EXPERIMENTAL PROCEDURES:

H-Ras **Sequencing**

Genomic DNAs were extracted from mouse skin tumors using a DNeasy® Tissue Kit (Qiagen). The sequences of *H-Ras* exon1 and 2 were amplified by PCR and the PCR products were purified and sequenced. The primers used for amplification are: exon1-Back: 5'-ctaagtgtgcttctcattggcaggt-3'; exon1-Forward: 5'-cctctggcaggtaggcagagctcac-3'; exon2-Back: 5'-ccactaagcctgttgtgttttgcag-3'; exon2-Forward: 5'-ctgtactgatggatgtcctcgaagga-3'.

Cell Culture

Immortalized *Hsf1*+/+ and -/- mouse embryonic fibroblasts (MEFs) were gifts from Dr. Ivor J. Benjamin. EcoPack2-293 cells were purchased from Invitrogen. All cell lines except the MCF-10A, PHME, HME and HMLER were maintained in DMEM supplemented with 10% FBS, 4mM L-glutamine, and streptomycin/penicillin. In amino acid-deprivation experiments, cells were cultured in DMEM (without L-glutamine and L-leucine, MP Biomedicals) supplemented with 10% FBS and either 4mM L-glutamine or 105mg/L L-leucine. The PHME (purchased from Cambrex), HME and HMLER cells (a gift from Tan Ince, Brigham and Women's Hospital, Boston) were cultured in MEGM (Cambrex). In glucose-deprivation experiments, cells were cultured in DMEM (without D-glucose, Invitrogen) supplemented with 10% FBS and D-glucose.

Immunoblotting

Whole-cell protein extracts were prepared by using cold lysis buffer consisting of: 100 mM NaCl, 30 mM Tris-HCl (pH 7.6), 1% NP-40, 30 mM sodium fluoride, 1 mM EDTA, 1 mM sodium vanadate, and Complete protease inhibitor cocktail tablet (Roche Diagnostics). Samples were incubated on ice for 30 min and supernatants were recovered by centrifuging at 14,000 rpm at 4°C for 10 min. Protein concentrations were determined by BCA method (Pierce Biochemical). Proteins were separated on NuPAGE® Novex gels and transferred to PROTRAN® nitrocellulose membrane (Whatman). Membranes were blocked with 5% non-fat dry milk in PBS (pH 7.4) and washed with PBS (pH 7.4)

containing 0.1% Tween-20. Membranes were probed with the following antibodies: HSF1 Ab4 (RT-629-P, NeoMarkers), HSF1 (4356, Cell Signaling Technology), KSR1 (ab11719-50, Abcam), RPL28 (SC-14151, Santa Cruz Biotechnology), RPL26 (A300-685A, Bethyl Laboratories), RPS6 (2217, Cell Signaling Technology), β-Actin (4970, Cell Signaling Technology), Phospho-p70 S6 Kinase (Thr389) (9234, Cell Signaling Technology), Phospho-(Ser/Thr) PKA Substrate (9621,Cell Signaling Technology), Phospho-S6 Ribosomal Protein (Ser235/236) (2211, Cell Signaling Technology), HSP72 (SPA-810, Stressgen), and GAPDH (MAB374, Chemicon).

Viral Production and Transduction

Genes encoding GFP, *H-Ras^{V12D}, PDGF-B*, myristoylated AKT1, *v-SRC*, *c-MYC*, and *LTA* were cloned into the retroviral vector pBABE-zeo. Stable virus packaging cell lines were prepared by transfecting individual plasmids into EcoPack2-293 cells using Lipofectamine (Invitrogen) followed by zeocin selection. Lentiviral pLKO.1-puro shRNA constructs were obtained from the RNAi Consortium. Lentiviruses were produced by transiently co-transfecting individual shRNAs with plasmids encoding ΔVPR and VSV-G together into 293T cells using Fugene 6 (Roche). Viral supernatants were collected and passed through 0.45μm syringe filters. Viral transduction was achieved by incubating target cells with viral supernatants containing 5μg/L polybrene (Sigma) overnight.

