Supplementary Figures and Tables

Figure S1. Profile of differentially expressed genes following exposure of WT and *hsp110*$^{-/-}$ mice to sham or TBI. Brain tissue encompassing the injured site (3mm coronal of the ipsilateral cortex centered around the impact site) of sham or TBI-treated WT or *hsp110*$^{-/-}$ 8-10 week-old male mice (n=5 mice each) were subjected to total RNA isolation and Affymetrix gene expression microarray analyses (for detail see Method section). We found 270 significantly differentially expressed genes between the groups which showed >1.5-fold change (log2 expression).

(A) Heatmap visualization of 100 significantly regulated gene sets ($p<0.05$) are presented. Right: 50 up and 50 down-regulated transcripts. Color indicates gene expression values with "red" being the up, and "blue" being the down-regulated genes. The gene identity is indicated in the left hand side for the WT sham versus WT TBI group. Also see Table S3.

(B) Top and bottom panels: qRT-PCR to verify the gene expression microarray result for a selected list of genes. 1. WT sham, 2. *hsp110*$^{-/-}$ sham, 3. *hsp70i*$^{-/-}$ sham, 4. WT TBI, 5. *hsp110*$^{-/-}$ TBI, 6. *hsp70i*$^{-/-}$ TBI. Data is presented as mean+/-SD (n=3 mice). *p<0.05, **$p<0.01$, ***$p<0.001$. ns=not significant.

Middle panels: ELISA was performed to detect the level of GH in the brain tissue and serum for the indicated groups. Briefly, a 96 well plate was coated with 50μl anti-rat GH (monkey) (AFP411S, NIDDK Program (Torrance, CA) for 16 hours at 4°C. The plate was incubated with blocking buffer (5% non-fat dry milk in PBST (0.05% tween-20)) for 2 hours at 25°C. Two-fold serial dilution of mouse GH (AFP-10783B, NIDDK-NHPP) in PBST (plus 0.2% BSA) was used to generate a standard curve. After washing, the bound standards and samples were detected with 50μl rabbit antiserum against rat GH (AFP5672099, NIDDK-NHPP). After washing, the

bound complex was incubated with 50µl of horseradish-peroxidase-conjugated anti-rabbit IgG. The TMB was used as a substrate to develop color. The reaction was stopped by 2N sulfuric acid and read at 450nm. Data is presented as mean+/-SD (n=3 mice). ***p<0.01, ***p<0.001.*

(C) Selected biological pathways associated with significantly up or down-regulated gene sets (fold change >1.0 and < 1.0) were generated using the 'Biological Pathways' subset of Gene Ontology included in the DAVID System (DAVID, http://david.abcc.ncifcrf.gov/). Blue bars indicate number of gene sets involved in specific biological processes and red bars indicate the level of significance (negative log10 of p-values). See Table S4 for the complete list of biological pathways and gene sets.

Figure S2. P-Tau is reduced following treatment of TBI-treated mice with Hsp70 inducers.

(A-C) WT mice were treated with TBI and then with Celastrol or BGP-15 (as in Figures 3, 4 and 5). *Hsp110*$^{-/-}$ mice were treated with TBI alone. Brain cell lysates were subjected to immunoblotting using the indicated antibodies. βActin is loading control. Quantification of the data is indicated in panels (B-C). Data is presented as mean +/-SD (n=3 mice per group). *$p<0.05$,**$p<0.01$,***$p<0.001$.

(D-E) WT mice were treated with TBI and then with Celastrol or BGP-15 as in A. IHC analyses using antibody to total-Tau or P-Tau (pS202) are presented. Quantification of the data is presented in panel E. Data is presented as mean+/-SD (n=3 mice). **$p<0.01$,***$p<0.001$. ns=not significant. DAPI (blue) represents nuclei staining.

