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Supplemental Data

Article

Hepatocyte Necrosis Induced by Oxidative Stress and IL-1α Release Mediate Carcinogen-Induced Compensatory Proliferation and Liver Tumorigenesis

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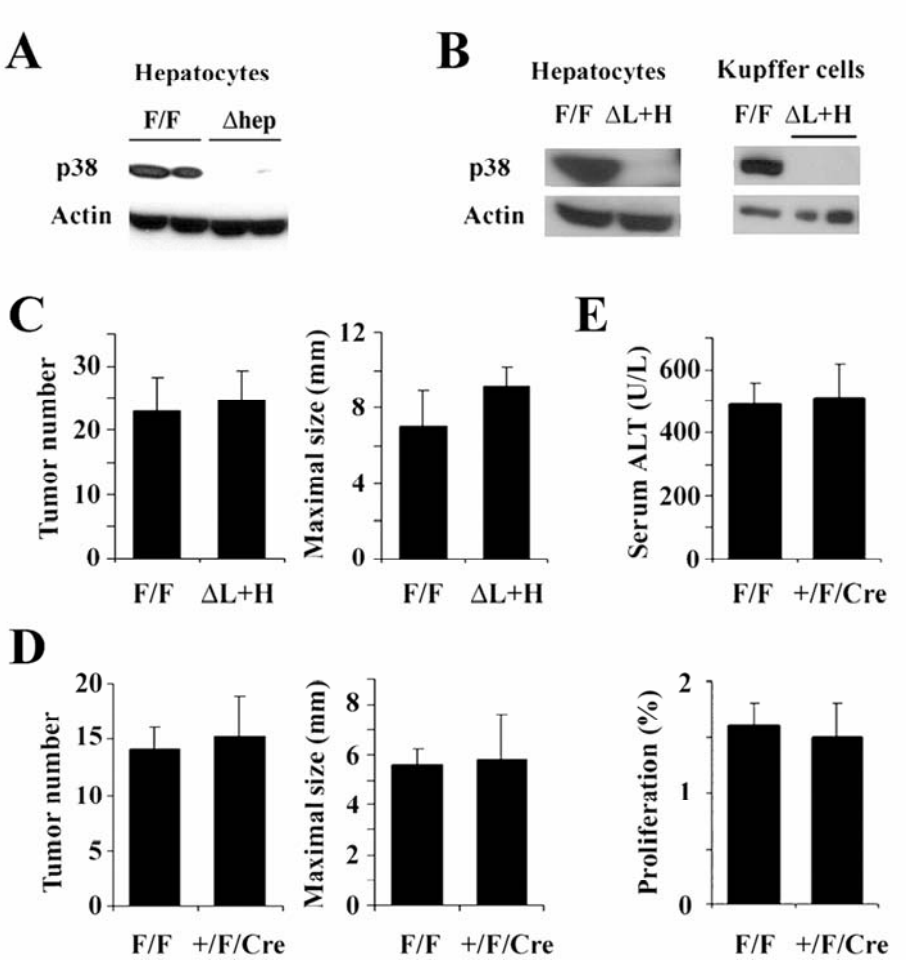


Figure S1. Characterization of p38α Conditional Deletions

(A) Extracts of primary hepatocytes from *p38α*$^{\Delta hep}$ and *p38α*$^{F/F}$ control mice were analyzed by immunoblotting with the indicated antibodies.

(B) Extracts of primary hepatocytes and KC from *p38α*$^{\Delta L+H}$ and *p38α*$^{F/F}$ control mice were analyzed by immunoblotting with the indicated antibodies.

(C) Tumor multiplicity (>0.5 mm) and maximal tumor sizes (diameters) in livers of male *p38α*$^{F/F}$ (F/F, n = 5) and *p38α*$^{\Delta L+H}$ (n = 8). Results are means ± SEM.

(D) Tumor multiplicity (>0.5 mm) and maximal tumor sizes (diameters) in livers of male *p38α*$^{F/F}$ (F/F, n = 15) and *p38α* $^{+/F}$*/Alb-Cre* (n = 4) mice. Results are means ± SEM.

(E) ALT in serum was measured 48 hrs after DEN injection and extent of compensatory proliferation was determined by BrdU labeling. Results are means ± SEM (n = 3).

