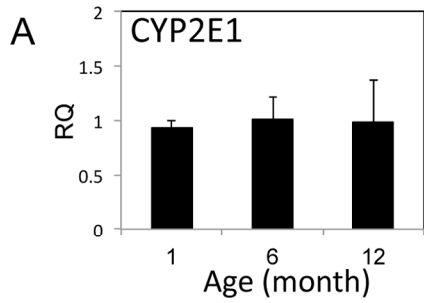
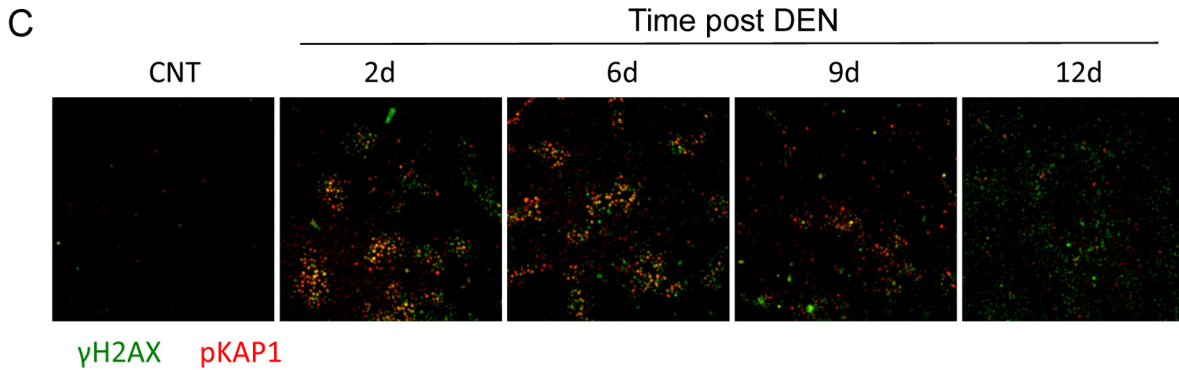