Figure S3. Quantification of the Figure 6 immunoblots showing differential p53 target gene expression. WT, *hsp110*$^{-/-}$ or *hsp70i*$^{-/-}$ mice were treated with sham or TBI and received

vehicle or Celastrol at 30 minutes and 6 hours post-TBI. 24 hours after TBI, brain tissues encompassing the injured sites (3mm coronal of the ipsilateral cortex centered around the impact site) were processed for immunoblotting and immunoblot were quantitated as described in the method section. Samples 1-9 are as follows: 1. WT sham, 2. *hsp110*$^{-/-}$ sham, 3. *hsp70*$^{-/-}$ sham, 4. WT TBI, 5. *hsp110*$^{-/-}$ TBI, 6. *hsp70i*$^{-/-}$ TBI, 7. WT TBI+ Celastrol, 8. *hsp110*$^{-/-}$ TBI+Celastrol, 9. *Hsp70i*$^{-/-}$ TBI +Celastrol. Data presented as mean +/- SD.*$p<0.05$, **$p<0.01$. ns=not significant. n=3 mice per group.

Table S1. Gene signature list. Data includes complete gene set and includes comparison of WT sham versus WT TBI, *hsp110*$^{-/-}$ sham versus *hsp110*$^{-/-}$ TBI, WT sham versus *hsp110*$^{-/-}$ sham and WT TBI versus *hsp110*$^{-/-}$ TBI.

Table S2. Gene signature list for significantly expressed genes. Data includes 270 differentially expressed genes (>1.5 fold change) and includes comparison of WT TBI versus WT sham, *hsp110*$^{-/-}$ sham versus *hsp110*$^{-/-}$ TBI, WT sham versus *hsp110*$^{-/-}$ sham and WT TBI versus *hsp110*$^{-/-}$ TBI.

Table S3. Gene signature list and their putative function for the 50 up and 50 down-regulated expressed genes that are presented in the heatmap. Comparison of WT sham versus WT TBI, *hsp110*$^{-/-}$ sham versus *hsp110*$^{-/-}$ TBI, WT sham versus *hsp110*$^{-/-}$ sham and WT TBI versus *hsp110*$^{-/-}$ TBI are indicated.

Table S4. Selected gene ordering according to the biological processes. Biological processes significantly enriched in the set of genes identified by Affymetrix microarray analyses as up- or down-regulated in WT sham versus WT TBI, *hsp110*$^{-/-}$ sham versus *hsp110*$^{-/-}$ TBI, WT sham versus *hsp110*$^{-/-}$ sham and WT TBI versus *hsp110*$^{-/-}$ TBI and classified in the DAVID program. According to DAVID, the threshold of minimum gene counts belonging to an annotation term has to be equal or greater than 2. That is, the pathway is not considered if there is only one gene involved.

