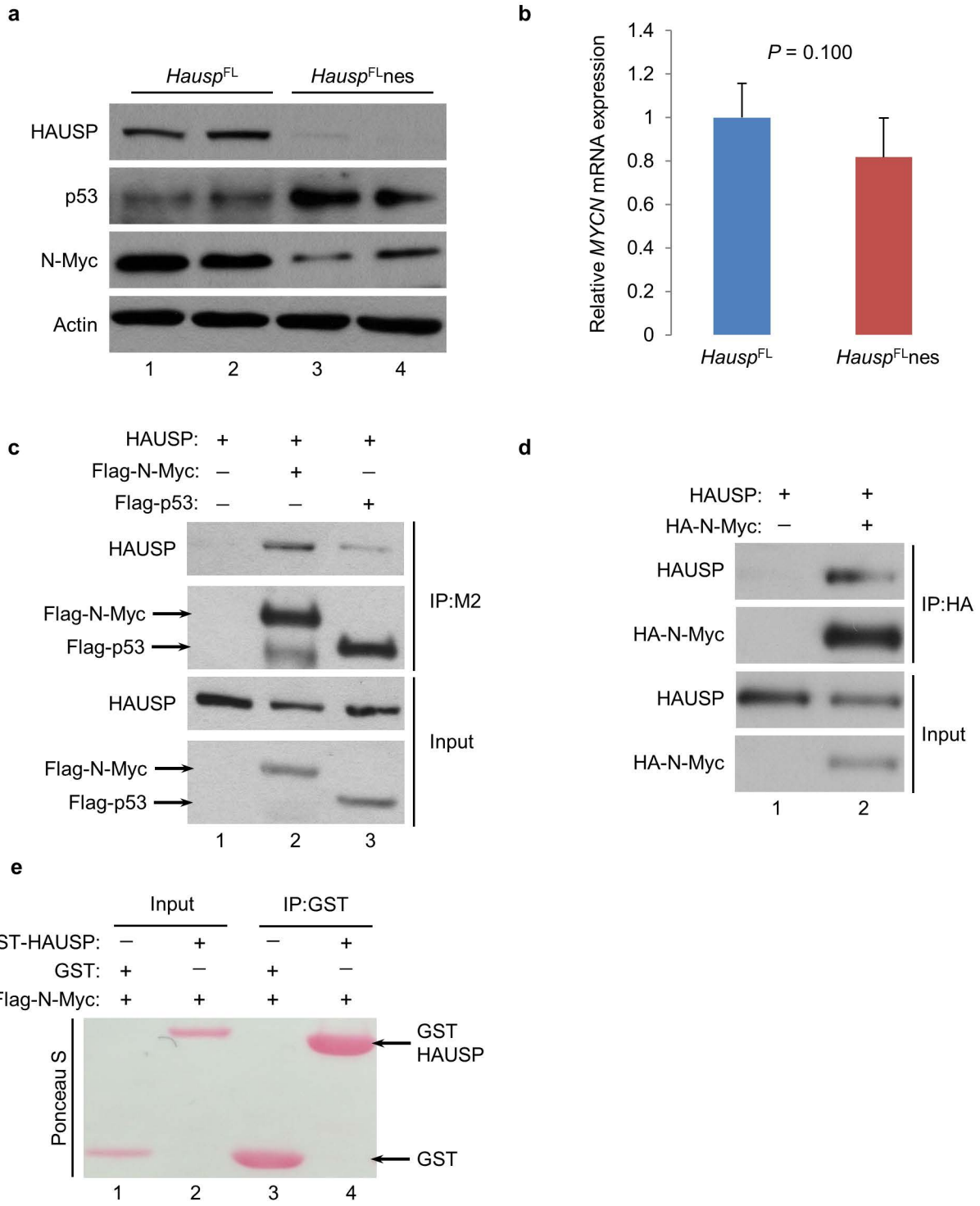
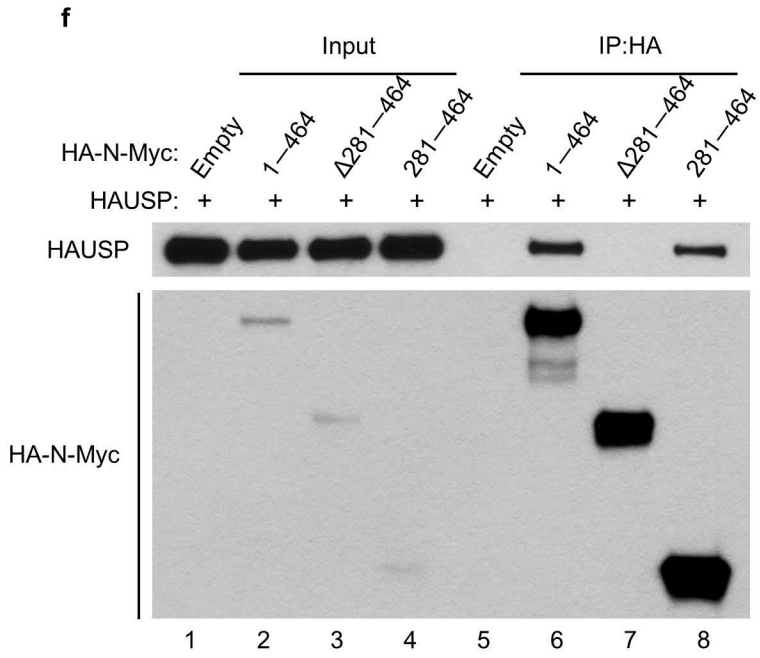
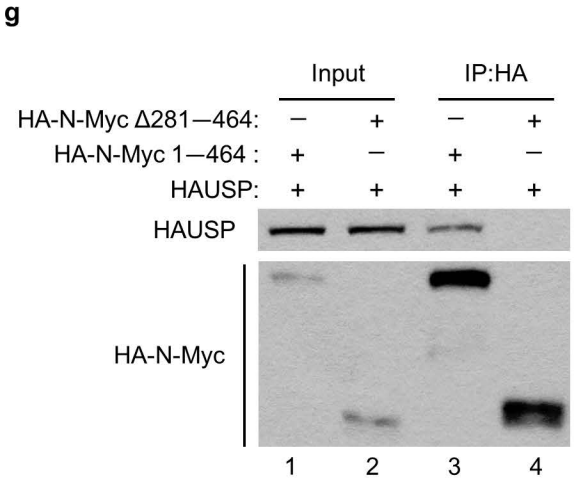
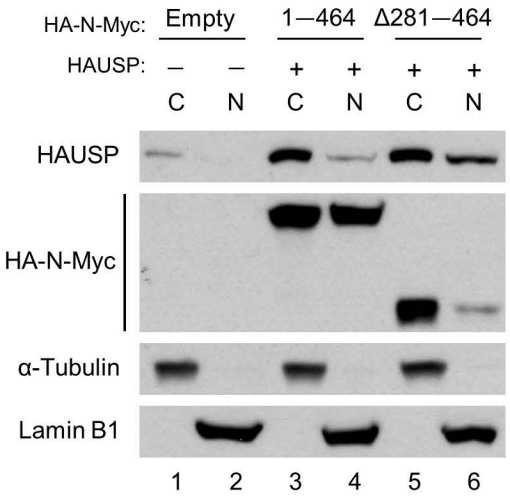