SUPPLEMENTARY MATERIAL

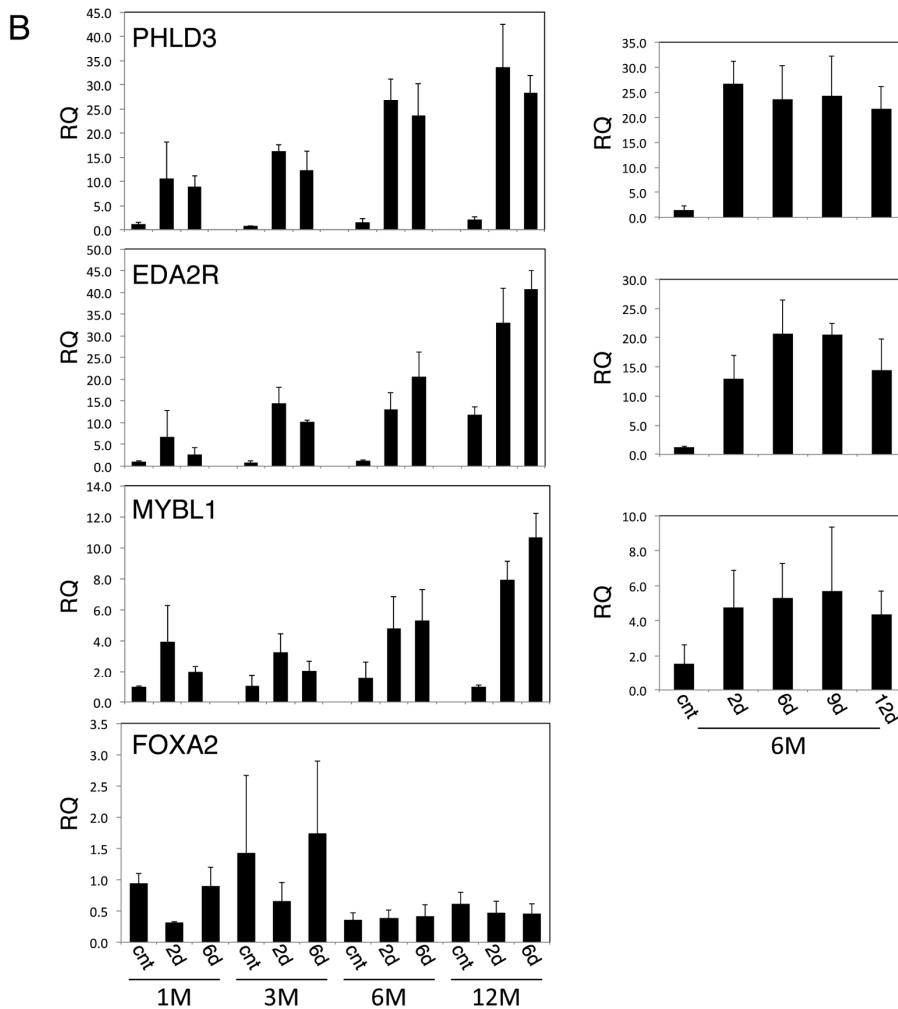
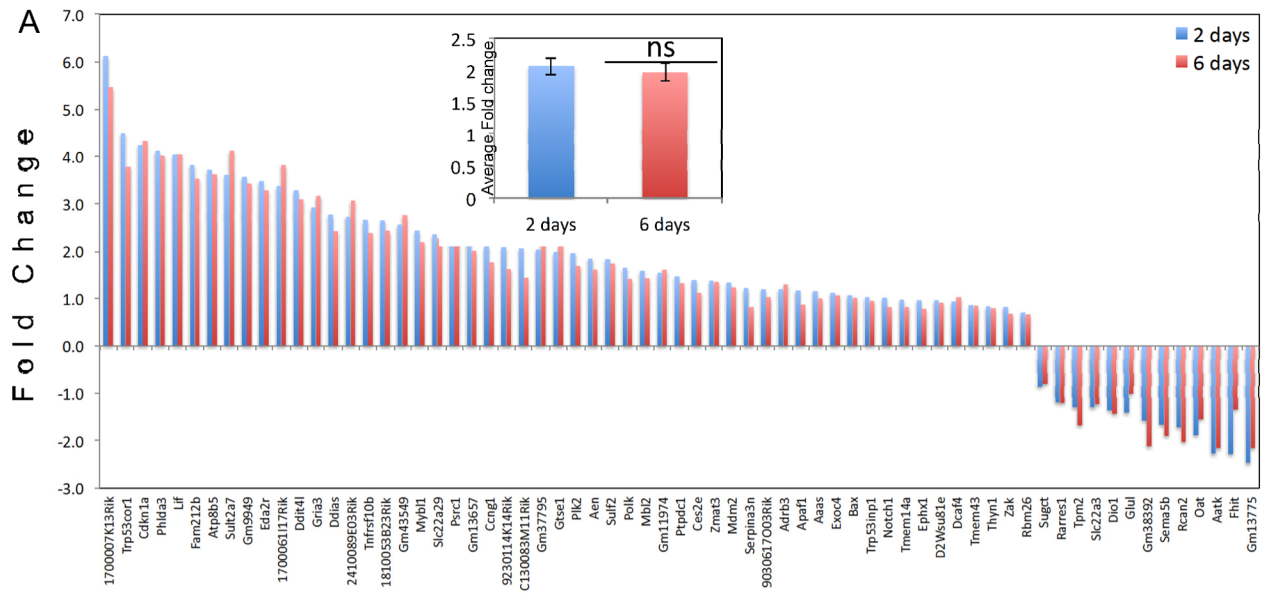