Lentiviral shRNA Sequences

The GFP targeting sequence is: 5'-GCAAGCTGACCCTGAAGTTCA-3'; the scramble sequence is: 5'-CCTAAGGTTAAGTCGCCCTCG-3'; the C2 targeting sequence is: 5'-GCTGCATACCTGCTGCCTTTA-3'; the C3 targeting sequence is: 5'-CCCTGAAGAGTGAGGACATAA-3'; the hA6 targeting sequence is: 5'-GCAGGTTGTTCATAGTCAGAA-3'; the hA8 targeting sequence is: 5'-CCAGCAACAGAAAGTCGTCAA-3'; and the hA9 targeting sequence is: 5'-GCCCAAGTACTTCAAGCACAA-3'.

Cell Cycle Analysis

5×10^4 cells were plated and treated with either methanol vehicle or 100nM rapamycin for 24 hours. After treatment, cells were detached and fixed with cold 70% ethanol overnight. After washing with PBS, cells were stained with Guava Cell Cycle reagent (Guava Technologies) for 30 min. The cell cycle distribution was determined by flow cytometry (Guava EasyCyte System, Guava Technologies) and further analyzed by the ModFit LT software (Verity Software House).

Measurement of Cell Size

Cells were harvested by trypsinization and diluted 1:40 with counting solution (Coulter Corp.). Cell diameters and volumes were measured using a particle size counter (Coulter Multisizer II).

Lactate and LDH Assay

Supernatant lactate levels were measured using the Lactate Assay Kit (BRSC, University at Buffalo) following the manufacture's instructions. For measuring enzymatic activity per cell, equal numbers of cells were pelleted, washed and extracted for protein to measure LDH activity using a commercially available kit (CytoTox 96 Non-radiooactive Cytotoxicity Assay, Promega).
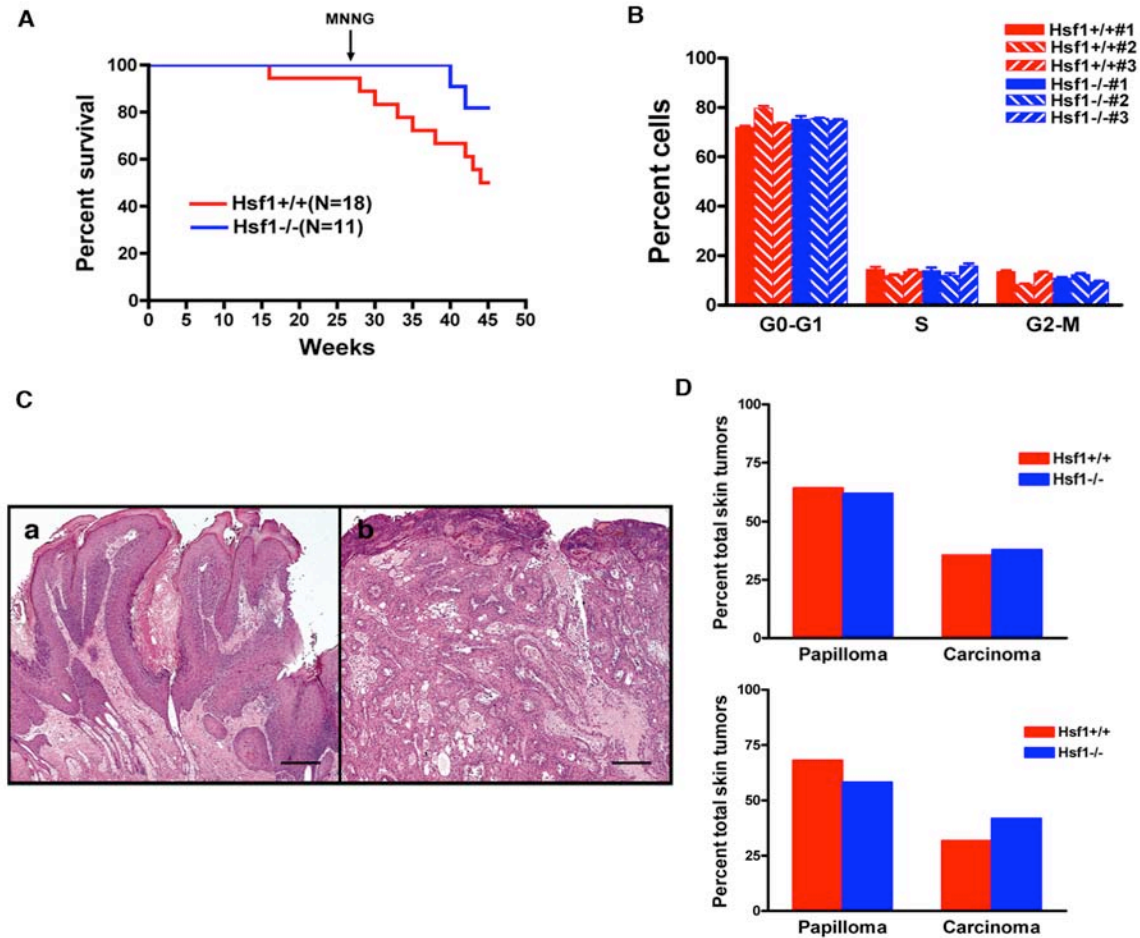
FIGURES:

Figure S1: **Mouse skin carcinogenesis. A:** The survival of *Hsf1*+/+ and -/- mice following skin carcinogenesis with secondary application of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) at week 27 after initial application of DMBA (median survival: *Hsf1*+/+ 45 weeks; *Hsf1*-/- undefined. P=0.0896, Logrank test). **B:** Primary Hsf1+/+ and -/- MEF share similar cell cycle distribution. Primary MEF were derived from embryos at day E13.5. Three independent lines for each genotype were used for cell cycle analysis, and triplicates of each line were analyzed (Mean±SD, N=3, p>0.05, two-way ANOVA). **C:** *Hsf1*+/+ and -/- mice developed both papillomas and squamous cell carcinomas. (a): a representative micrograph of papilloma. (b): a representative micrograph of squamous cell carcinoma. Scale bar=160μm. **D:** *Hsf1*+/+ and -/- mice share a similar incidence of papilloma and carcinoma. Skin tumors were classified by pathological examination of stained sections, and the incidence of benign versus malignant histology was calculated as a percentage of the total number of tumors

examined. The upper panel depicts the first experiment without secondary application of MNNG, the lower panel represents a second experiment that included MNNG application.
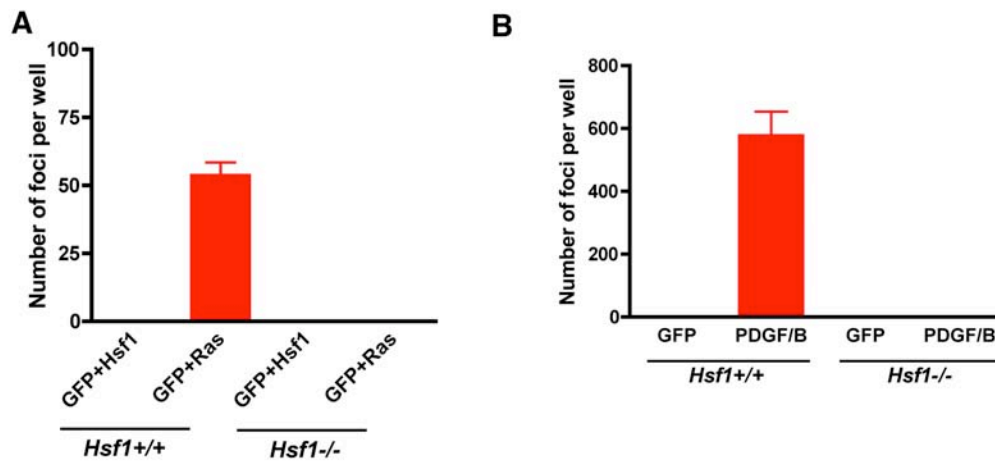
Figure S2: Germline *Hsf1* knockout prevents cellular transformation initiated by oncogenic *RAS* and *PDGF-B*. A: Quantification of foci driven by oncogenic H-RAS^{V12D} in *Hsf1*+/+ and -/- MEFs. Each viral transduction was performed in duplicate and the number of foci per well was counted using CellProfiler^{TM} software (Mean±SD, N=2). **B:** Quantification of foci driven by proto-oncogene *PDGF-B* in *Hsf1*+/+ and -/- MEFs (Mean±SD, N=2).
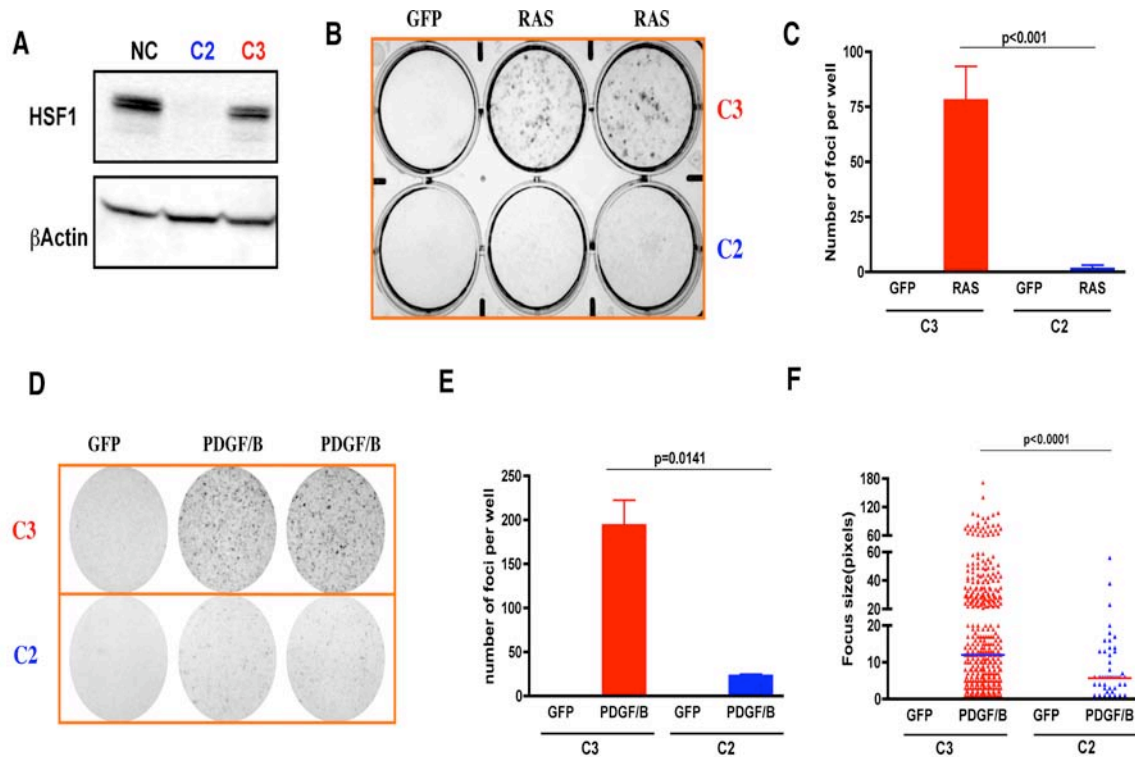
Figure S3: shRNAi-mediated *Hsf1* knockdown prevents cellular transformation initiated by oncogenic *RAS* and *PDGF-B*. A: Western blotting of HSF1 expression after lentiviral shRNAi transduction. NC: non-transduced control. C2, C3: two independent shRNAi constructs. **B:** C2-transduced MEFs are relatively resistant to focus formation driven by oncogenic *H-RAS*. Stable shRNA-transduced MEFs were plated and transduced with retroviruses encoding GFP or *H-RAS* ^{V12D}. **C:** Quantification of foci driven by oncogenic *H-RAS*^{V12D} in C2 and C3 MEFs (Mean±SD, N=4, p<0.001, Student's t