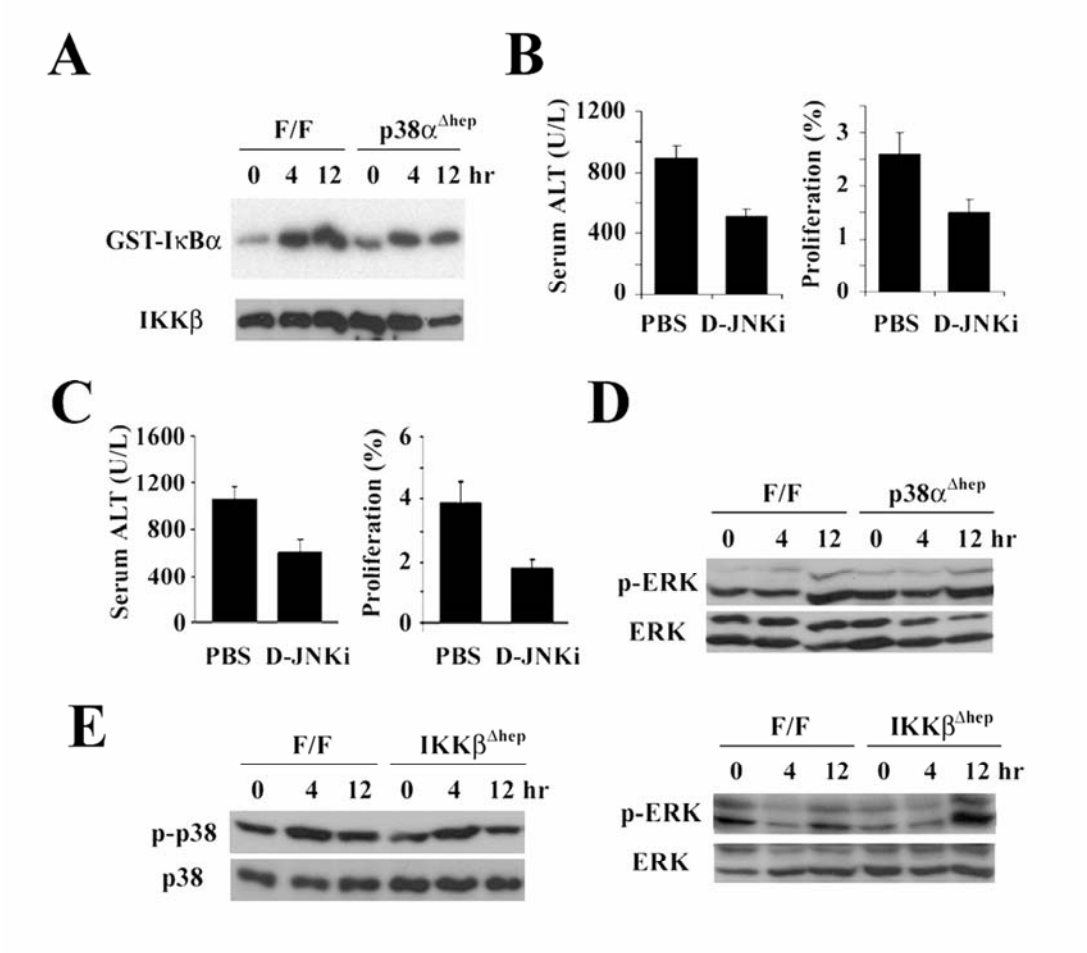


Figure S2. Effect of p38α Ablation on Activity of Other Protein Kinases

(A) Mice were given DEN and their livers isolated at the indicated times and homogenized. IKK activity was determined by immunocomplex kinase assay with GST-IκBα as a substrate. Protein recovery was determined by immunoblotting.

(B and C) Effects of JNK inhibitor (D-JNKi) on cell death and compensatory proliferation. Serum ALT levels and extent of compensatory proliferation determined by BrdU labeling were examined in *p38α*$^{\Delta hep}$ (B) and *Ikkβ*$^{\Delta hep}$ mice (C) 48 hrs after DEN administration. Either PBS or D-JNKi (20 mg/kg) was given before DEN treatment. Results are means $\pm$ SEM (n = 4).

(D and E) Mice were given DEN and their livers isolated at the indicated times and homogenized. ERK and p38 phosphorylation were analyzed by immunoblotting.

