Supplemental materials comprised of 8 figures, their legends, 2 tables, and additional experimental methods.

Itemized list of figures and their relevance to manuscript

Supplemental Figure 1: Supports statements in the text about the diverse functionality of HSF1-interacting proteins and how candidate genes for shRNA knockdown were selected.

Supplemental Figure 2: Positive control experiment for HSF1 nuclear translocation following heat shock and quantitation of immunoblot in Figure 2B.

Supplemental Figure 3: In support of Figure 4, panel A presents total survival as impacted by deaths from both tumor-related and non-tumor related causes for control HSF1 mice (*NPcis* +/+, *Hsf1+/+; NPcis* +/+, *Hsf1*-/-). Panel B presents the effects of *Hsf1* compromise on the tumor spectrum of *NPcis* mice. Panel C presents a positive correlation between glioma incidence and survival time.

Supplemental Figure 4: Histological evidence that diverse tumor types arise in *NPcis* mice as claimed in the text.

Supplemental Figure 5: Supports the assertion that *Hsf1* compromise prolongs the tumor-free survival of *NPcis* mice. Panel A and B present insignificant effect of the genetic modifier *Nstr2* on the survival of *NPcis* mice. Panel C-F present insignificant effect of sex on the survival of *NPcis* mice.

Supplemental Figure 6: Supports the assertions that inhibiting HSP90 results in KSR1 destabilization in Figure 7 and that HSF1 overexpression increases the levels of KSR1 and ERK phosphorylation in Figure 8.

Supplemental Figure 7: Immunohistochemical data supporting a key conclusion in text that HSF1 is preferentially over-expressed by the malignant elements within an MPNST.

Supplemental Figure 8: Immunohistochemical evidence supporting the claim that ERK is activated to a much greater extent in MPNST cells than in normal nerve or benign neurofibroma.

Supplemental Table 1: Summary of all the *NPcis* mice included in this study.

Supplemental Table 2: Summary of D15mit111 and D19mit59 genotyping results.

Supplemental methods:

Cells, tissues and reagents

Human MPNST cell lines sNF96.2 (CRL-2884), sNF94.3 (CRL-2886) and sNF02.2 (CRL-2885) were purchased from the American Type Culture Collection (ATCC). S462 (33) and 90-8TL (34) cells were generously provided by Karen Cichowski (Brigham and Women's Hospital, Boston, Massachusetts, USA). Primary MEF cultures were established from day E13 transgenic embryos by enzymatic digestion and mechanical dissociation. All cell cultures were maintained under 5% CO2 in DMEM (ATCC) supplemented with 10% fetal bovine serum (Sigma).

Immunoblotting and immunostaining for HSF1 were performed with a cocktail of 3 rat monoclonal antibodies (Ab4, Thermo-Fisher Scientific) while phosphoserine 326 HSF1 was detected by rabbit monoclonal antibody (Epitomics). Antibodies for total ERK, phospho-ERK, cleaved PARP, cleaved Caspase 7, tubulin, and lamin A/C were from Cell Signaling Technologies. KSR1 rabbit monoclonal antibody (EPR2421Y) was from Millipore. Neurofibromin antibody was from Santa Cruz Biotechnologies. HSP90 and HSP72 antibody were from Assay Designs (SPA-810) and GAPDH from Chemicon (MAB374). Monoclonal antibody-HRP conjugate recognizing polyubiquitinylated proteins was from Enzo Life Sciences (FK2H).

U0126, Radicicol and MG132 were purchased from Sigma. Withaferin A was purchased from Chromadex. CI-1040 was purchased from Selleck Chem. Cytoplasmic and nuclear fractions were prepared using the NE-PER Nuclear protein Extraction Kit from Pierce according to manufacturer' instructions.

Heat-shock reporter cells

Reporter cells were generated by infecting NIH-3T3 cells with a lentiviral vector encoding a fusion protein consisting of enhanced GFP fused to firefly luciferase under control of *HSP70B'* promoter

elements (14). The plasmid encoding the fusion protein was generously provided by Khalid Shah (Massachusetts General Hospital, Boston, Massachusetts, USA). The recombinant reporter was packaged by co-transfection of 293T cells with pCMV-VSVG and pCMV-deltaVPR. To isolate a homogenous population of high responding cells, a transduced culture was heat shocked at 42°C for 1hr, then processed 8hrs later by fluorescence activated cell sorting (FACS). Prior to use for shRNA screening, cells were reverse selected by FACS to eliminate a minority population of cells constitutively expressing the reporter in the absence of induction.

High throughput production of lentiviral shRNA supernatants

Transfection-quality DNA encoding hairpin constructs in the pLKO.1 lentiviral backbone was prepped using 96-well PureLink kits (Invitrogen). Average yields of 4 mg DNA/well, were quantified using a Pico-Green assay (Molecular Probes), and normalized robotically in each plate. Lentiviral supernatants were made in 96-well format by transfecting packaging cells (293T) with a three-plasmid system (http://www.broad.mit.edu/genome_bio/trc/rnai.html).

Lentiviral infection of reporter cells

Screening was performed in Optimem medium (Invitrogen) supplemented with 2.5% FBS. Reporter cells were seeded at a density of 2,000 cells/well into 384-well assay plates and allowed to adhere for 24 hr prior to infection in the presence of polybrene (8 μg/ml) via robotic transfer of shRNA lentiviral supernatant (4 μl/well) from 96-well viral production plates. The following day, medium was replaced with fresh Optimem. All infections were performed in duplicate with an additional replicate performed to confirm infection efficiency by adding selection antibiotic during the medium change post infection (puromycin, 2 μg/ml). Five days post-infection relative viable cell number per well was monitored using resazurin prior to measurement of reporter induction using One-Glo luciferase assay reagent (Promega, 10 μl/well).