test). **D:** C2-transduced MEFs are relatively resistant to focus formation driven by *PDGF-B*. **E:** The number of foci per well was quantified (Mean±SD, N=2, p=0.0141, Student's t test) and the size of foci was measured (Fig S2D). **F:** C2-transduced MEFs formed smaller size foci. The size of foci was measured using CellProfiler^{TM} software (The horizontal bars indicate geometric means. p<0.0001, Mann Whitney test).
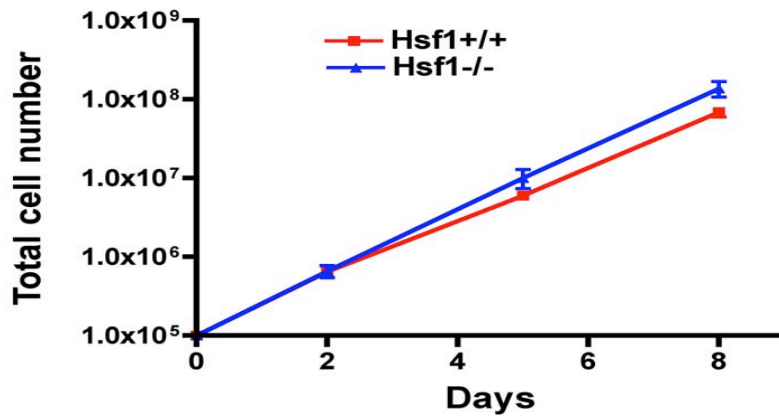
Figure S4: Immortalized *Hsf1+/+* and -/- MEFs share similar growth kinetics. 1×10^5 cells were plated each well in 6-well plates and cultured for 2 days. Cells were trypsinized and counted using a hematocytometer. 1×10^5 cells were re-plated and grown for 3 days, and the counting and re-plating were repeated. The total cell numbers were calculated and plotted (Mean±SD, N=6).
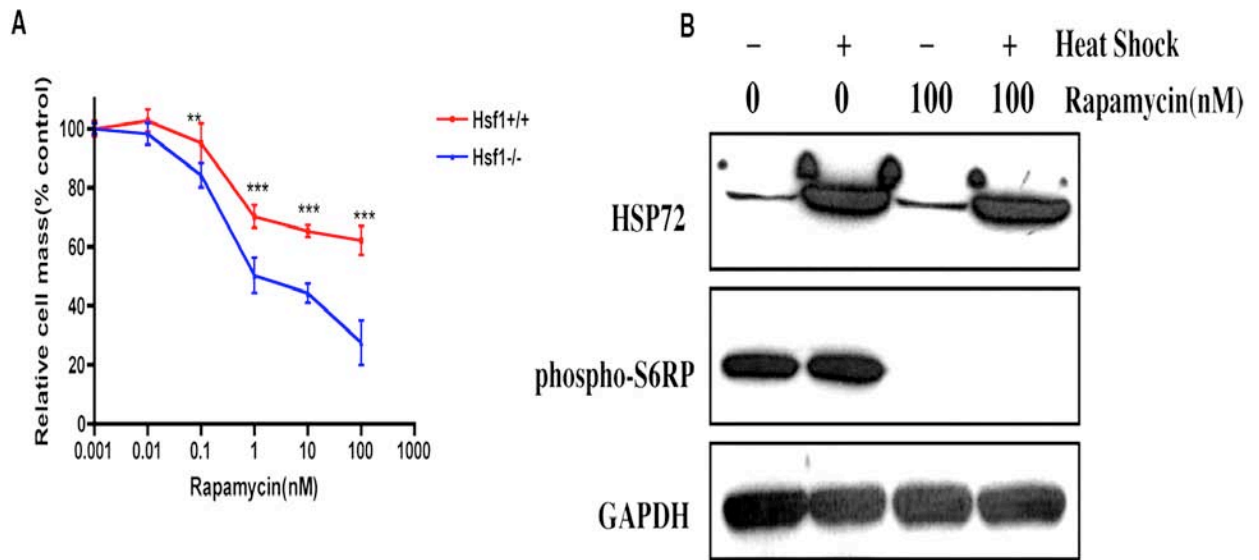
Figure S5: *Hsf1-/-* **MEFs are more sensitive to rapamycin. A:** Rapamycin causes a more dramatic decrease in total cell mass in *Hsf1-/-* cells. Cells were exposed to a series of rapamycin concentrations as indicated for 5 days. After fixation, the total cell mass in each well was determined by staining cellular