**B**

General_Gene_Details		Normalized_Counts_per_Sample				log2FoldChange	lfcSE	C_6M / C_3W			Significance C_6M / C_3W
Ensembl_Gene_ID	Associated_Gene_Name	C_3W_1	C_3W_2	C_6M_1	C_6M_2			stat	pvalue	padj	
ENSMUSG00000060407	Cyp2a12	17486.641	20720.850	11402.358	13498.510	-0.617	0.135	-4.560	5.107E-06	6.159E-04	yes
ENSMUSG00000091867	Cyp2a22	1038.661	1209.649	33.972	35.589	-5.012	0.428	-11.709	1.149E-31	2.632E-28	yes
ENSMUSG00000074254	Cyp2a4	8867.257	1104.807	12.133	12.604	-8.654	1.239	-6.984	2.875E-12	1.398E-09	yes
ENSMUSG00000030483	Cyp2b10	539.903	1124.189	211.110	40.037	-2.734	0.813	-3.362	7.750E-04	2.988E-02	yes
ENSMUSG00000040660	Cyp2b9	2004.064	1766.457	4.853	2.966	-8.952	0.949	-9.429	4.148E-21	5.118E-18	yes
ENSMUSG00000025004	Cyp2c40	4688.527	9038.446	129.820	166.822	-5.528	0.367	-15.072	2.493E-51	2.000E-47	yes
ENSMUSG00000062624	Cyp2c67	3124.012	4374.294	7160.749	8046.020	1.020	0.221	4.620	3.833E-06	4.879E-04	yes
ENSMUSG00000074882	Cyp2c68	9516.546	12652.414	3832.735	3773.889	-1.543	0.236	-6.526	6.769E-11	2.857E-08	yes
ENSMUSG00000092008	Cyp2c69	2722.597	4554.023	30.332	54.125	-6.408	0.446	-14.353	1.018E-46	4.084E-43	yes
ENSMUSG00000023963	Cyp39a1	2693.495	2759.373	463.471	368.492	-2.714	0.335	-8.114	4.916E-16	3.943E-13	yes
ENSMUSG00000054417	Cyp3a44	69.244	115.414	6.066	8.156	-3.676	0.677	-5.431	5.603E-08	1.362E-05	yes
ENSMUSG00000061292	Cyp3a59	2779.799	4266.809	707.339	1289.350	-1.818	0.329	-5.517	3.451E-08	8.516E-06	yes
ENSMUSG00000066071	Cyp4a12a	2491.784	5445.622	8244.203	8639.165	1.089	0.339	3.211	1.324E-03	4.240E-02	yes
ENSMUSG00000078597	Cyp4a12b	440.553	940.055	2920.353	2962.762	2.091	0.449	4.660	3.160E-06	4.087E-04	yes
ENSMUSG00000039519	Cyp7b1	663.338	1494.220	4651.696	9310.903	2.694	0.389	6.922	4.455E-12	2.042E-09	yes