Primer sequences for qRT-PCR:

Primers	Sequence 5' to 3'
Ms_HSPA1A-F	ATGGACAAGGCGCAGATCC
Ms_HSPA1A-R	CTCCGACTTGTCCCCCAT
Ms_HSP10-F	AGTTTCTTCCGCTCTTTGACAG
Ms_HSP10-R	TGCCACCTTTGGTTACAGTTTC
Ms_Dnajb1-F	TTCGACCGCTATGGAGAGGAA
Ms_Dnajb1-R	CACCGAAGAACTCAGCAAACA
Ms_Dnajb2-F	ACCACACGCAGAATCATGGAG
Ms_Dnajb2-R	CTAGTGCCAGGTCATCTGGGA
Ms_Hspb8-F	TCCCGTGCTCCTACCCAAG
Ms_Hspb8-R	GCTGTCAAGTCGTCTGGAAAAG
Ms_HspA4L-F	TTCCTCAACTGCTACATCGCT
Ms_HspA4L-R	AATTGCCCAGTTAATGTCCTTGA
Ms_Hsp90AA1-F	AATTGCCCAGTTAATGTCCTTGA
Ms_Hsp90AA1-F	CGTCCGATGAATTGGAGATGAG

Primer sequences for SSLP genotyping:

Primers	Sequence 5' to 3'
D15mit111-F	GTTTCAGAAGGCAATGTCTGG
D15mit111-R	GCTCAGTGCTAATCTCTGACTCC
D19mit59-F	CTCTAACTATCCTCTGACCTTCACA
D19mit59-R	TTTTAAGCAGAACATTGAGGACC

Lentiviral shRNA knockdown

Lentiviral shRNA plasmids targeting mouse *Nf1* (NM_010897) and human *Hsf1* (NM_005526) were obtained from the Broad Institute RNAi platform and are deposited in the Open Biosystems collection under the following TRCID numbers (bold font indicates text references used for each plasmid): Nf1:TRCN0000034339, and TRCN0000034342; HSF1:TRCN0000007480 (**hA6**), TRCN0000007483 (**hA9**). Control hairpins targeting **GFP** (5'-GCAAGCTGACCCTGAAGTTCA-3') and a scrambled

sequence with no known homology to any human gene (**Scram**; 5'-CCTAAGGTTAAGTCGCCCTCG-3') have been described previously (5).

HSF1 trimer cross-linking:

Equal amounts of extracted nuclear proteins were incubated with 1mM EGS (ethylene glycol bis[succinimidylsuccinate] (Thermo-Fisher Scientific) at RT for 30 min and the reactions were quenched by 50mM glycine for 15 min. The cross-linked proteins were subjected to SDS-PAGE and immunoblotted for HSF1.

siRNA Knockdown

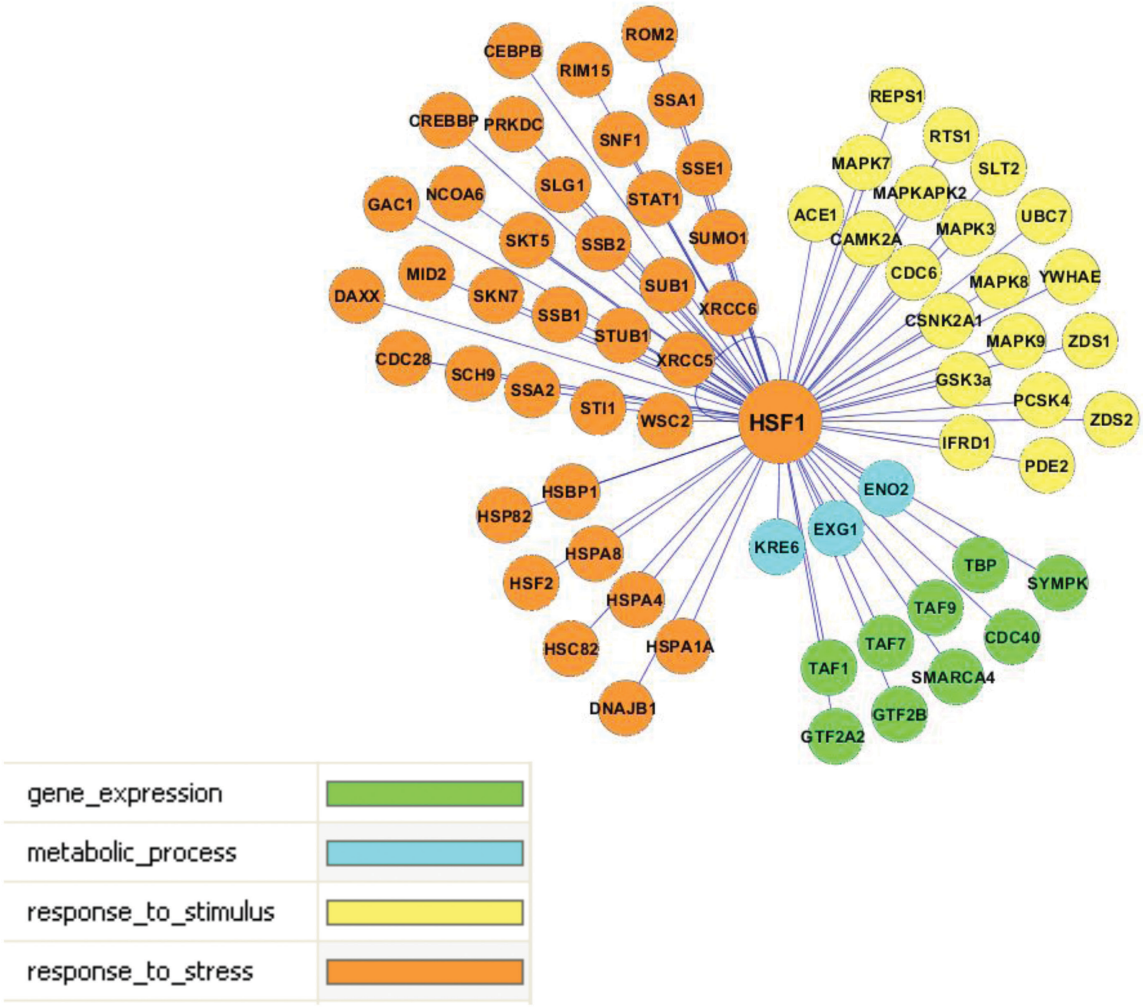
KSR1-targeting siRNAs and a non-targeting siRNA were ordered from Dharmacon. siRNAs were transiently transfected into cells at 25nM final concentration using Lipofectamine® RNAiMAX reagent following the instructions (Invitrogen).