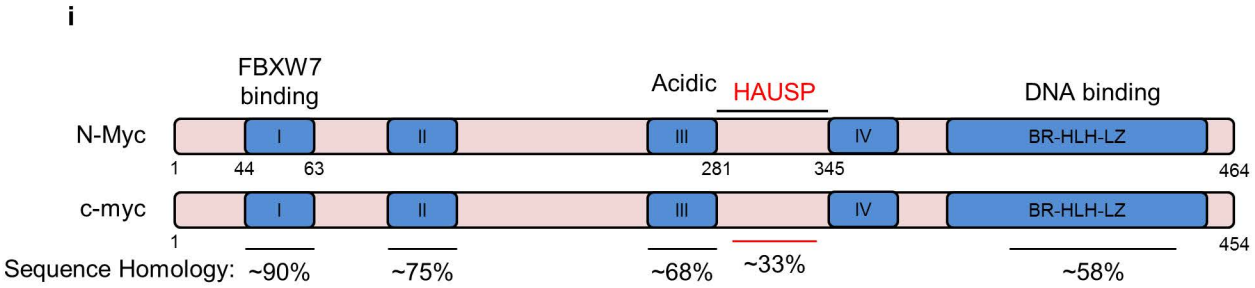

a
Hausp^FL
Hausp^FL-nes
HAUSP
p53
N-Myc
Actin
1
2
3
4
b
Relative MYCN mRNA expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
P = 0.100
Hausp^FL
Hausp^FL-nes
c
HAUSP: + + +
Flag-N-Myc: – + –
Flag-p53: – – +
HAUSP
Flag-N-Myc
Flag-p53
IP:M2
HAUSP
Flag-N-Myc
Flag-p53
Input
1
2
3
d
HAUSP: + +
HA-N-Myc: – +
HAUSP
HA-N-Myc
IP:HA
HAUSP
HA-N-Myc
Input
1
2
e
Input
IP:GST
GST-HAUSP: – + – +
GST: + – + –
Flag-N-Myc: + + + +
Ponceau S
GST
HAUSP
GST
1
2
3
4

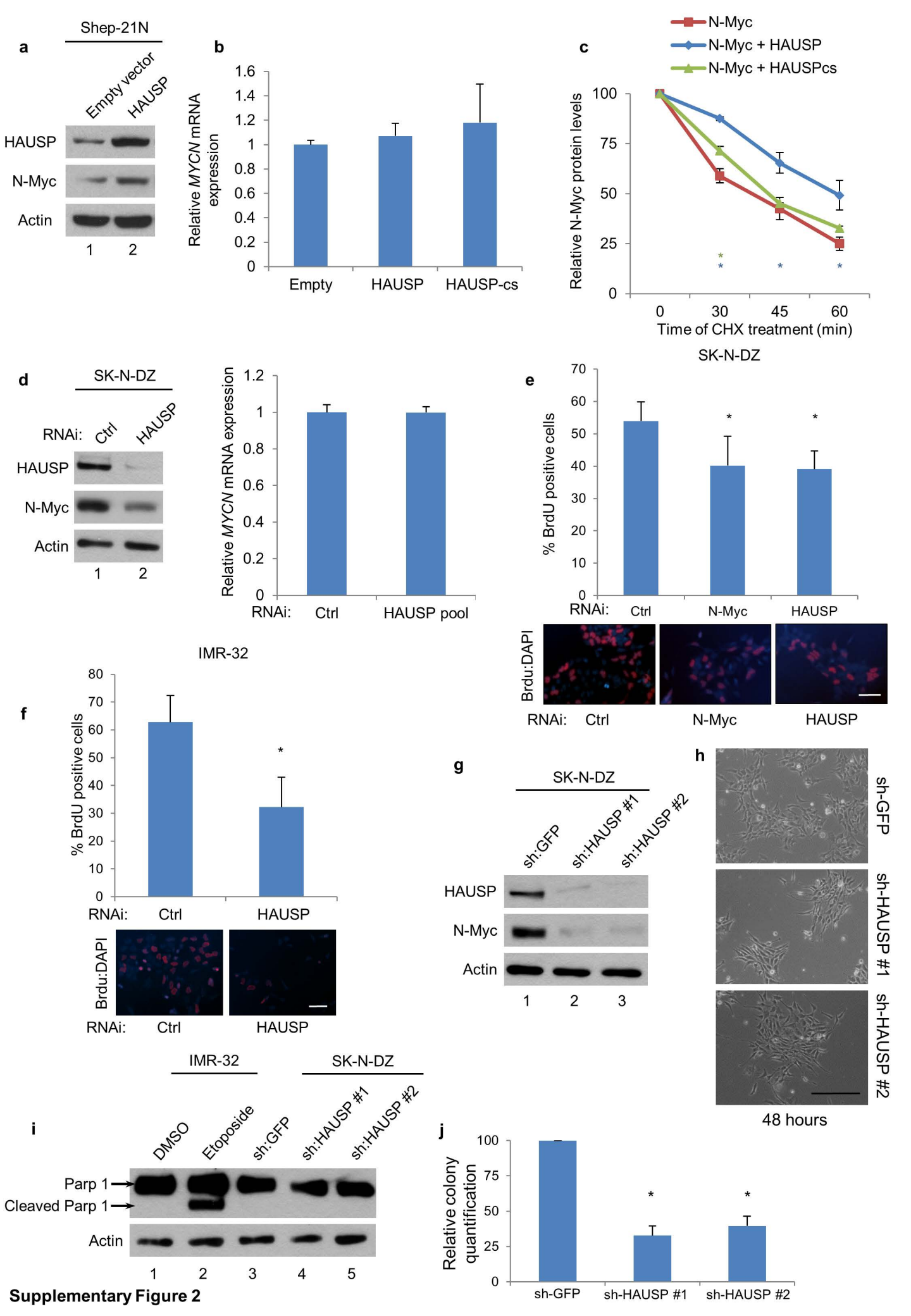




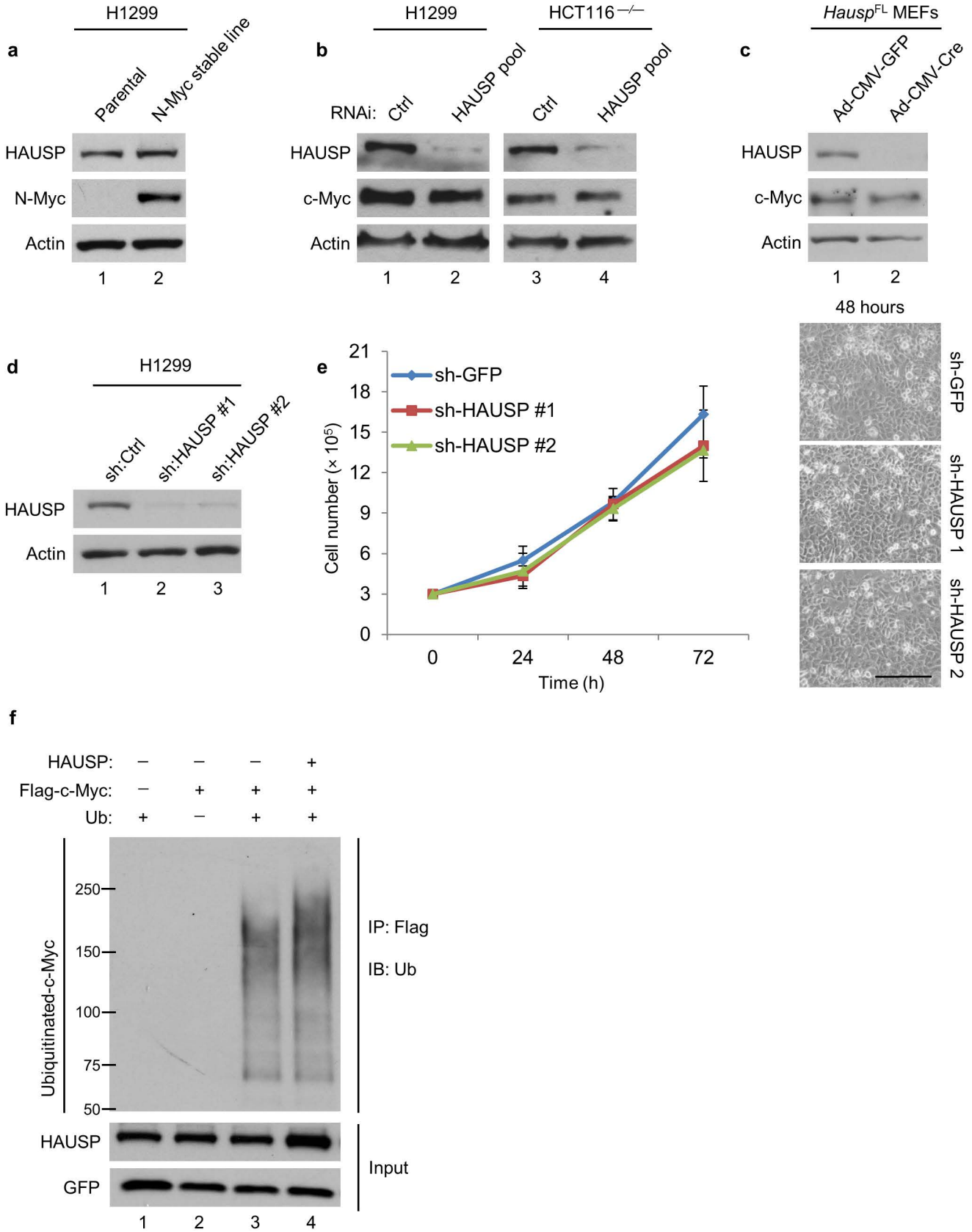