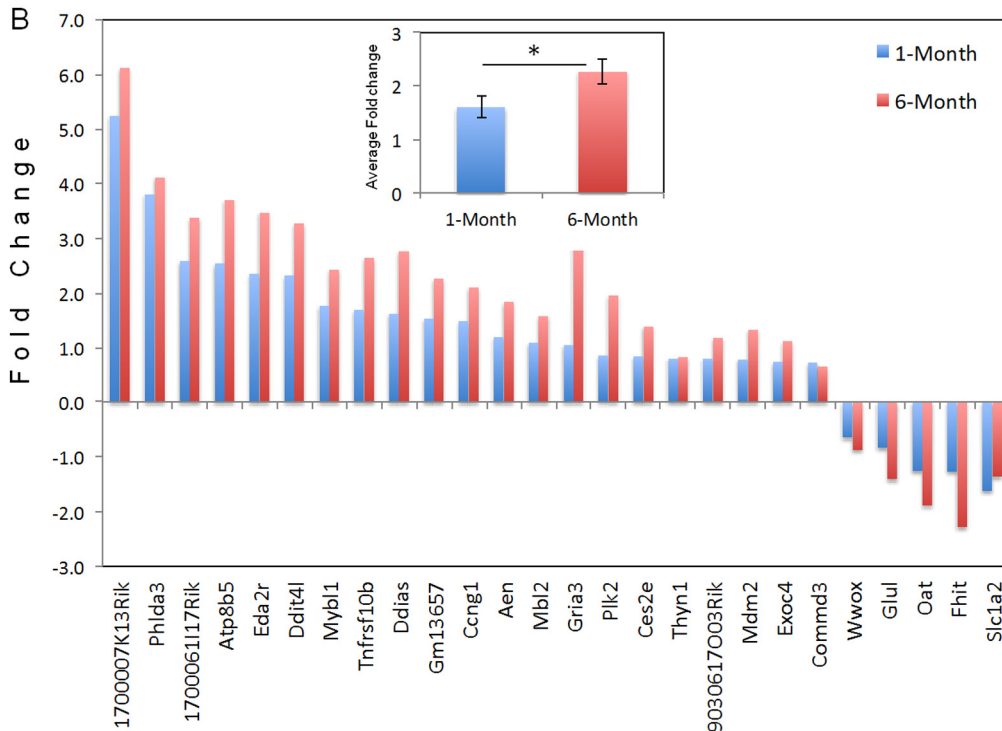
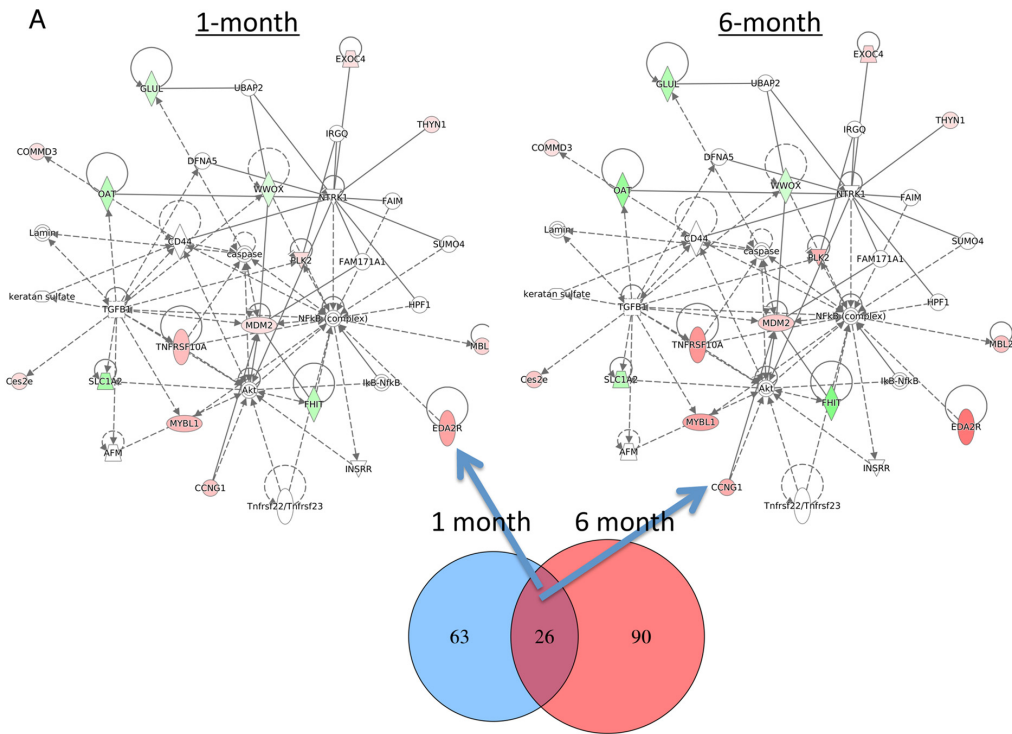


**Supplementary Figure S1. Delayed DNA damage response in 6-month-old mice.** (A) RT-qPCR analysis of CYP2E1 expression in RNA extracts from frozen liver tissues harvested from mice at the indicated ages. (B) A table presenting RNAseq data for the 15 CYP genes that show differential expression between 1 and 6 month-old mice. 11 genes were downregulated with age and only five genes were upregulated. (C) Six-month-old mice were injected with DEN and the levels of residual damage before and after 2, 6, 9 and 12 days were determined as above. Representative images of  $\gamma$ H2AX (green) and pKAP-1 (red) staining are shown.



**Supplementary Figure S2. Gene expression profiles following DEN treatment.** (A) Plot depicting the fold-change of 67 transcripts that are differentially expressed both in 2 days (blue bars) and 6 days (red bars) after DEN treatment in 6-month-old mice. Inset shows the average fold changes of all 67 genes  $\pm$ SD. ns. non-significant. (B) qRT-PCR analysis of genes representing differential gene expression patterns in response to DEN treatment. Left panels: The level of gene expression in 1, 3, 6 and 12-month old mice before and 2 and 6 days after DEN treatment. Right panels: the level of gene expression before and 2, 6, 9 and 12 days after DEN treatment in 6-month-old mice.