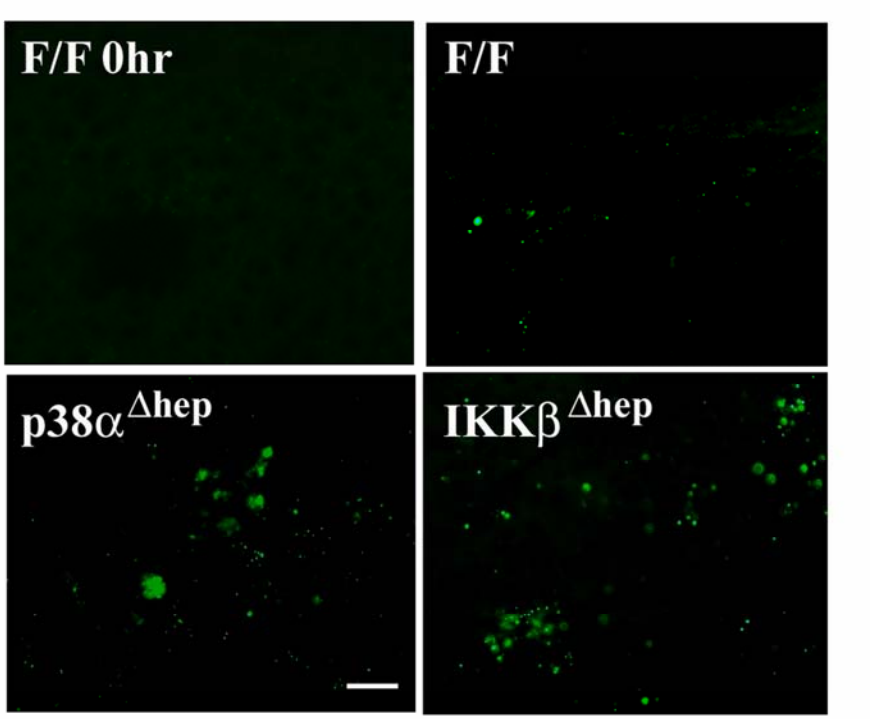
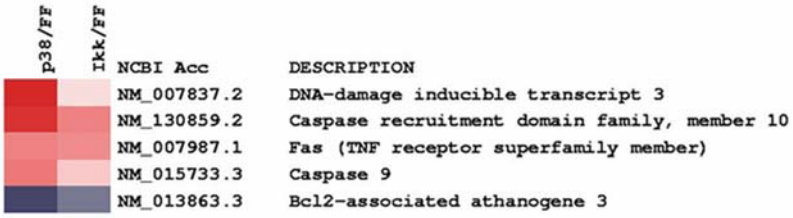


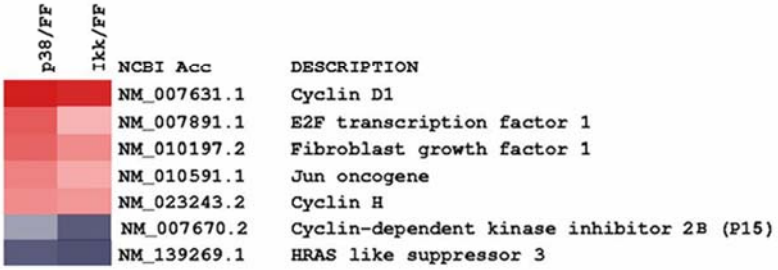
Figure S3. Enhanced H_2O_2 Accumulation in *p38α*$^{\Delta hep}$ and *Ikk*β$^{\Delta hep}$ Mice

Liver cryosections prepared before (0 hr) and 6 hrs after DEN injection into mice of the indicated genotypes were incubated with 5 μM 5-[and-6]-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate for 30 min at 37ºC. Cells staining positively for the oxidized dye were identified by fluorescent microscopy. Scale bar = 50 μm.