Simple sequence length polymorphism (SSLP) genotyping

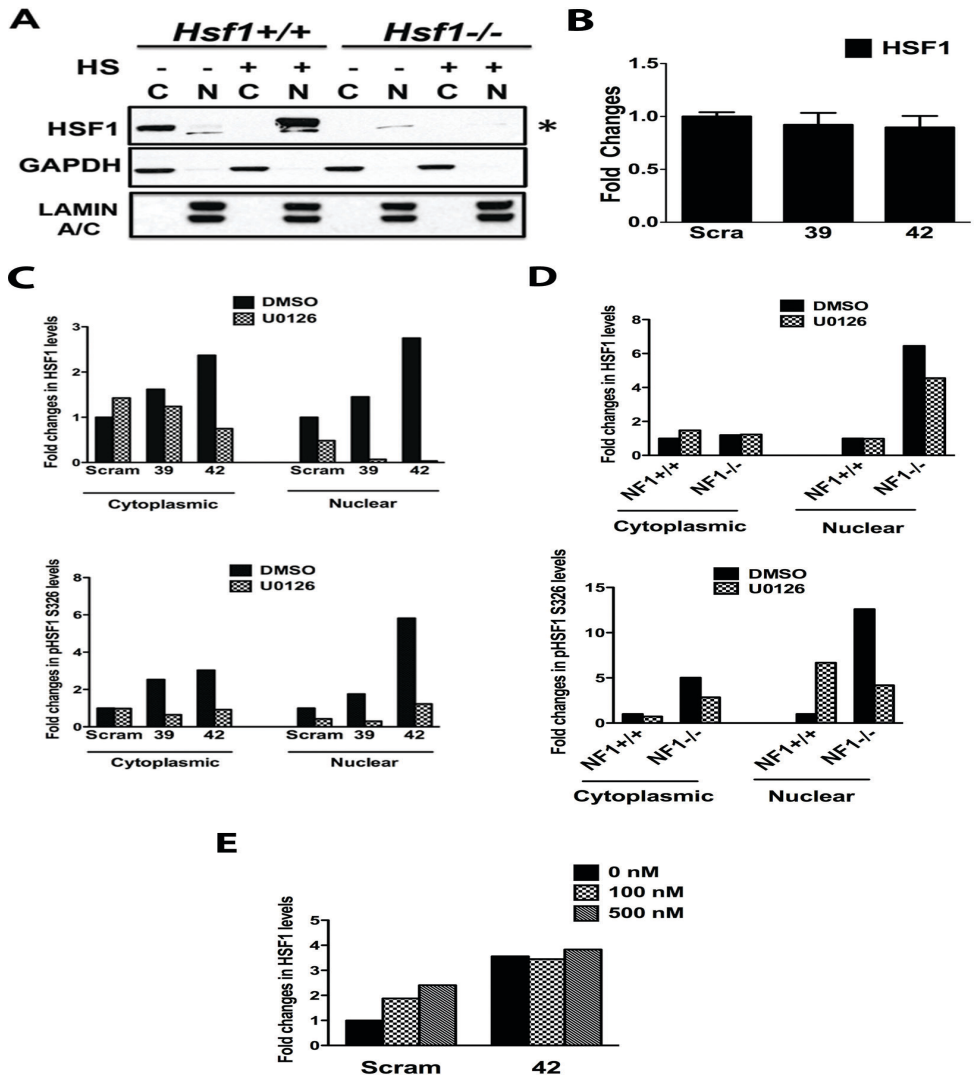
Genomic DNAs were extracted from frozen tumor tissues or mouse tails. Regular PCR was applied to genotype *Nstr1* (D19mit59 marker) and *Nstr2* (D15mit111 marker) loci. PCR products were separated on 3% agarose gels. Genomic DNAs derived from inbred 129/SvJ or Babl/cJ mice (The Jackson Laboratory DNA Resource) were used to serve as positive controls.

Image densitometry

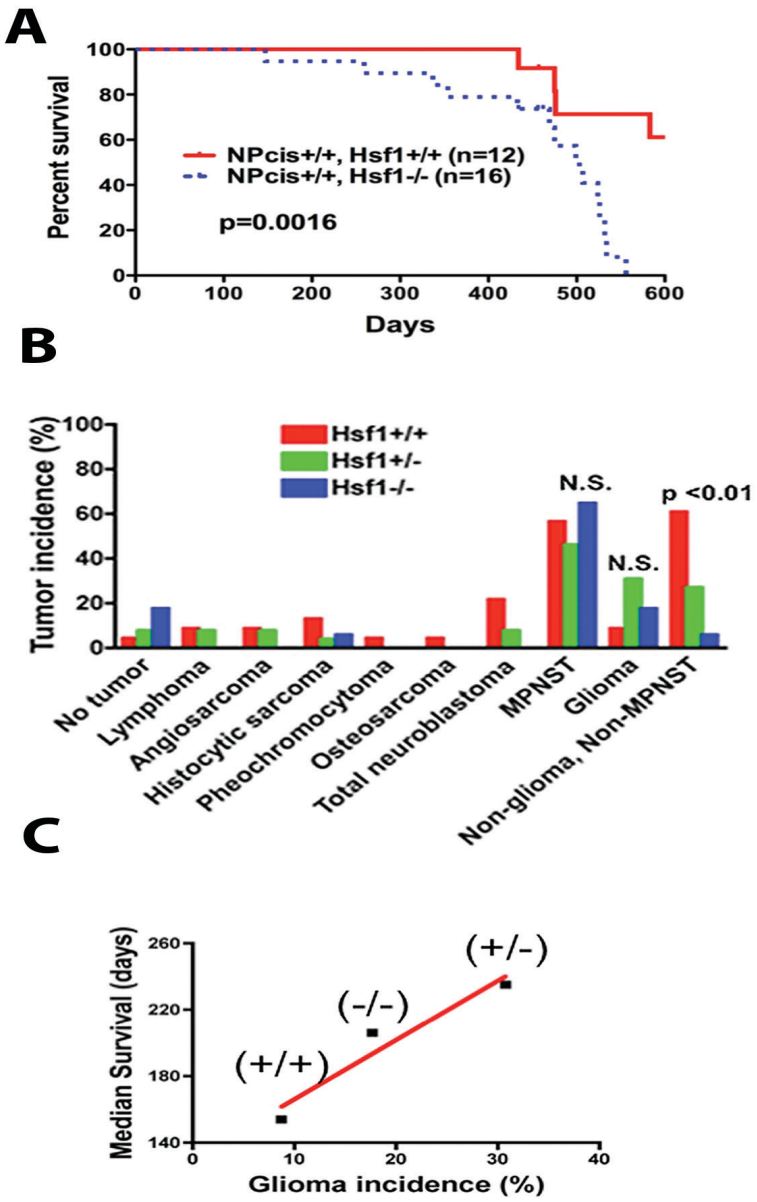
All digital western blotting images were quantitated by the free software package ImageJ according to the instructions.