Network1\_Cancer organismal injury and abnormality and cell cycle



**Supplementary Figure S3. DEN induce a robust transcriptional response in 6-month-old mice. (A)** Age-related IPA enriched interaction network for 26 differentially expressed transcripts 2 days after DEN that are common for both 1- and 6-month-old mice. Each gene is colored according to the directionality of expression; red-upregulated, green-downregulated. Color intensity is proportional to the fold change of that particular gene. **(B)** Graph depicting the fold-change of all 26 transcripts that are differentially expressed in both 1-month (blue bars) and 6-month old mice (red bars) 2 days after DEN treatment. Inset shows the average fold changes of all genes for the two ages  $\pm$ SD. \*  $p < 0.05$ .

A

Upstream Regulators (Activated)	Activation z-score	p-value of overlap	Target molecules in dataset
cisplatin	2.651	3.25E-05	CCNG1, ENDOG, FOS, GDF15, JUND, MDM2, OAT, PHLDA3, PLK2, ZNF174
IL1B	2.387	5.63E-03	DDIAS, FOS, GOS2, GDF15, IL1R1, JUND, SLC1A2, TNFRSF10A
hydrogen peroxide	2.328	2.93E-03	DDIAS, FOS, GOS2, GDF15, IL1R1, JUND, SLC1A2, TNFRSF10A