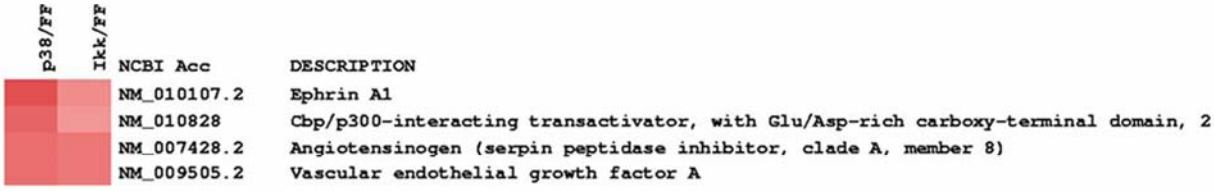
Cell Death



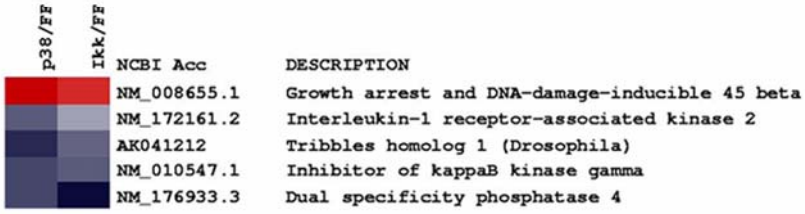
Cell Cycle and Transformation



Angiogenesis



Protein Kinases



Heat Shock Response

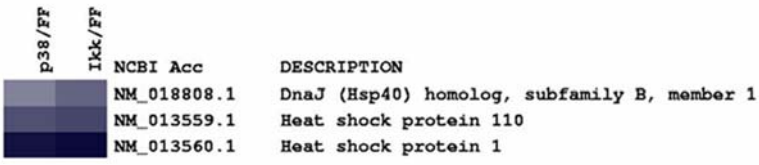


Figure S4.

Figure S4. Expression Profiling of $p38\alpha^{\Delta hep}$, $Ikk\beta^{\Delta hep}$, and Control Livers (n = 3)

Total liver RNA was extracted 4 hrs after DEN administration and gene expression profiling was conducted using microarray analysis with whole-genome oligonucleotide arrays. Genes whose expression was altered by more than 1.5-fold with a p-value < 0.05 upon p38α or IKKβ deletion were categorized as shown. Positive or negative numbers represent fold-changes in $p38\alpha^{\Delta hep}$ or $Ikk\beta^{\Delta hep}$ livers relative to $p38\alpha^{F/F}$ or $Ikk\beta^{F/F}$ livers, respectively.

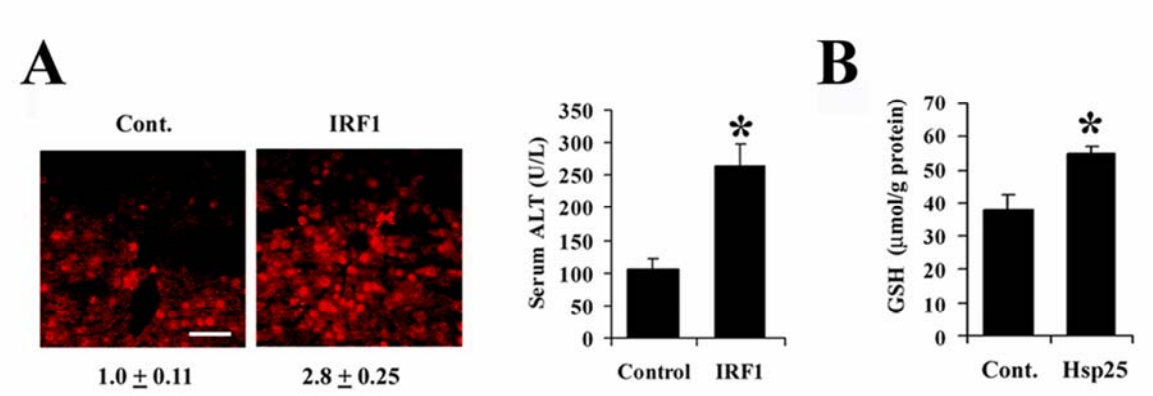


Figure S5. Effects of IRF1 and Hsp25 on ROS, Liver Damage, and GSH

(A) $p38\alpha^{F/F}$ mice were infected with adenovirus expressing IRF1 or a control adenovirus and after 20 hrs were injected with DEN. Liver cryosections prepared 8 hrs later were incubated with 2 mM dihydroethidine hydrochloride for 30 min at 37ºC. Cells staining positively for the oxidized dye were identified by fluorescent microscopy. Scale bar = 50 μm. The numbers at the bottom are mean fluorescent intensity ± SEM. Serum ALT levels were also measured 8 hrs after DEN injection. Results are means ± SEM (n = 4). *p < 0.05 vs. control mice.

(B) $p38\alpha^{\Delta hep}$ mice were infected with adenovirus expressing Hsp25 or a control adenovirus. After 20 hrs the mice were given DEN and 24 hrs later liver lysates were prepared and GSH content was measured. Results are means ± SEM (n = 4). *p < 0.05 vs. control mice.