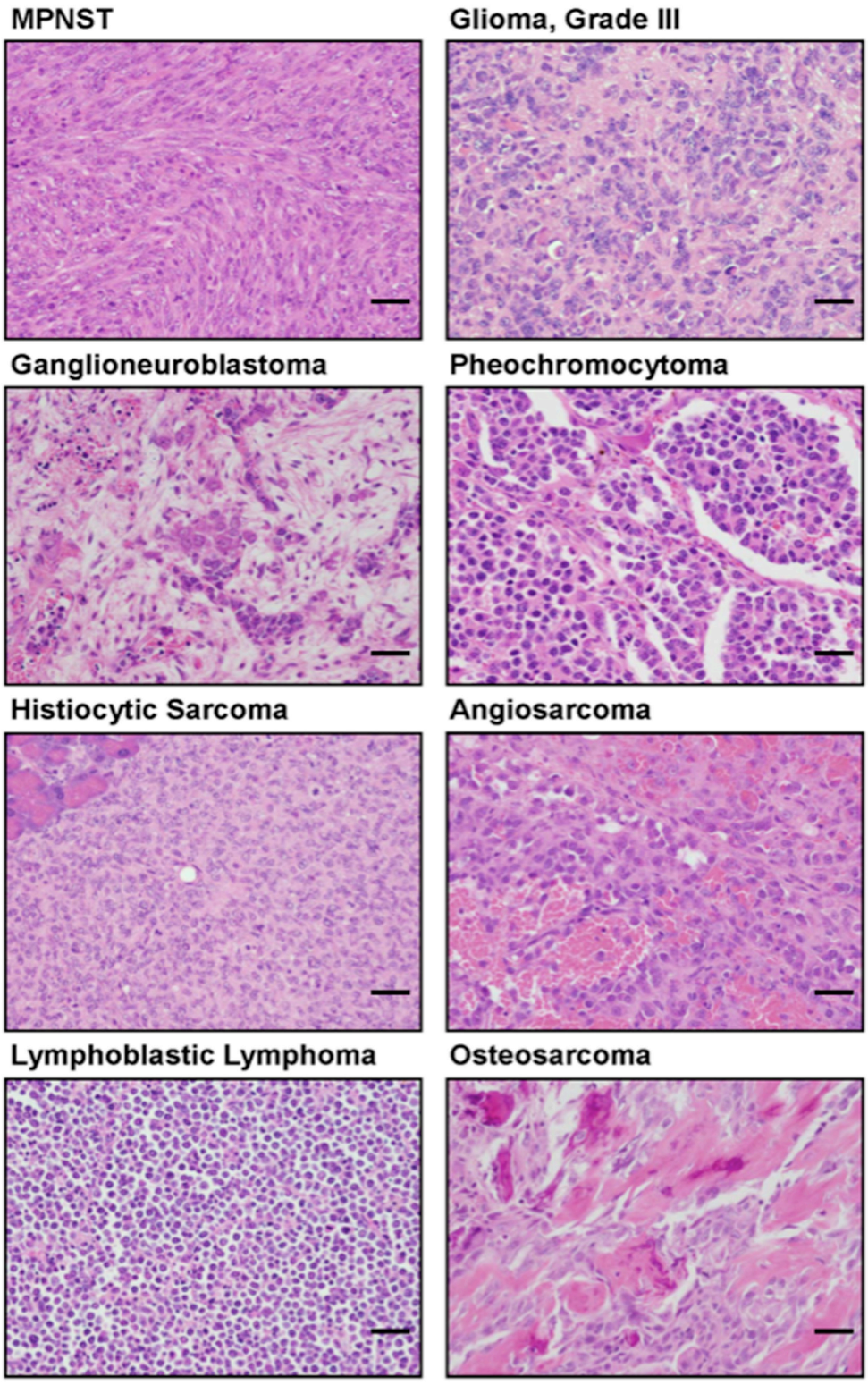
Supplemental Figure 1: Immediate interacting partners of HSF1 as mined from the iHOP interactome database.