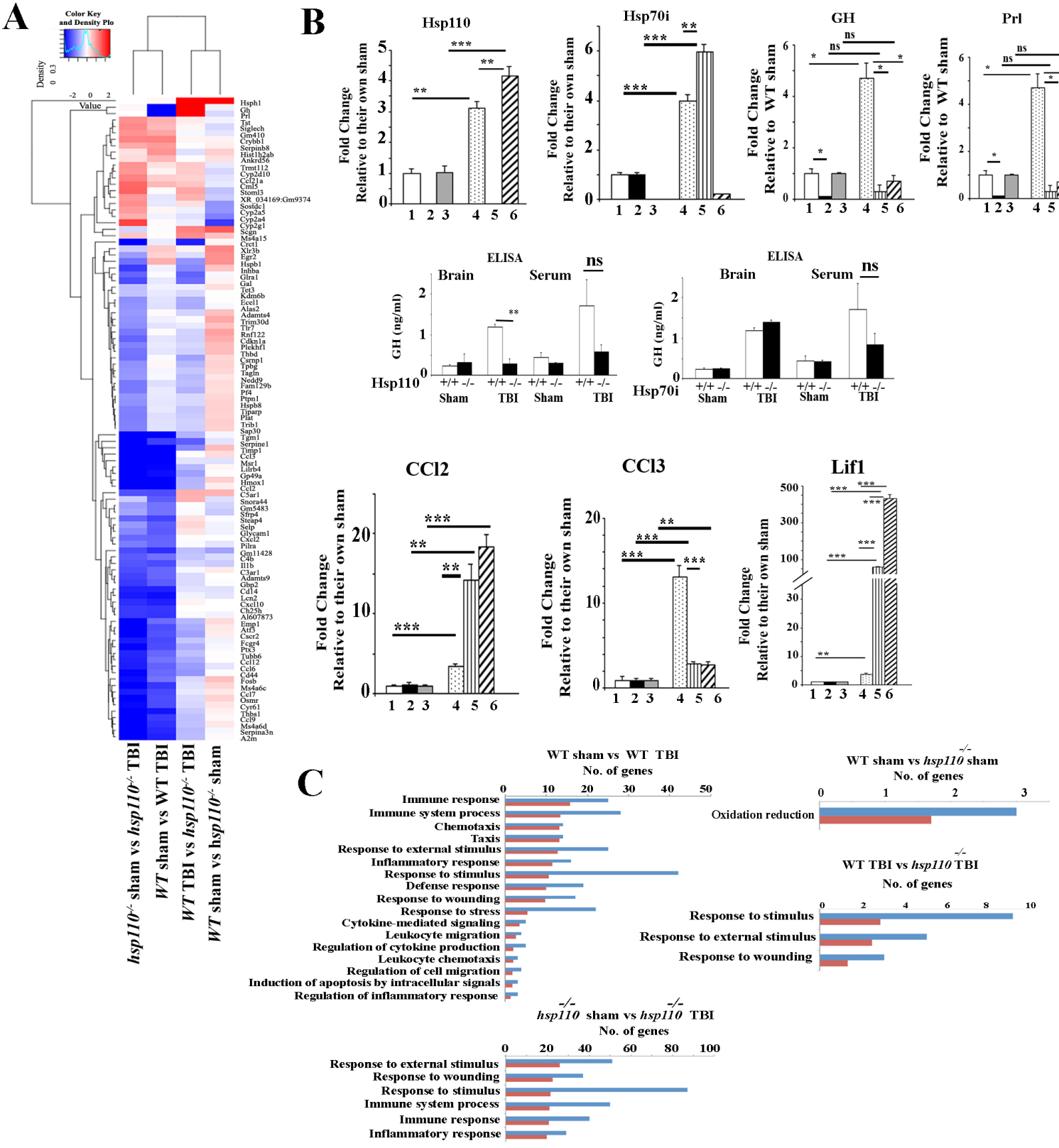
A
Color Key and Density Plo
Density
Value
hsp110-/- sham vs hsp110-/- TBI
WT sham vs WT TBI
WT TBI vs hsp110-/- TBI
WT sham vs hsp110-/- sham
B
Hsp110
Hsp70i
GH
Prl
Fold Change Relative to their own sham
Fold Change Relative to WT sham
ELISA
Brain
Serum
ns
GH (ng/ml)
Hsp110
Hsp70i
+/+ -/-
Sham
TBI
CCl2
CCl3
Lif1
C
WT sham vs WT TBI
No. of genes
Immune response
Immune system process
Chemotaxis
Taxis
Response to external stimulus
Inflammatory response
Response to stimulus
Defense response
Response to wounding
Response to stress
Cytokine-mediated signaling
Leukocyte migration
Regulation of cytokine production
Leukocyte chemotaxis
Regulation of cell migration
Induction of apoptosis by intracellular signals
Regulation of inflammatory response
-/-
WT sham vs hsp110 sham
No. of genes
Oxidation reduction
-/-
WT TBI vs hsp110 TBI
No. of genes
Response to stimulus
Response to external stimulus
Response to wounding
-/-
-/-
hsp110 sham vs hsp110 TBI
No. of genes
Response to external stimulus
Response to wounding
Response to stimulus
Immune system process
Immune response
Inflammatory response