proteins with sulforhodamine B. Data are presented as relative cell mass by normalizing the values of rapamycin-treated wells against those of solvent-treated wells (Mean±SD, N=5, ** $p<0.01$, *** $p<0.001$, two-way ANOVA). **B:** Rapamycin does not inhibit the heat shock response. *Hsf1+/+ MEF* were treated with methanol or 100nM rapamycin followed by heat stress at 43ºC for 30 min. The cells were recovered at 37ºC for 8 hours before harvesting for western blotting.
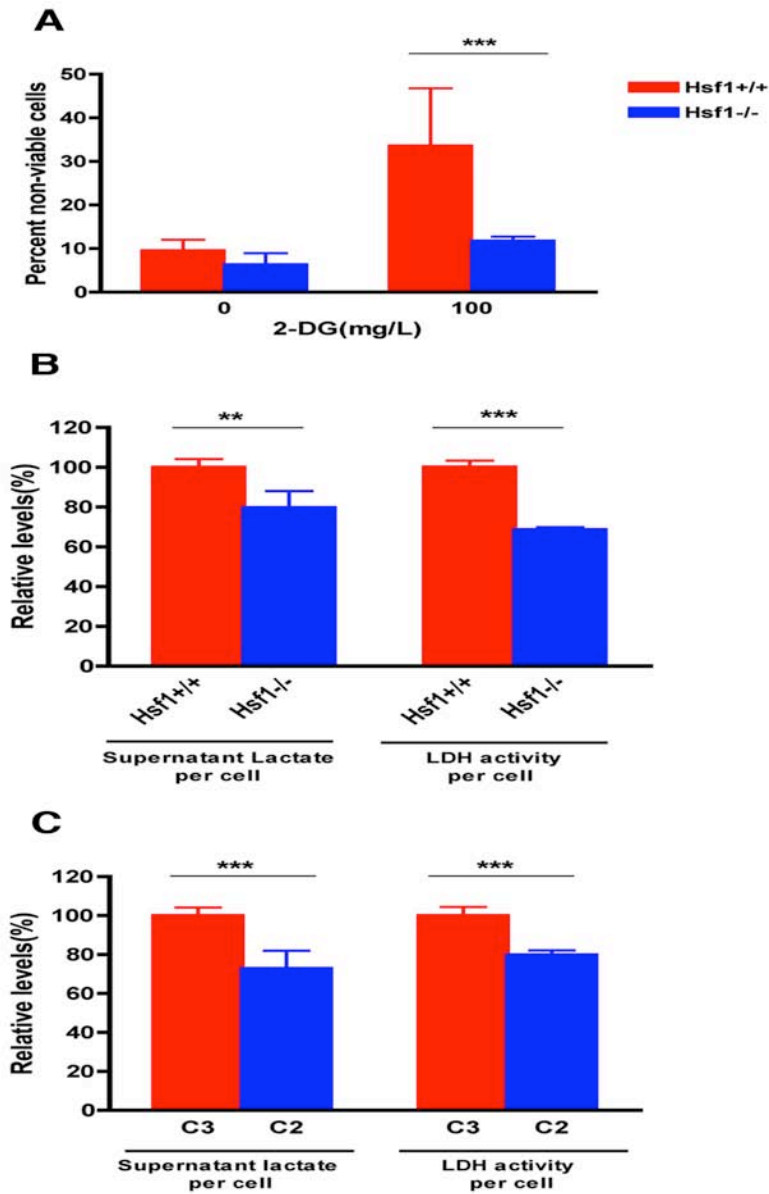
Figure S6: HSF1 modulates glucose metabolism. A: *Hsf1+/+* MEFs are more sensitive to the survival-inhibitory effect of 2-DG. Cells were incubated in 96-well plates with either PBS or 2-DG (100mg/L)–containing medium for 3 days. The number and viability of cells in each well were determined by flow cytometry. Data are presented as percent non-viable cells (Mean±SD, N=5, *** $p<0.001$, two-way ANOVA). **B and C:** HSF1 regulates LDH activity and lactate production. Cells were cultured in 96-well plates with glucose-rich medium. After 4 days, supernatants were harvested to measure lactate levels and cells were fixed and stained with Hoechst 33342 to determine the relative cell