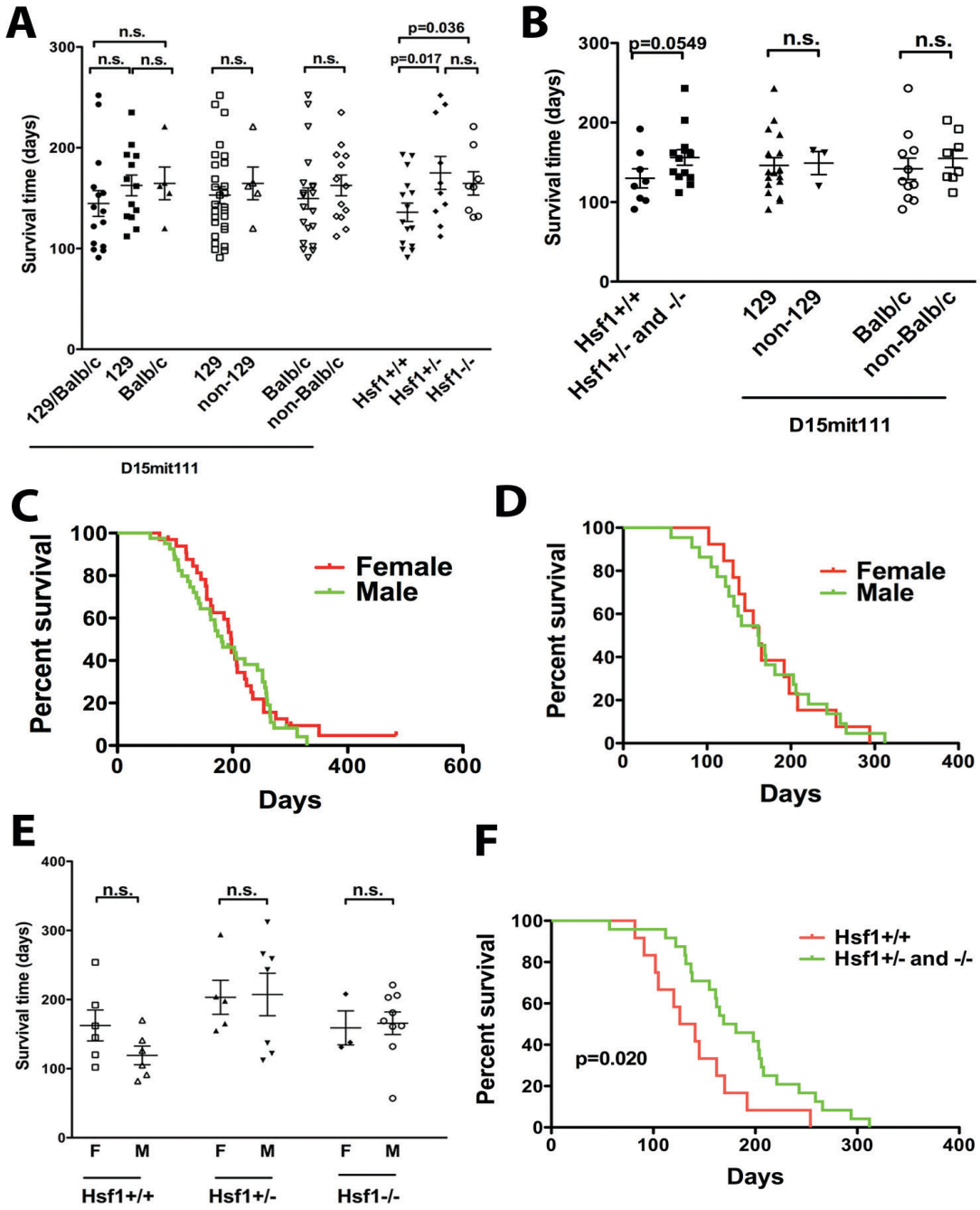
Supplemental Figure 2: (**A**) Immunoblot demonstrating marked HSF1 translocation from the cytoplasm to nucleus following classical heat-shock (43^{0}C for 30 min) of *Hsf1*+/+ wild-type MEF. *Hsf1*-/- MEFs were analyzed in parallel as a specificity control for HSF1 immunoblotting. The cytosolic protein GAPDH and nuclear lamins A/C were blotted as controls for the fractionation procedure. (**B**) HSF1 mRNA levels in *Nf1*-knockdown cells. (**C** and **D**) Densitometric quantitation of immunoblot (Figure 2B and 2D) by ImageJ software showing reduction of HSF1 nuclear localization and pHSF1 Ser326 following U0126 treatment. The densities of cytoplasmic and nuclear HSF1 and pHSF1 Ser326 were normalized against the densities of LDH and Lamin A/C respectively. The normalized protein levels of NF1-deficient cells (#39 and 42 or *Nf1-/-* cells) relative to those of the control cells (Scramble or *Nf1+/+* cells) were expressed as fold changes. (**E**) Densitometric quantitation of HSF1 levels in Figure 2H.