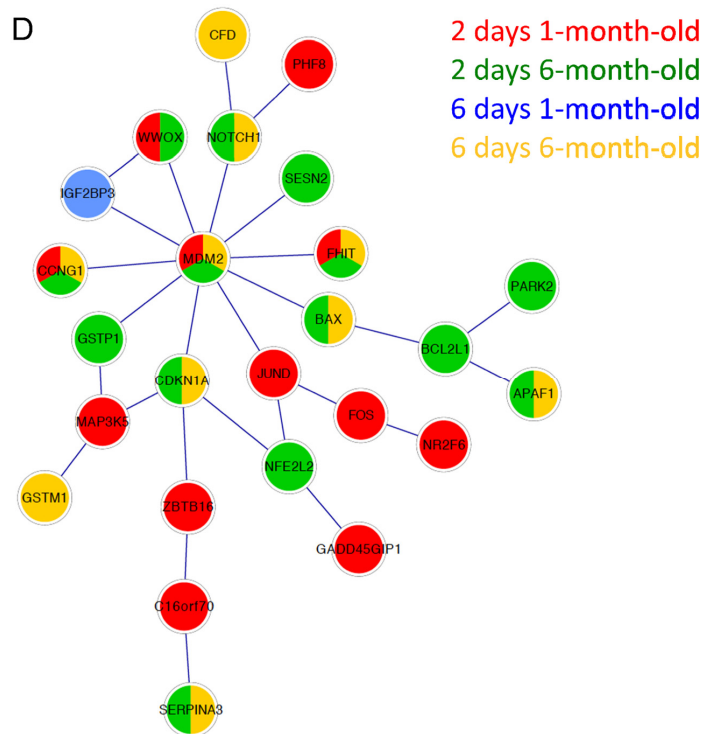
B

Upstream Regulators (Activated)	Activation z-score	p-value of overlap	Target molecules in dataset
TP53	2.000	3.57E-04	ADRB3, AEN, APAF1, BAX, CCNG1, CDKN1A, DDIAS, DDIT4L, EDA2R, EPHX1, FAM212B, GAS6, GLUL, GNA14, GTSE1, LIF, MDM2, MYBL1, NOTCH1, OAT, PARK2, PHLDA3, PLK2, POLK, PSRC1, SERPINA3, SESN2, TMEM43, TNFRSF10A, TP53INP1, Tpm2, ZMAT3
TNF	3.937	1.53E-20	AATK, ADRB3, APAF1, BAX, BCL2L1, BTG2, CDKN1A, DDIAS, DIO1, DUSP6, LIF, MBL2, NFE2L2, NOTCH1, PLK2, RCAN2, SERPINA3, SLC1A2, TNFRSF10A, TP53INP1, ZAK
CDKN2A	3.239	3.08E-08	AK1, APAF1, BAX, BTG2, CCNG1, CDKN1A, GTSE1, MDM2, POLK, TP53INP1, ZMAT3
IL1B	2.639	5.97E-05	BAX, BCL2L1, BTG2, CDKN1A, CYP8B1, DDIAS, DIO1, GAS6, LIF, NFE2L2, SERPINA3, SLC1A2, TNFRSF10A
NFE2L2	2.597	4.62E-05	APOA4, BCL2L1, CDKN1A, Cyp2a12/Cyp2a22, EPHX1, GNA14, NFE2L2, OAT, SERPIN A3
methyl methanesulfonate	2.596	5.47E-10	BTG2, CCNG1, CDKN1A, LIF, MDM2, PHLDA3, TP53INP1
doxorubicin	2.434	6.15E-07	APAF1, BAX, BCL2L1, BTG2, CDKN1A, DDIAS, MDM2, NFE2L2, PSRC1, TNFRSF10A, TP53INP1
1,2-dithiol-3-thione	2.433	2.56E-04	APOA4, Cyp2a12/Cyp2a22, EPHX1, GNA14, NFE2L2, SERPINA3
topotecan	2.415	1.29E-05	CDKN1A, FHIT, MDM2, PARD3B, PARK2, PLK2, SESN2
FGF2	2.401	6.56E-04	BAX, BCL2L1, BTG2, CDKN1A, GRIA3, MDM2, NOTCH1
benzo(a)pyrene	2.400	3.62E-04	BAX, CDKN1A, EPHX1, GNA14, MDM2, ZMAT3
CHEK2	2.400	3.75E-11	APAF1, BAX, BCL2L1, CCNG1, CDKN1A, MDM2
KRAS	2.236	1.72E-05	BCL2L1, CDKN1A, DUSP6, GLUL, MDM2, NFE2L2, SERPINA3, TNFRSF10A, Tpm2
idarubicin	2.236	1.34E-08	BAX, CDKN1A, MDM2, SESN2, TP53INP1
arsenite	2.236	8.37E-05	CDKN1A, FHIT, MDM2, NFE2L2, VWOX
NFKB (complex)	2.219	8.28E-06	BAX, BCL2L1, CDKN1A, DIO1, GAS6, LIF, MBL2, MDM2, MYBL1, NOTCH1, PLK2, SERPINA3
SLC29A1	2.200	1.34E-08	BAX, CCNG1, CDKN1A, MDM2, TP53INP1
gentamicin	2.196	3.85E-03	BAX, BTG2, CCNG1, DIO1, MDM2
paclitaxel	2.186	3.58E-06	BAX, BCL2L1, BTG2, CCNG1, CDKN1A, DUSP6, MDM2, TNFRSF10A, TP53INP1
cytarabine	2.183	8.82E-06	BAX, CDKN1A, MDM2, SESN2, TP53INP1