number in each well. The supernatant lactate levels were then normalized against the cell numbers. For measuring enzymatic activity per cell, equal numbers of cells were extracted for protein to measure LDH activity using a commercially available kit (CytoTox 96 Non-radiooactive cytotoxicity assay, Promega). Data are presented as relative levels by setting the values of either *Hsf1*+/+ or C3 MEF as 100% (Mean±SD, lactate N=5, LDH N=4, ** $p<0.01$, *** $p<0.001$, Student's t test).
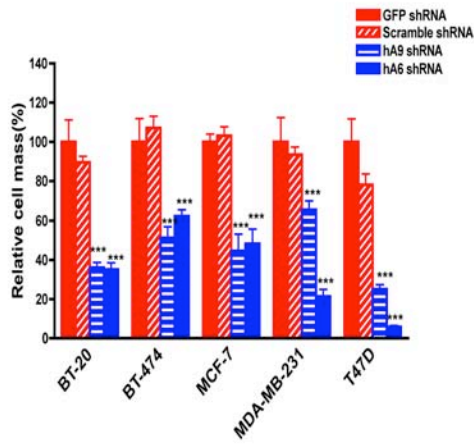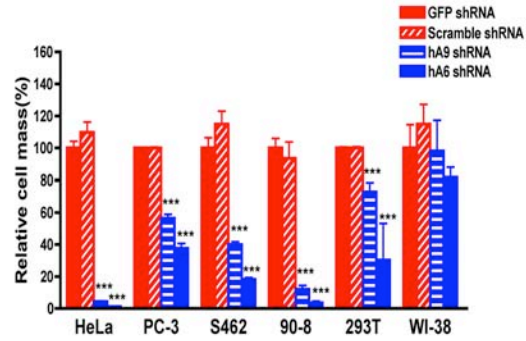
Figure S7: HSF1 maintains tumorigenicity. A: HSF1 compromise results in a decrease in cell number in primary human breast cancer cell lines. 4 days after viral infection, cells were fixed and stained with 0.4% sulforhodamine B solution. The changes in cell number are reflected by the changes in cell biomass. The relative cell mass of each transduction group was calculated by normalizing the values against those of GFP shRNA-transduced group (Mean±SD, N=5, * $p < 0.05$, *** $p < 0.001$, two-way ANOVA). **B**: HSF1 compromise results in a decrease in cell number in human malignant cell lines of other histological origins (Mean±SD, N=5, * $p < 0.05$, *** $p < 0.001$, two-way ANOVA).
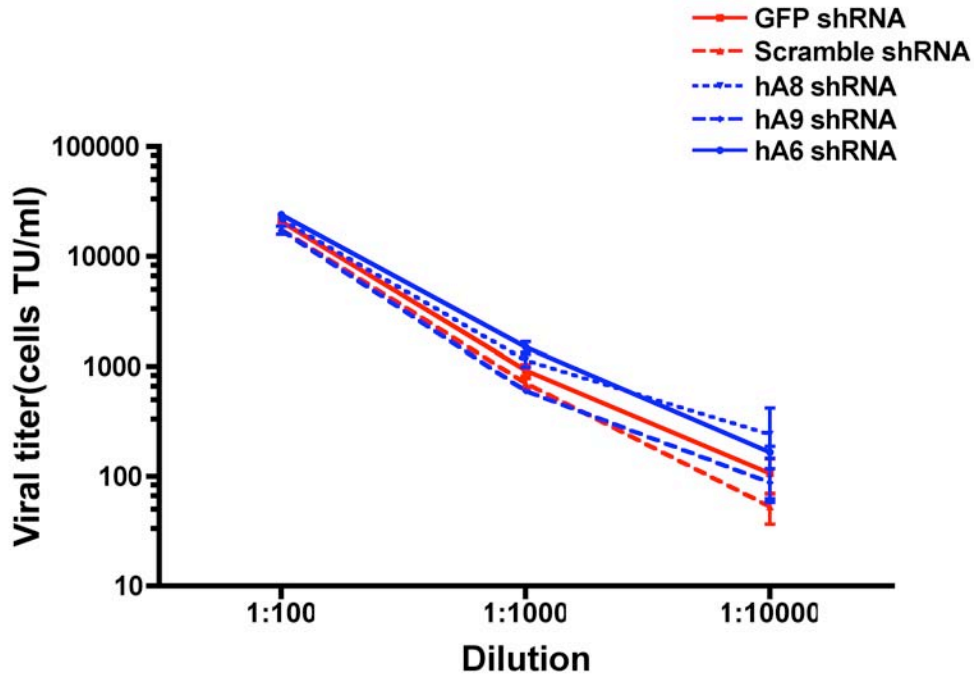
Figure S8: Viral supernatants have similar biological titers. C3H 10T1/2 cells were plated in 96-well format at 5000 cells/well. Viral supernatants were diluted in DMEM and 100μl of such supernatants were incubated with 10T1/2 cells overnight. Each dilution was transduced in quadruplicates. After being cultured in fresh medium for 24hr, cells were