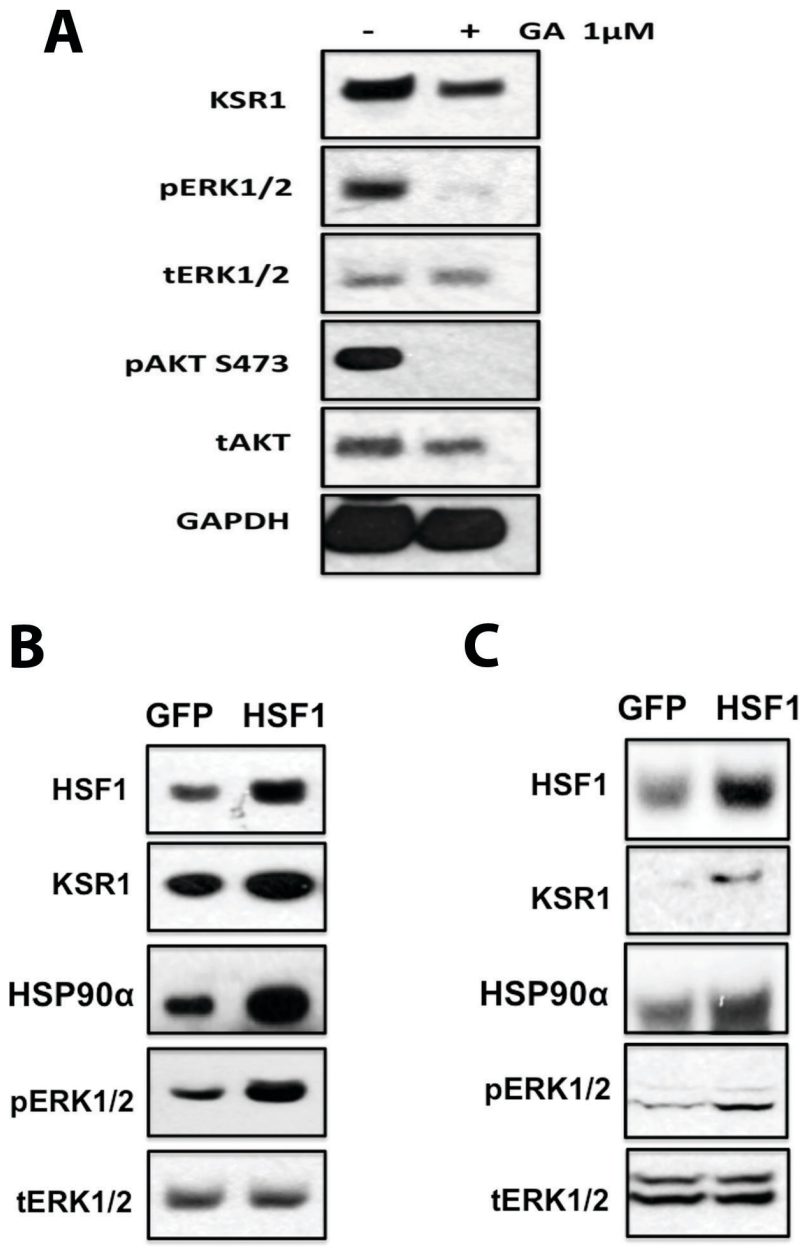
Supplemental Figure 3: (**A**) Kaplan-Meier analysis shows that *Hsf1*-/- mice have shorter overall survival than *Hsf1*+/+ mice in the absence of *Nf1* alterations. (**B**) The incidence of most spontaneously arising tumor types in *NPcis* mice is modified by *Hsf1*. Compared to *Hsf1* wild-type mice, the combined incidence of tumor histologies other than MPNST and glioma is significantly decreased in *Hsf1* null and hemizygous knockout mice (Chi-square test). (**C**) The occurrence of glioma positively correlates with survival, which is prolonged by *Hsf1*-deficiency (r^2=0.9283).



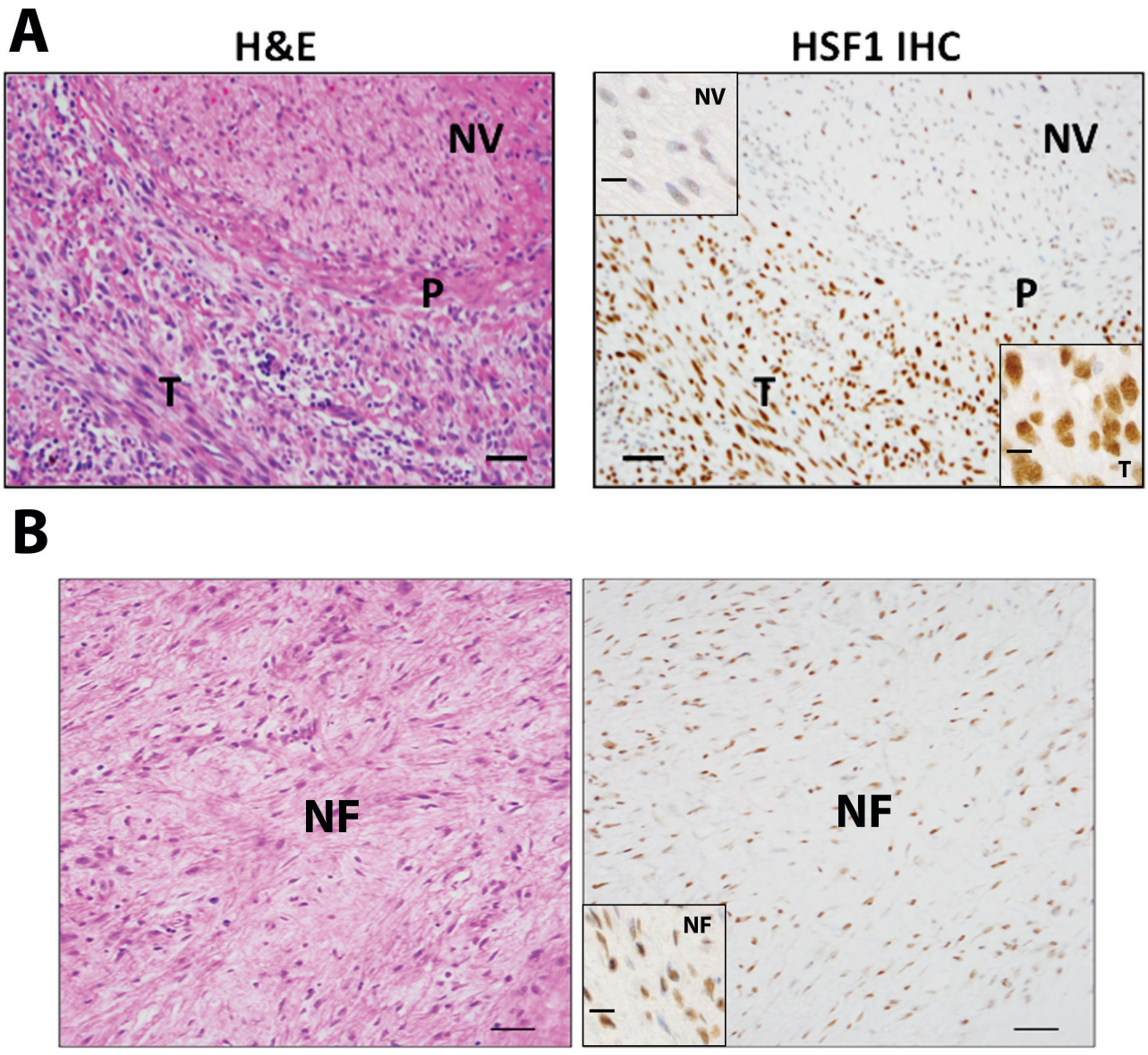
Supplemental Figure 4: *NPcis* mice develop a broad range of malignant tumors. Representative photomicrographs of H&E-stained sections demonstrate characteristic histologies for the indicated tumor types. All images were acquired at the same magnification. Scale bar, 50 μm.



