metabolism	4
Cell adhesion	4
Cell differentiation	4
DNA repair	4
G-protein coupled receptor protein signaling pathway	4
Lipid metabolism	4
Protein biosynthesis	4
Protein folding	4
Protein transport	4
Immune response	3
Induction of apoptosis by intracellular signals	3
Integrin-mediated signaling pathway	3
Intracellular protein transport	3
Protein targeting	3
Protein ubiquitination	3
Regulation of apoptosis	3
Rho protein signal transduction	3
Transcription	3
Ubiquitin cycle	3

Sup. Table VI - Differentially-expressed genes in tumorigenic compared to non-tumorigenic p53.S389A bladders after long-term exposure to 2-AAF

Symbol	Acc.Nr.	Fold Change Tumorigenic
Myh11	NM_013607	-8,2
Gsta3	NM_010356	-7,7
Adh1	NM_007409	-6,8
Acta2	NM_007392	-4,9
Actg2	NM_009610	-4,6
4631426E05Rik	AK014536	-4,6
Ugt1a6	U16818	-4,6
Upk1b	AF073956	-4,6
Upk3a	AF222750	-4,4
Acta1	X03766	-4,3
Myl9	AK007972	-4,3
Gsta2	NM_008182	-3,9
Aldh3a1	NM_007436	-3,7
1110032A04Rik	AF365876	-3,7
Ldh2	NM_008492	-3,6
Cyb5	NM_025797	-3,5
Des	L22550	-3,4
Ndrg2	NM_013864	-3,4
Tcea3	D00926	-3,2
Smtn	NM_013870	-3,2
Tnnt2	NM_011619	-3,2
Depdc6	BC004774	-3,1
Upk1a	AK004014	-3,0
Prom2	AF128113	-3,0
4631408O11Rik	AK014514	-2,9
Nqo1	U12961	-2,9
Gstm2	NM_008183	-2,9
4632417N05Rik	AK014586	-2,9
Ephx1	NM_010145	-2,9
Cyp1b1	NM_009994	-2,9
Pdlim3	NM_016798	-2,8
mt-Nd5	AK018737	-2,8
C030033M19Rik	AK021118	-2,8
-	U50959	-2,8
Coch	NM_007728	-2,8
Atp2a2	NM_009722	-2,7
Tagln	NM_011526	-2,7
Art3	AK016257	-2,7
Fmo2	AK009753	-2,6
Rnf128	AB041548	-2,6
Homer2	AB017136	-2,6
2200001I15Rik	AK008614	-2,6
Ogn	AK014259	-2,6
Sytl2	NM_031394	-2,5
Sod1	M35725	-2,4
-	NM_010354	-2,4
Tcf21	NM_011545	-2,4
Lmod1	AF237627	-2,4
Prelp	AK020180	-2,3
Synpo2	AK004418	-2,3
P2rx1	NM_008771	-2,3
Scnn1g	NM_011326	-2,2
Sntg2	AF367760	-2,2
Akap12	AB020886	-2,2
Fbln5	NM_011812	-2,1
Sh3bgr	NM_015825	-2,1
Abcc3	AK006128	-2,1
Col14a1	AJ131395	-2,1
S3-12	NM_020568	-2,1
1110003E01Rik	BC009097	-2,1
Upk2	NM_009476	-2,1
Tm4sf6	NM_019656	-2,1
Gstt3	BC003903	-2,1
Tnxb	X73959	-2,1
Igfbp6	NM_008344	-2,0
Pitx2	NM_011098	-2,0
0610010I15Rik	AK002481	-2,0
Slc16a7	NM_011391	-2,0
Gata3	NM_008091	-2,0
Sgpl1	NM_009163	-2,0
Jph2	AK003288	-2,0
9530014B07Rik	AK020556	-1,9
2610207I16Rik	NM_024255	-1,9
IV ADH	NM_009626	-1,9
1810041L15Rik	AK007745	-1,9
Pltp	NM_011125	-1,9
Cbr1	NM_007620	-1,9
Pcp4l1	AK002772	-1,9
Fmo5	AK017179	-1,9
Sorbs1	AK010043	-1,8
Aldh3b1	AK005615	-1,8
Rassf3	BC011511	-1,8
Gjb6	NM_008128	-1,8
Slc2a4	NM_009204	-1,7
Mylk	AF314149	-1,7
Pparg	NM_011146	-1,7
1110032A03Rik	AJ250230	-1,7
Pdk2	AF267660	-1,7
A930010G16Rik	AK020843	-1,7
4930469P12Rik	AF412298	-1,7
Trpv4	AF208026	-1,7
Fnbp1	AK004278	-1,7
Dscr5	NM_019543	-1,7
Slc12a7	NM_011390	-1,7
Bmp4	NM_007554	-1,7