selected with 5μg/ml puromycin for 3 days. Cell number in each well was then determined by adding the CellTiter-Blue® reagent diluted in DMEM. The wells without viral transduction and antibiotics selection serve as the 100% transduction control, and the titers of each viral supernatant are expressed as: the number of cells transduction unit per ml (cells TU/ml).
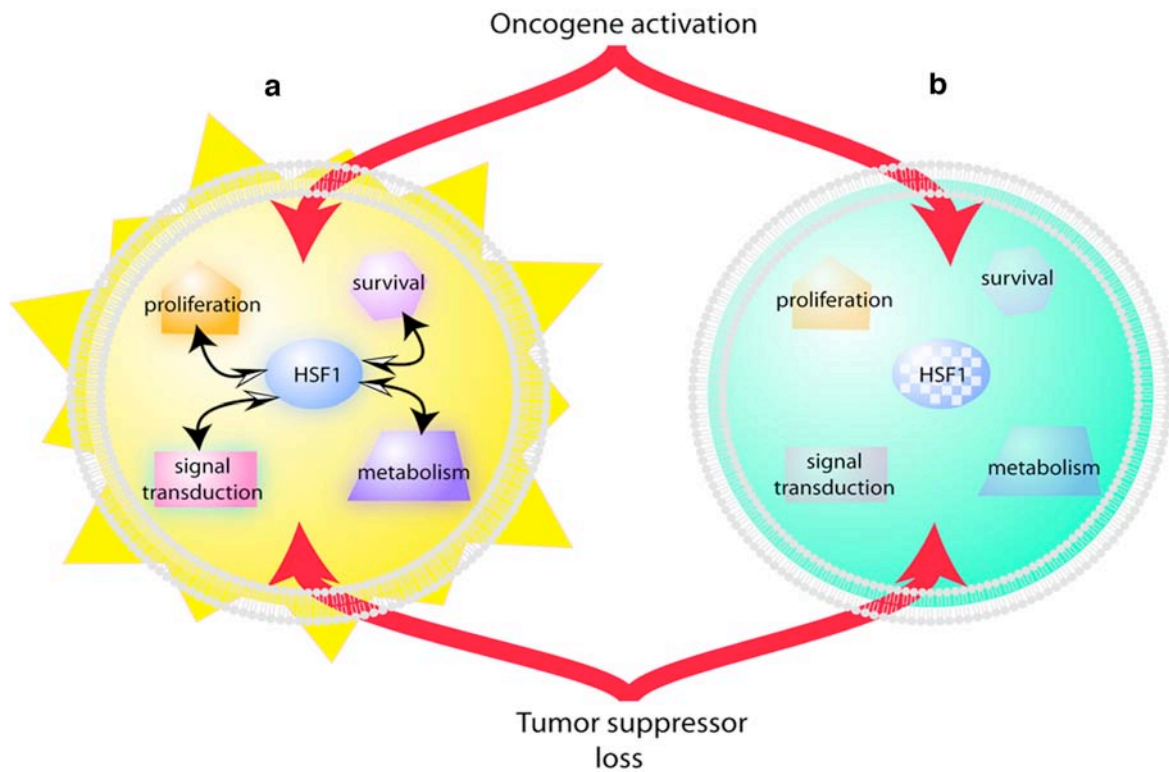
Figure S9: HSF1 modulates oncogenesis by orchestrating a diverse network of cellular functions. (a): With intact HSF1 function, cells accommodate the stressful alterations imposed by either oncogene activation or tumor suppressor inactivation by modulating diverse cellular responses, such as proliferation, survival, signaling transduction, and metabolism. Ironically, the robustness of a healthy cell is exploited by oncogenic stimuli to accomplish the process of malignant transformation. (b): In contrast, with impaired HSF1, cells are unable to adapt efficiently because of disarrayed cellular responses. As a result, such cells demonstrate relative resistance to malignant transformation.

Table S1: Mutations of *H-Ras* in mouse skin tumors.

ID#	Hsf1	Codon 12/13	Codon 61
1	+/+	gga/ggc	caa(Gln)→cta(Leu)
2	+/+	gga/ggc	caa(Gln)→cga(Arg)
3	+/+	gga/ggc	caa(Gln)→ctt(Leu)
4	+/+	gga/ggc	caa(Gln)→cta(Leu)
5	+/+	gga/ggc	caa(Gln)→cta(Leu)
6	+/+	gga/ggc	caa(Gln)→cta(Leu)
7	+/+	gga/ggc	caa(Gln)→cta(Leu)
8	+/+	gga/ggc	caa(Gln)→cta(Leu)
9	-/-	gga/ggc	caa(Gln)→ct/g/a(Leu/Arg)
10	-/-	gga/ggc	caa(Gln)→cta(Leu)
11	-/-	gga/ggc	caa(Gln)→cta(Leu)
12	-/-	gga/ggc	caa(Gln)→cta(Leu)
13	-/-	gga/ggc	caa(Gln)→cg/t/a(Arg/Leu)
14	-/-	ggc(Gly)→cgc(Arg)	caa