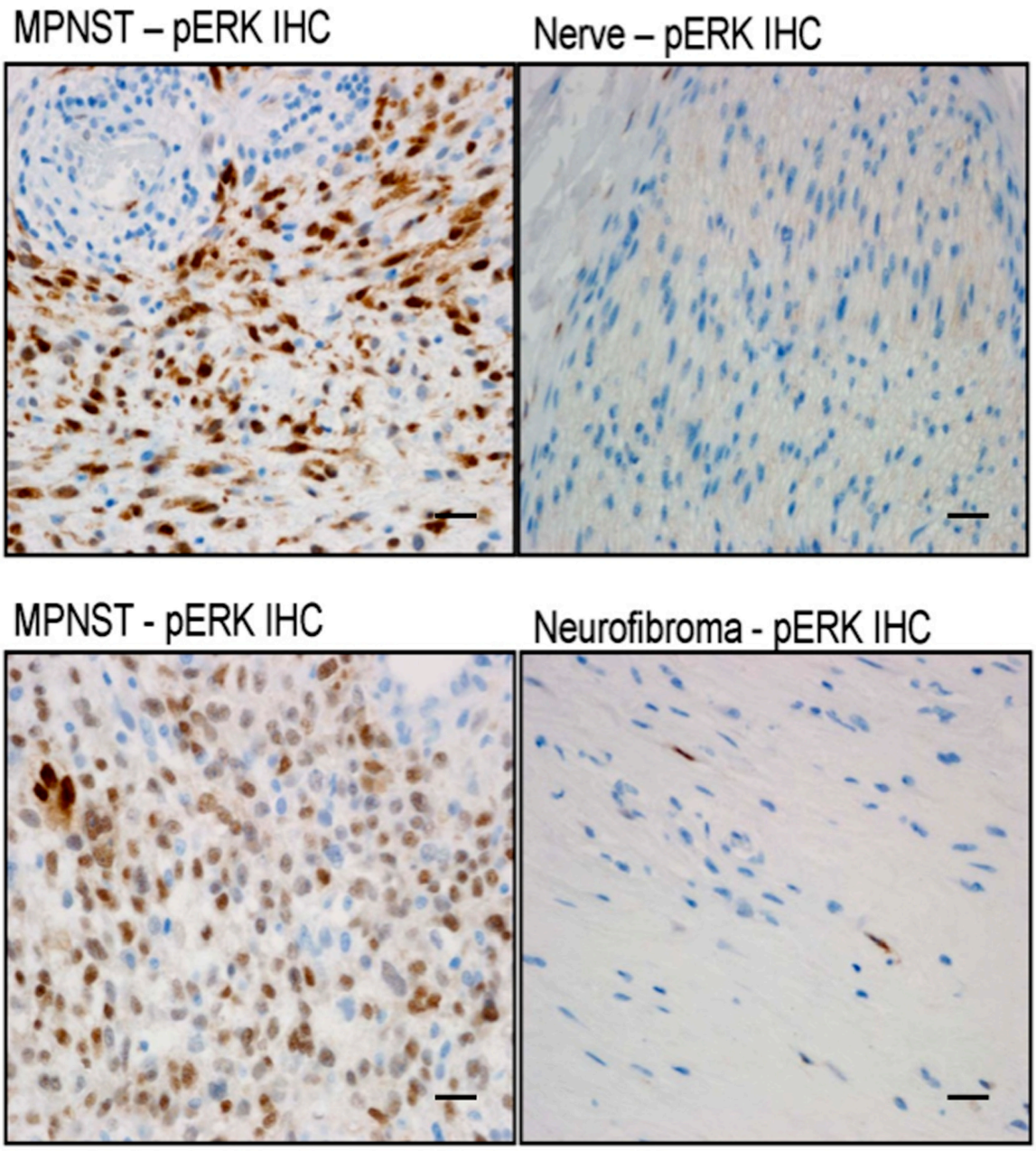
Supplemental Figure 5: (**A**) The previously mapped genetic modifier on chromosome 15, *Nstr2*, did not significantly affect the survival of *NPcis* mice. Total 33 tumors harvested from all 3 *Hsf1* experimental groups were genotyped using the simple-sequence length polymorphism (SSLP) marker D15mit111 to distinguish between the 129/SvJ and Balb/cJ genetic backgrounds. These mice are divided into groups based on D15mit111 genotypes and their survival times are compared (Student's t-test, one-tailed). (**B**) Mice that developed MPNSTs are divided into groups based on D15mit111 genotypes and their survival times are compared (Student's t-test, one-tailed). (**C**) Sex did not significantly affect the survival of all *NPcis* mice. (**D**) Sex did not significantly affect the survival of *NPcis* mice that developed MPNSTs. (**E**) Sex also did not significantly affect the survival time of mice developing MPNSTs within the same *Hsf1* genotypic group (Student's t-test, two-tailed). (**F**) In contrast, *Hsf1*-deficient mice that developed MPNSTs had a longer survival than *Hsf1* wild-type mice that developed MPNSTs (p=0.025, Log-rank test).



Supplemental Figure 6: **(A)** Decreased levels of KSR1, phospho-ERK1/2, and AKT following HSP90 inhibition. 90-8TL cells were treated overnight with 1μM geldanamycin and harvested for immunoblotting. (**B** and **C**) HSF1 overexpression increases the levels of HSP90α, KSR1 and phospho-ERK1/2 in HEK293 cells (B) and in immortalized MEFs (C). HSF1 overexpression was achieved by either transient transfection in HEK293 cells or stable retroviral infection in MEFs.