Figure S1

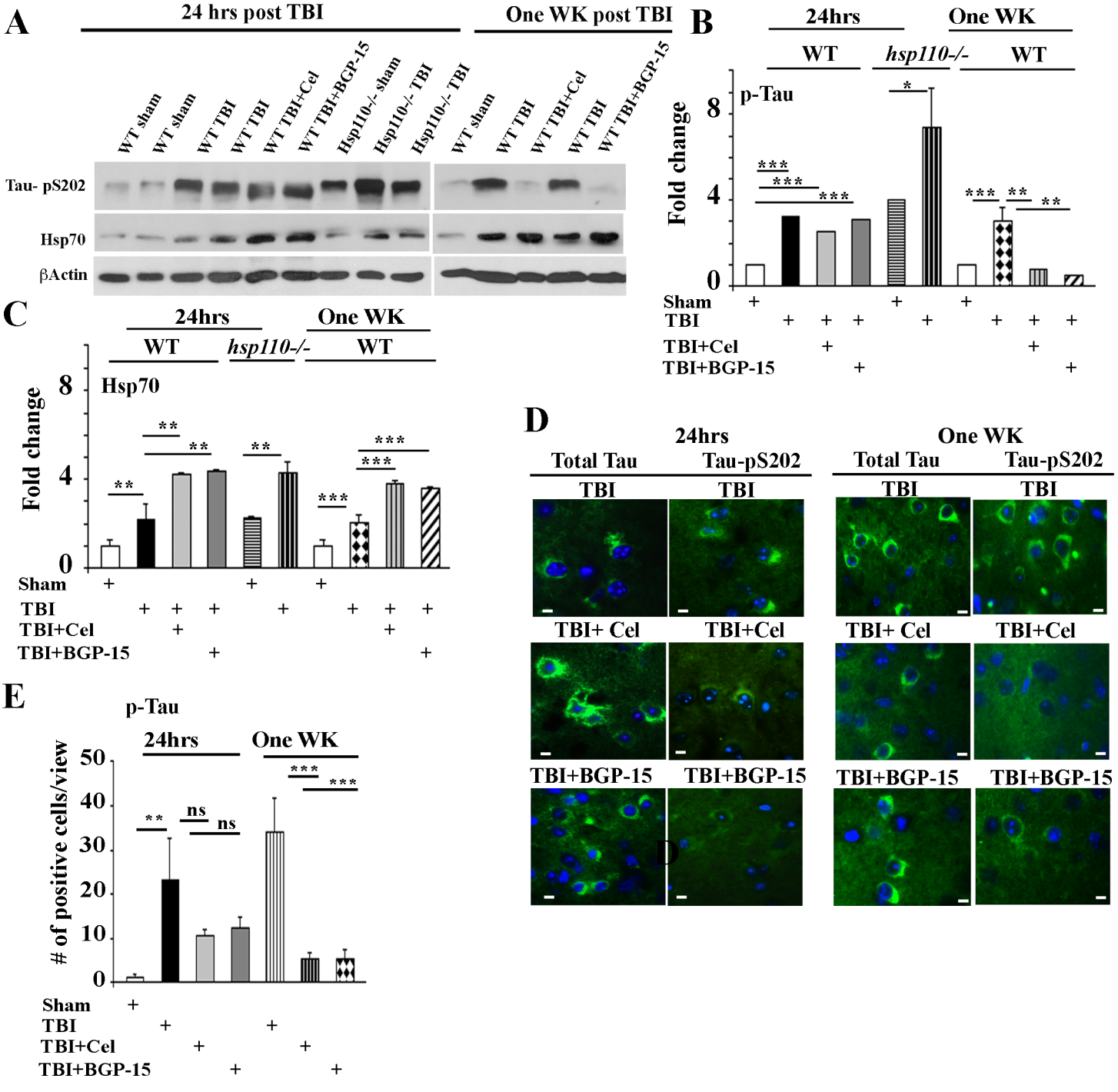
A
24 hrs post TBI
One WK post TBI
WT sham
WT sham
WT TBI
WT TBI
WT TBI+Cel
WT TBI+BGP-15
Hsp110-/- sham
Hsp110-/- TBI
Hsp110-/- TBI
WT sham
WT TBI
WT TBI+Cel
WT TBI
WT TBI+BGP-15
Tau- pS202
Hsp70
βActin
B
24hrs
One WK
WT
hsp110-/-
WT
p-Tau
Fold change
Sham
TBI
TBI+Cel
TBI+BGP-15
C
24hrs
One WK
WT
hsp110-/-
WT
Hsp70
Fold change
Sham
TBI
TBI+Cel
TBI+BGP-15
D
24hrs
One WK
Total Tau
Tau-pS202
Total Tau
Tau-pS202
TBI
TBI
TBI
TBI
TBI+ Cel
TBI+Cel
TBI+ Cel
TBI+Cel
TBI+BGP-15
TBI+BGP-15
TBI+BGP-15
TBI+BGP-15
E
p-Tau
24hrs
One WK
# of positive cells/view
ns
ns
Sham
TBI
TBI+Cel
TBI+BGP-15