U2af1-rs1	NM_011663	-1,7
1810019D21Rik	AK007545	-1,7
Sort1	AK013673	-1,6
Sulf2	AK008108	-1,6
Capza3	NM_007605	-1,6
Prdx3	NM_007452	-1,6
Pdha2	NM_008811	-1,6
Lmo1	AJ296304	-1,6
1700021N21Rik	AK006224	-1,6
Sh3gl2	NM_019535	-1,6
Cntnap2	NM_025771	-1,6
Map3k7ip1	AK009321	-1,6
Sash1	AK003151	-1,6
Cds2	AK017677	-1,6
3110048E14Rik	AK014192	-1,6
-	NM_028785	-1,6
Cirbp	NM_007705	-1,6
Gpc6	AK020118	-1,6
Gpr124	AF378759	-1,5
Adcy9	NM_009624	-1,5
Akap8l	NM_017476	-1,5
2900060P06Rik	AK013739	-1,5
Slc14a1	AK012066	-1,5
2900041A09Rik	AK013631	-1,5
Arg2	NM_009705	-1,4
Ly6g6c	AF109905	-1,4
Traf5	NM_011633	-1,4
Hoxd10	NM_013554	-1,4
Asb2	AF155353	-1,4
2010316F05Rik	AK008574	-1,4
Map4k2	NM_009006	-1,4
8430421H08Rik	AK018430	-1,4
8430436L14Rik	AK018464	-1,3
Thbd	NM_009378	-1,3
Bcl2l14	AK016670	-1,3
Tekt2	NM_011902	-1,3
5930405J04Rik	AK013190	-1,3
1700009P17Rik	AK005804	-1,3
2310005P05Rik	NM_026189	-1,3
Dsip1	NM_010286	-1,3
1810044A24Rik	AK007766	-1,3
Pfn2	NM_019410	-1,3
Coh1	AK007749	-1,3
Tigd2	AK019456	-1,3
Tbx2	NM_009324	-1,3
D11Wsu68e	NM_026776	-1,2
Abcd2	NM_011994	-1,2
Scrn3	BC015296	-1,2
2900003A17Rik	AK013479	-1,2
Sox17	NM_011441	-1,2
1810013C15Rik	AK007472	-1,2
Slc29a2	NM_007854	-1,2
Pex11b	NM_011069	-1,2
Cltb	AK009844	-1,2
Aes	NM_010347	-1,2
6330407A06Rik	AK020091	-1,2
Ryr1	X83932	-1,1
5430400D12Rik	AK017243	-1,1
Ahrr	NM_009644	-1,1
Tscot	NM_021053	-1,1
Tpra40	NM_011906	-1,1
Tk2	NM_021028	-1,1
1700003F17Rik	AK005634	-1,1
1500004F05Rik	AK005145	-1,1
4930444E06Rik	AK015377	-1,0
D730003K21Rik	AK021326	-1,0
4932412D23Rik	AK016521	-1,0
BC005561	BC005561	-1,0
-	U29236	-1,0
Chek2	NM_016681	-1,0
1700026G02Rik	AK006382	1,0
2310005A03Rik	AK009160	1,0
-	U37877	1,0
Nfia	U57633	1,1
Letmd1	AK003833	1,1
Slco6d1	AK014872	1,1
4921525L17Rik	AK014968	1,1
4930459C07Rik	AK015487	1,1
4930554H23Rik	AK019764	1,1
Fgf14	NM_010201	1,1
Dntt	NM_009345	1,1
Hrh4	AF358859	1,1
-	BC006036	1,2
Clps	NM_025469	1,2
Vrk2	BC013520	1,2
Tnfrsf23	AJ278264	1,2
1700120G07Rik	AK007216	1,2
En2	NM_010134	1,2
Rbm22	AK018452	1,2
2310015D24Rik	AK009355	1,2
Gtf2a1lf	NM_023630	1,2
1810053B23Rik	AK007854	1,2
2600001B17Rik	AK011126	1,2
Suv39h2	AF149205	1,2
Tulp4	AF219945	1,2
6030442E23Rik	AK020061	1,2
Serpinh1	J05609	1,2
2700003A03Rik	AK012205	1,2
Wdr12	NM_021312	1,2
1500005K14Rik	AK005164	1,2
3930401E15Rik	AK013936	1,2
-	X56613	1,2
Psg28	AF113598	1,3
Smurf2	AK012060	1,3
Seh1l	AK011330	1,3
Zfp51	AB010357	1,3
Rbl1	U27177	1,3
Fbxo28	AK018321	1,3
Dusp10	AB037908	1,3
C030039E19Rik	AK021134	1,3
4930431P22Rik	AK015281	1,3
Hoxb13	NM_008267	1,4
Cenph	NM_021886	1,4
Tbc1d1	NM_019636	1,4
Drd1a	AK021124	1,4
Sag	NM_009118	1,4
Prps2	NM_026662	1,4
Pigt	AK019717	1,4
Phca	AK004287	1,4
-	NM_029207	1,4
Skil	NM_011386	1,4