Supplemental Figure 7: HSF1 is highly overexpressed in human MPNST cells from surgical resection specimens. (**A**) H&E stain and HSF1 immunostain of a MPNST tumor section at the same magnification. Scale bar, 50 μm. The cells within the endoneurium of the nerve (NV) and the perineurium (P) show low level expression of HSF1 compared with the malignant cells. Scale bar for the insets, 10 μm. (**B**) H&E stain and HSF1 immunostain of a neurofibroma section at the same magnification. Scale bar, 25 μm. Many cells within benign neurofibroma (NF) show intermediate level expression of HSF1. Scale bar for the inset, 10 μm.



Supplemental Figure 8: Phospho-ERK (pERK) is markedly increased in human MPNSTs compared to normal nerve and benign neurofibroma. Left panels: Representative photomicrographs of tissue sections from two human MPNSTs stained by IHC for pERK (brown signal). Right panels: Sections of nerve and neurofibroma stained under identical conditions show much less pERK immunoreactivity. All sections were counterstained with Mayer hematoxylin (blue) and imaged at identical magnification. Scale bar, 25 μm.

Supplemental Table 1: Summary of all NPcis mice.

ID#	Hsf1	sex	survival days	Tumor types
195	+/+	M	174	Histiocytic sarcoma
197	+/+	F	119	ganglioneuroblastoma
200	+/+	F	197	Autolysis
287	+/+	M	258	Angiosarcoma
288	+/+	M	256	Osteosarcoma,Histiocytic sarcoma,glioma,Angiosarcoma
289	+/+	M	183	ganglioneuroblastoma , Glioma
292	+/+	M	98	Neuroblastoma
297	+/+	F	153	Pheochromocytoma, Rhabdomyosarcoma
300	+/+	M	91	MPNST
302	+/+	M	105	MPNST
307	+/+	F	102	MPNST
308	+/+	F	145	MPNST
311	+/+	F	193	Lymphoblastic lymphoma
330	+/+	M	126	MPNST
373	+/+	M	141	MPNST
378	+/+	M	82	MPNST
382	+/+	F	192	MPNST
388	+/+	M	192	No tumor
408	+/+	F	155	Neuroblastoma
437	+/+	M	170	MPNST
489	+/+	F	120	MPNST, lung adenoma
491	+/+	F	254	MPNST, Lymphoblastic lymphoma
619	+/+	F	162	MPNST
716	+/+	M	99	Composite tumor: Ganglioneuroblastoma/MPNST
124	+/-	F	204	MPNST
126	+/-	M	106	Lymphoma and Angiosarcoma
127	+/-	M	265	Neurofibroma
128	+/-	F	235	Histiocytic Sarcoma, Glioma
130	+/-	F	350	Glioma
131	+/-	F	165	MPNST
132	+/-	M	252	Lymphoblastic Lymphoma
139	+/-	F	185	ganglioneuroblastoma
146	+/-	F	88	No Tumor
148	+/-	M	144	Composite tumor: Ganglioneuroblastoma/MPNST
150	+/-	F	73	Autolysis
151	+/-	F	224	Glioma, lung adenoma
152	+/-	F	155	MPNST
156	+/-	M	272	Autolysis
157	+/-	M	122	MPNST
163	+/-	M	101	No tumor
186	+/-	M	265	GLIOMA
187	+/-	M	243	MPNST, Glioma
189	+/-	M	252	Glioma
198	+/-	F	294	MPNST, Glioma
202	+/-	F	275	Autolysis
203	+/-	M	329	Autolysis
290	+/-	M	112	MPNST
389	+/-	M	137	MPNST
399	+/-	M	266	MPNST
400	+/-	M	312	MPNST
698	+/-	M	260	Glioma
717	+/-	M	259	MPNST
738	+/-	F	198	MPNST
742	+/-	F	198	ANGIOSARCOMA
134	-/-	M	169	MPNST
135	-/-	M	206	MPNST
159	-/-	F	301	No tumor
188	-/-	M	221	MPNST
196	-/-	M	162	MPNST
296	-/-	M	181	MPNST
316	-/-	F	254	Autolysis
371	-/-	F	207	Autolysis
374	-/-	M	161	MPNST
404	-/-	F	221	Histiocytic sarcoma, Glioma
407	-/-	F	131	MPNST
439	-/-	M	57	MPNST
506	-/-	M	293	No tumor
631	-/-	M	203	MPNST
702	-/-	F	138	MPNST
704	-/-	F	208	MPNST, Glioma
706	-/-	M	132	MPNST
710	-/-	F	484	No tumor
711	-/-	F	232	Glioma

Supplemental Table 2: Genotypes of tumors for SSLP markers.

ID#	Hsf1 genotype	D15mit111		D19mit59		Survival days	MPNST
		129/SvJ	Balb/cJ	129/SvJ	Balb/cJ		
197	+/+	+	-	+	-	119	
289	+/+	+	-	+	-	183	
292	+/+	+	+	+	-	98	
297	+/+	+	+	+	-	153	
300	+/+	+	+	+	-	91	Y
302	+/+	+	+	+	-	105	Y
307	+/+	+	+	+	-	102	Y
311	+/+	+	-	+	-	193	
330	+/+	+	+	+	-	126	Y
373	+/+	+	+	+	-	141	Y
382	+/+	+	-	+	-	192	Y
408	+/+	-	+	+	-	155	
489	+/+	-	+	+	-	120	Y
619	+/+	-	+	+	-	162	Y
716	+/+	+	+	+	-	99	
128	+/-	+	-	+	-	235	
131	+/-	-	+	+	-	165	Y
132	+/-	+	+	+	-	252	
139	+/-	+	+	+	-	185	
148	+/-	+	-	+	-	144	
152	+/-	+	+	+	-	185	Y
157	+/-	+	+	+	-	122	Y
187	+/-	+	+	+	-	243	Y
290	+/-	+	-	+	-	112	Y
389	+/-	+	+	+	-	137	Y
134	-/-	+	-	+	-	169	Y
196	-/-	+	-	+	-	162	Y
374	-/-	+	+	+	-	161	Y
404	-/-	-	+	+	-	221	
407	-/-	+	-	+	-	131	Y
631	-/-	+	-	+	-	203	Y
702	-/-	+	-	+	-	138	Y
706	-/-	+	-	+	-	132	Y