C

Upstream Regulators (Activated)	Activation z-score	p-value of overlap	Target molecules in dataset
TP53	3.838	6.55E-18	ADRB3, AEN, APAF1, BAX, CCNG1, CDKN1A, DDIAS, DDIT4L, EDA2R, EPHX1, FAM212B, GLUL, GSTM1, GTSE1, LIF, MDM2, MYBL1, NOTCH1, OAT, PHLDA3, PLK2, POLK, PSRC1, SERPINA3, TMEM43, TNFRSF10A, TP53INP1, Tpm2, ZMAT3
TNF	3.011	2.01E-07	AATK, ADRB3, APAF1, BAX, CDKN1A, CFD, DDIAS, DIO1, FAM198B, GPD1, LIF, MBL2, NOTCH1, PLK2, RCAN2, SERPINA3, TNFRSF10A, TP53INP1, ZAK
CDKN2A	2.922	1.52E-07	APAF1, BAX, CCNG1, CDKN1A, GTSE1, MDM2, POLK, TP53INP1, ZMAT3
lipopolysaccharide	2.645	8.99E-03	BAX, CDKN1A, CFD, GPD1, GTSE1, LIF, MBL2, NOTCH1, PLK2, SERPINA3, Tpm2
doxorubicin	2.497	1.68E-05	APAF1, BAX, CDKN1A, DDIAS, MDM2, PSRC1, TNFRSF10A, TP53INP1
IFNG	2.441	4.15E-03	BAX, CDKN1A, DDIAS, DIO1, GLUL, LIF, MDM2, NOTCH1, RARRES1, TNFRSF10A
methyl methanesulfonate	2.401	3.09E-09	CCNG1, CDKN1A, LIF, MDM2, PHLDA3, TP53INP1
ethanol	2.401	5.19E-05	BAX, CDKN1A, EPHX1, GSTM1, MDM2, ZMAT3
benzo(a)pyrene	2.401	5.19E-05	BAX, CDKN1A, EPHX1, GSTM1, MDM2, ZMAT3
mitomycin C	2.374	2.70E-08	BAX, CDKN1A, MDM2, PSRC1, TP53INP1, ZMAT3
SLC29A1	2.219	2.25E-09	BAX, CCNG1, CDKN1A, MDM2, TP53INP1
ETS1	2.215	4.33E-04	BAX, CCNG1, CDKN1A, MDM2, MGAT5
NR112	2.207	7.00E-05	CDKN1A, GLUL, GSTM1, Gstm3, Mup1 (includes others)
CHEK2	2.186	8.53E-10	APAF1, BAX, CCNG1, CDKN1A, MDM2
doxifluridine	2.176	1.21E-09	BAX, CCNG1, CDKN1A, MDM2, TP53INP1
ethanol	2.156	4.26E-03	BAX, CDKN1A, EPHX1, Gstm3, TP53INP1
cisplatin	2.067	4.03E-08	BAX, CCNG1, CDKN1A, CLSTN3, GSTM1, MDM2, NOTCH1, OAT, PHLDA3, PLK2, PSRC1, TP53INP1, ZMAT3
ZBTB33	2.000	9.52E-08	APAF1, BAX, CDKN1A, TNFRSF10A
RASSF1	2.000	1.15E-04	CDKN1A, LIF, PLK2, TP53INP1
INH A	2.000	2.53E-04	BAX, EPHX1, TP53INP1, Tpm2
idarubicin	2.000	2.68E-07	BAX, CDKN1A, MDM2, TP53INP1

**Supplementary Figure S4. Upstream regulators and protein interaction network analysis.** IPA upstream regulator analysis for differentially expressed genes 2 days after DEN treatment in 1-month (A) and 6-month-old mice (B; top 20 are shown) as well as 6 days after DEN in 6-month-old mice (C).



**Supplementary Figure S4. Upstream regulators and protein interaction network analysis.** (D) Protein:protein interaction network constructed for the differentially expressed genes of the various comparisons. Nodes representing interacting proteins and were colored as a pie chart based on their differential expression in any of the four indicated comparisons.

**Supplementary Table 1. Oligonucleotide Sequences for qRT-PCR.**

Target gene	Forward Primer	Reverse Primer
<i>MYBL1</i>	5'-GAAACCGTTGGGCCGAGATT-3'	5'-CCATCTTGTAATAGCCCTCCTG-3'
<i>Eda2r</i>	5'-CACACTGCATAGTCTGCCCTC-3'	5'-GCCTTCTGGACCCGATTGA-3'
<i>Foxa2</i>	5'-CATGGGACCTCACCTGAGTC-3'	5'-CATCGAGTTCATGTTGGCGTA-3'
<i>PHLDA3</i>	5'-CCGTGGAGTGCCTAGAGAG-3'	5'-CCAGGGTGATCTGAGCGTT-3'
<i>Cyp2e1</i>	5'-CCACCAGCACAACTCTGAGATA-3'	5'-CCCAATAACCCTGTCAATTTCTT-3'
<i>HPRT</i>	5'-GCGATGATGAACCAGGTTATGA-3'	5'-ATCTCGAGCAAGTCTTTCAGTCCT-3'