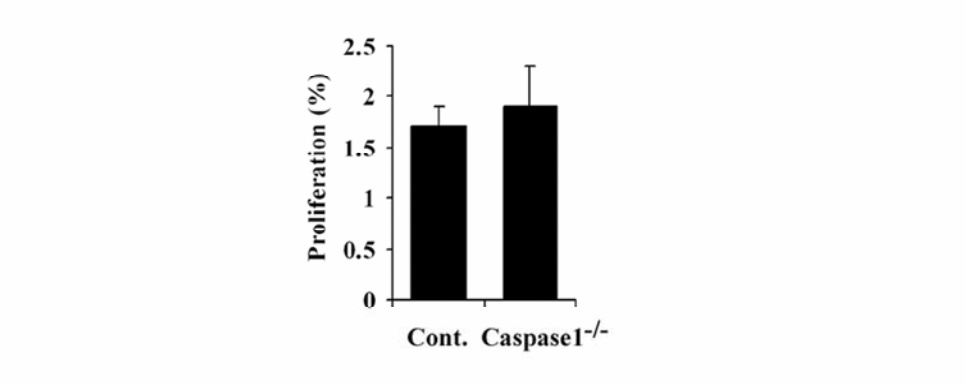


Figure S6. Compensatory Proliferation in *Caspase-1*$^{-/-}$ Mice

Extent of compensatory proliferation was determined by BrdU labeling in control and *Caspase-1*$^{-/-}$ mice 48 hrs after DEN injection. Results are means $\pm$ SEM (n = 3).

Table S1. Genes with Altered Expression Levels in *p38α*$^{\Delta hep}$ and *Ikkβ*$^{\Delta hep}$ Livers

Gene	Accession Number	Description	p38α$^{\Delta/F}$	IKKβ$^{\Delta/F}$
14262	NM_008030.1	Flavin containing monooxygenase 3	4.9688	7.4163
17873	NM_008655.1	Growth arrest and DNA-damage-inducible 45 beta (Gadd45β)	3.2574	2.288
104943	NM_027828.2	RIKEN cDNA 9030611O19 gene	2.9666	1.8347
269344	NM_145973.2	adult retina cDNA, RIKEN clone:A930015D22 productprotein	2.681	1.4873
12443	NM_007631.1	Cyclin D1	2.3348	2.2221
16362	NM_008390.1	Interferon regulatory factor 1	2.3031	1.2747
13198	NM_007837.2	DNA-damage inducible transcript 3 (Chop)	2.1638	1.4516
13122	NM_007824.2	Cytochrome P450, family 7, subfamily a, polypeptide 1	1.9945	2.5534
13555	NM_007891.1	E2F transcription factor 1	1.8644	1.2745
14102	NM_007987.1	Fas (TNF receptor superfamily member)	1.4666	1.2777
22339	NM_009505.2	Vascular endothelial growth factor A	1.45	1.4061
277753	NM_177406.2	Cytochrome P450, family 4, subfamily a, polypeptide 12	-3.9136	-5.5372
15507	NM_013560.1	Heat shock protein 1 (Hsp25)	-2.5401	-1.2962
15368	NM_010442.1	Heme oxygenase (decycling) 1	-1.8324	-1.9963
232174	NM_175475.2	14, 17 days embryo head cDNA, RIKEN clone:3221401J18	-1.809	-2.4473
319520	NM_176933.3	Dual specificity phosphatase 4 (MKP2)	-1.7999	-2.8351
15505	NM_013559.1	Heat shock protein 110	-1.7509	-1.8271
12575	NM_007669.2	Cyclin-dependent kinase inhibitor 1A (p21)	-1.6014	-1.6667
69134	NM_183278.1	RIKEN cDNA 2200001I15 gene	-1.3865	-3.3427

Expression profiling of *p38α*$^{\Delta hep}$, *Ikkβ*$^{\Delta hep}$, and control livers (n = 3). Liver RNA extracted 4 hrs after DEN administration was analyzed using whole-genome oligonucleotide arrays. Genes whose expression is substantially and consistently altered upon deletion of p38α or IKKβ are listed. Positive and negative numbers represent fold changes in expression (up or down, respectively) in *p38α*$^{\Delta hep}$ or *Ikkβ*$^{\Delta hep}$ mice relative to the corresponding floxed strains.