Symbol	Acc.Nr.	Fold Change Tumorigenic
2010002N04Rik	AF313412	1,4
Gtf2f2	AK003999	1,4
-	U23095	1,5
2310008M10Rik	NM_025509	1,5
-	U50960	1,5
-	M12194	1,5
-	AF120320	1,5
Ggps1	NM_010282	1,5
2410042D21Rik	BC003216	1,5
Ddit4	AK017926	1,5
D10Ertd516e	AK009683	1,5
Ttk	NM_009445	1,5
2300006M17Rik	BC006061	1,5
2610206B13Rik	AK011896	1,5
Has3	NM_008217	1,5
-	AF290967	1,5
Psmd14	NM_021526	1,5
Pdcd6ip	NM_011052	1,5
Tlr2	NM_011905	1,6
Bmper	AK014221	1,6
Hoxb6	X56461	1,6
Rala	NM_019491	1,6
Tex292	X80433	1,6
Gpr87	NM_032399	1,6
-	AK006925	1,6
Jub	NM_010590	1,7
1700030C10Rik	AK006529	1,7
Sdad1	BC005751	1,7
Olfr64	NM_013616	1,7
BC003236	NM_030249	1,7
Psph	BC002251	1,7
-	NM_019509	1,7
Ccng2	NM_007635	1,7
Gjb4	NM_008127	1,7
Syncrip	AB035725	1,7
Nodal	NM_013611	1,7
Nolc1	BC003244	1,7
Sgol2	AK003555	1,7
Ttll4	AK014557	1,8
Nola2	NM_026631	1,8
6720460F02Rik	AK011345	1,8
Fgd6	AK016940	1,8
Ubqln1	NM_026842	1,8
Pole2	AF036898	1,8
Fosl1	NM_010235	1,8
Map4k4	NM_008696	1,8
Trip13	AK010336	1,8
Smarca5	AF325921	1,9
Agpt2	NM_007426	1,9
Pkp3	NM_019762	1,9
Bhlhb2	NM_011498	1,9
Irf6	NM_016851	1,9
Ch25h	NM_009890	1,9
1110015M06Rik	AK003729	1,9
AA545217	NM_013726	1,9
Rhoj	AJ276568	1,9
Mmp9	NM_013599	1,9
Dtr	NM_010415	1,9
Neb	U58108	1,9
Inpp5b	NM_008385	1,9
Celsr1	NM_009886	2,0
Lamb3	NM_008484	2,0
Ifrd1	NM_013562	2,0
Melk	NM_010790	2,0
1500012M23Rik	AK005238	2,0
S100a3	NM_011310	2,1
1700093E07Rik	BC013063	2,1
Plscr2	NM_008880	2,1
Shcbp1	NM_011369	2,1
Hk2	NM_013820	2,1
Igsf4a	NM_018770	2,1
Tnfrsf11b	NM_008764	2,1
Rab38	NM_028238	2,1
Gdf1	NM_008107	2,1
Serpinb1a	NM_025429	2,1
Rras2	NM_025846	2,2
Plscr1	NM_011636	2,2
2310076G05Rik	AK010197	2,3
Fnbp3	NM_018785	2,3
Tuba6	NM_009448	2,3
Cd44	M30655	2,3
Tm4sf1	NM_008536	2,4
Birc5	NM_009689	2,5
Sfn	NM_018754	2,5
Acate2	NM_019736	2,5
1200002N14Rik	AK004552	2,5
Trim15	AK007445	2,5
Slc14a2	AF258602	2,7
Timp1	NM_011593	2,7
Adam8	NM_007403	2,7
Vsnl1	NM_012038	2,7
Tgfbi	NM_009369	2,8
Sox2	NM_011443	2,9
Gm566	AF014453	2,9
E030027H19Rik	BC011340	2,9
Bnc1	U88064	3,1
Areg	NM_009704	3,1
Ctla2a	NM_007796	3,1
Serpine1	NM_008871	3,4
Pkp1	NM_019645	3,7
Sfrp1	NM_013834	4,1
Col17a1	NM_007732	4,3
Mmp13	NM_008607	4,6

Acc.Nr. = Accession Number

Sup. Table VII - Biological processes (i.e., GO categories) containing genes involved in 2-AAF induced tumor response in mouse bladders

GO category	Total	Corrected p-value
Metabolism	11	0,0044
Cell adhesion	10	0,0287
Muscle development	9	<0.001
Apoptosis	7	0,0456
Cytoskeleton organization and biogenesis	7	0,0005
Small GTPase mediated signal transduction	5	0,0366
Pattern specification	4	0,0064
Skeletal development	4	0,0023
Cell fate commitment	3	0,0064
Chromatin assembly or disassembly	3	0,0046
Chromatin modification	3	0,0324
DNA replication	3	0,0453
Glycolysis	3	0,0085
Inflammatory response	3	0,0465
Neuropeptide signaling pathway	3	0,0309
Perception of sound	3	0,0103
Regulation of growth	3	<0.001
Regulation of muscle contraction	3	0,0021