Figure S2

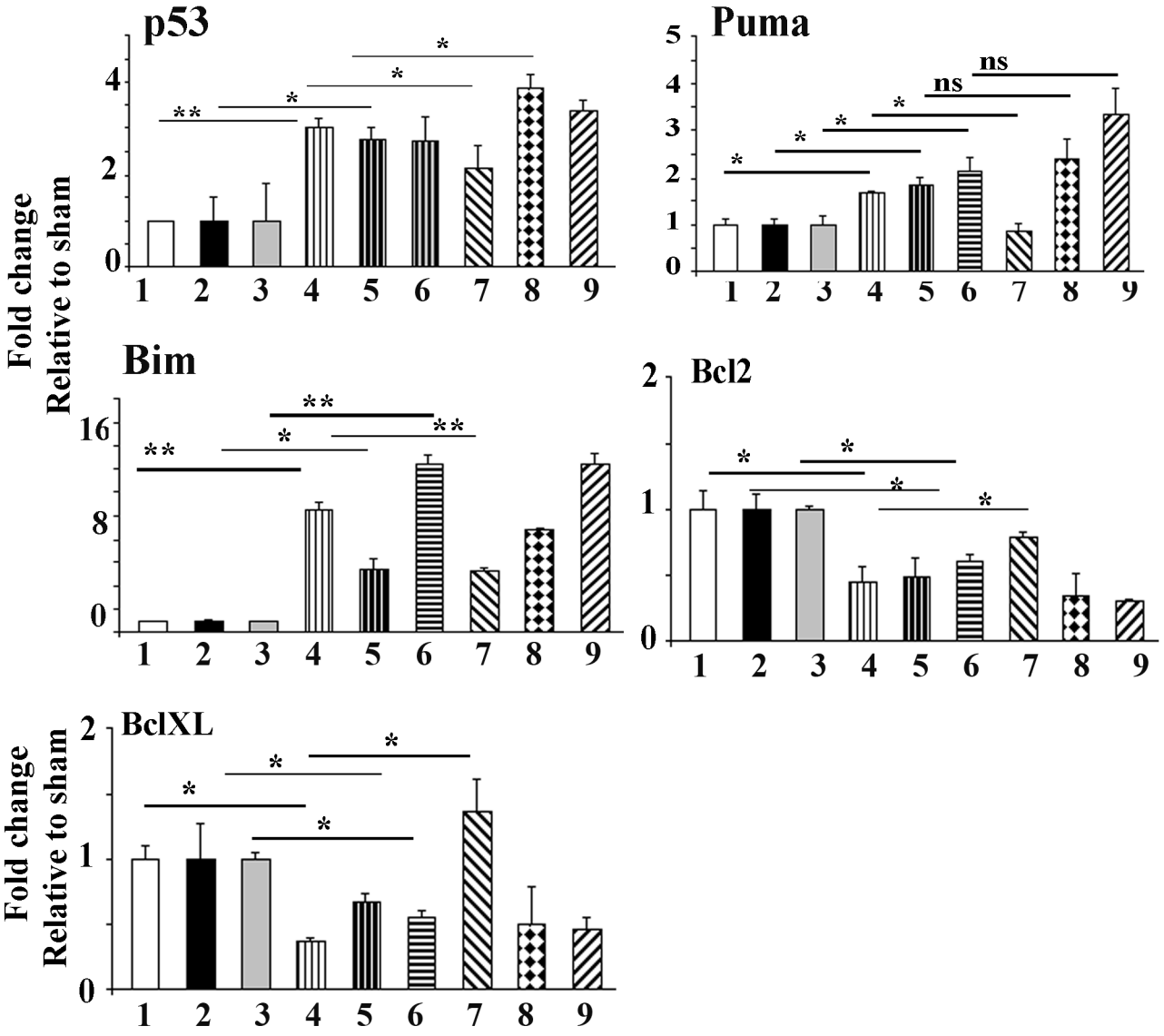
p53
Puma
Bim
Bcl2
BclXL
Fold change
Relative to sham
ns
1 2 3 4 5 6 7 8 9

Figure S3