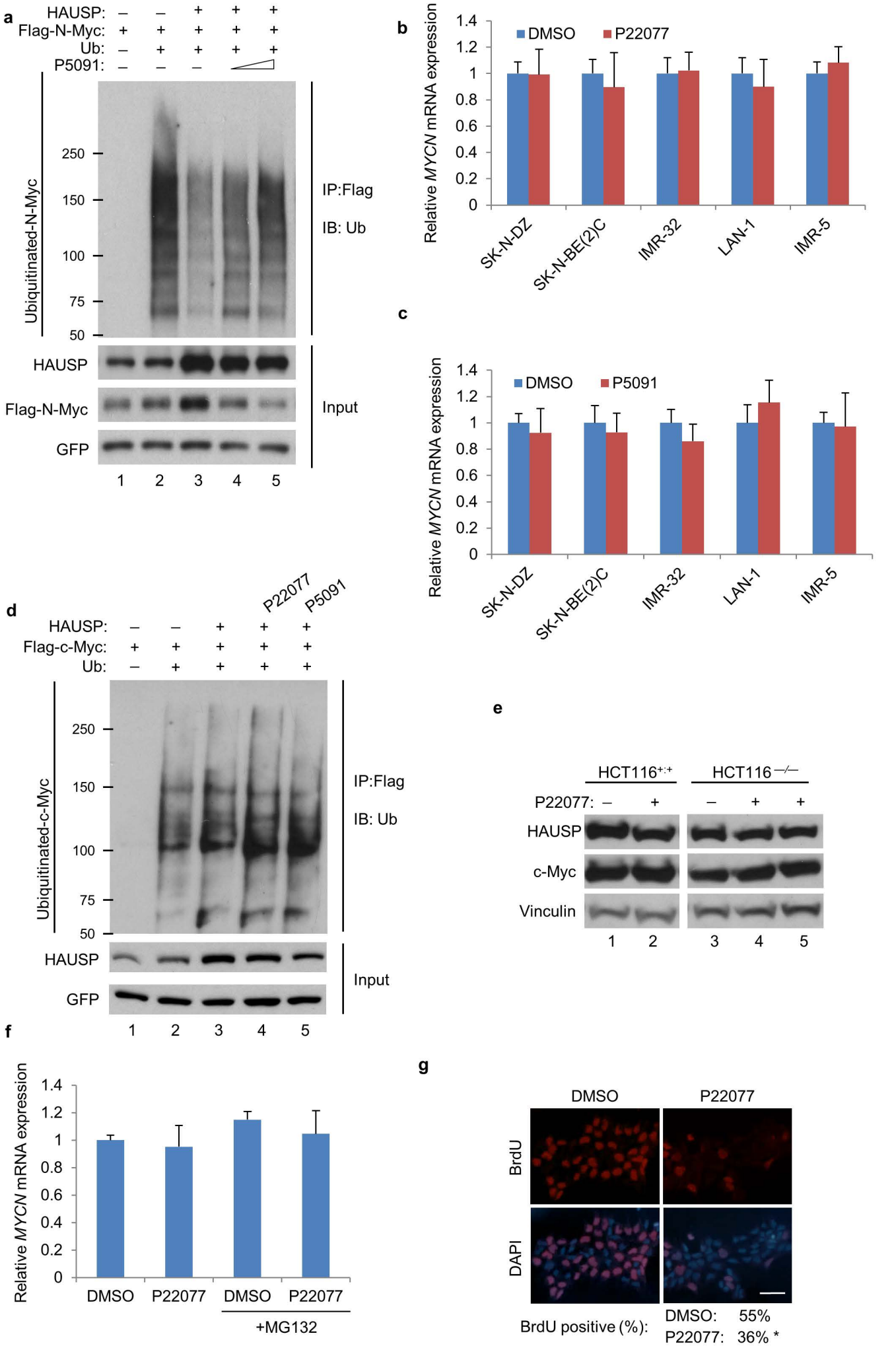
Supplementary Figure 1

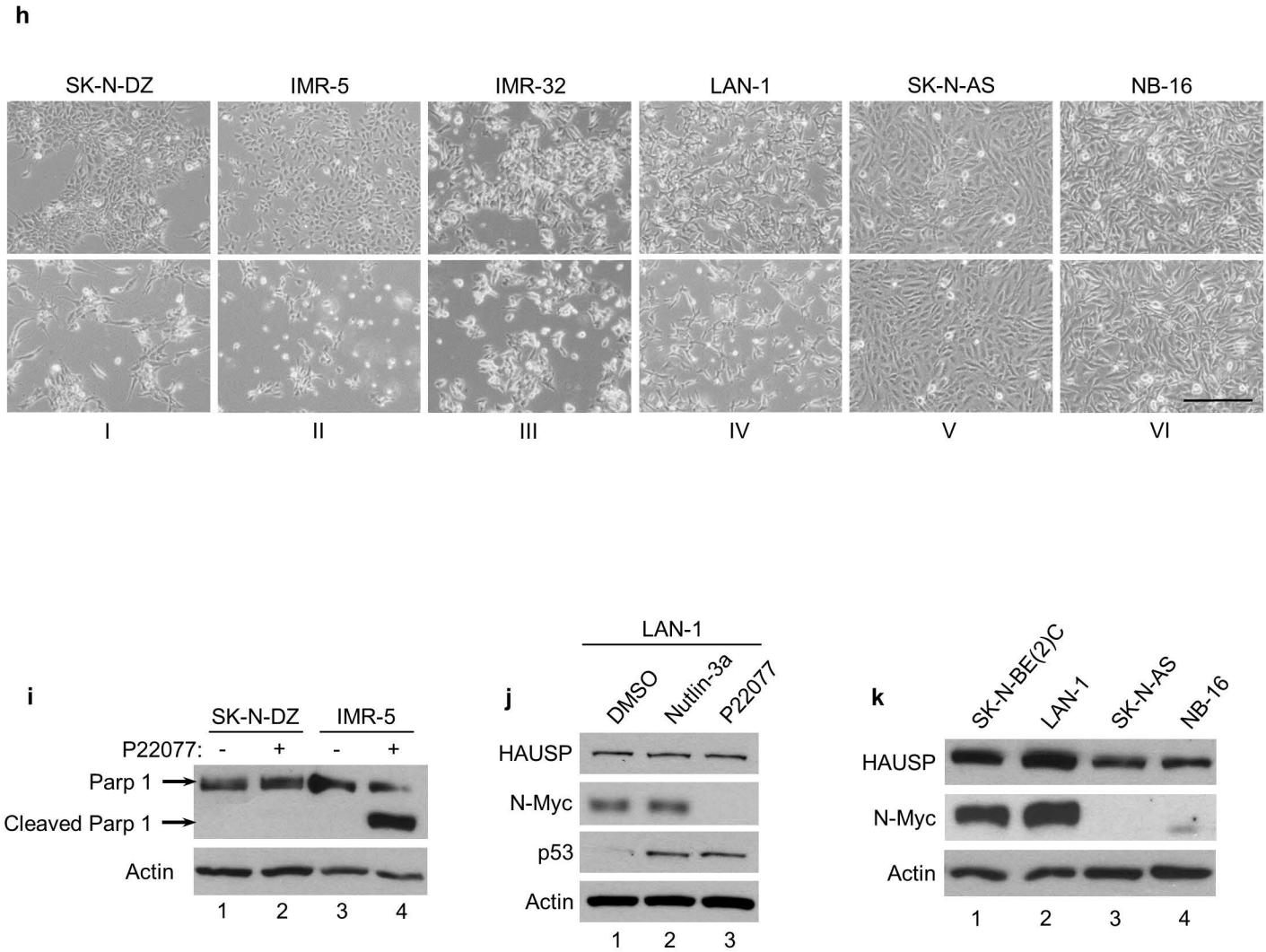


Supplementary Figure 2

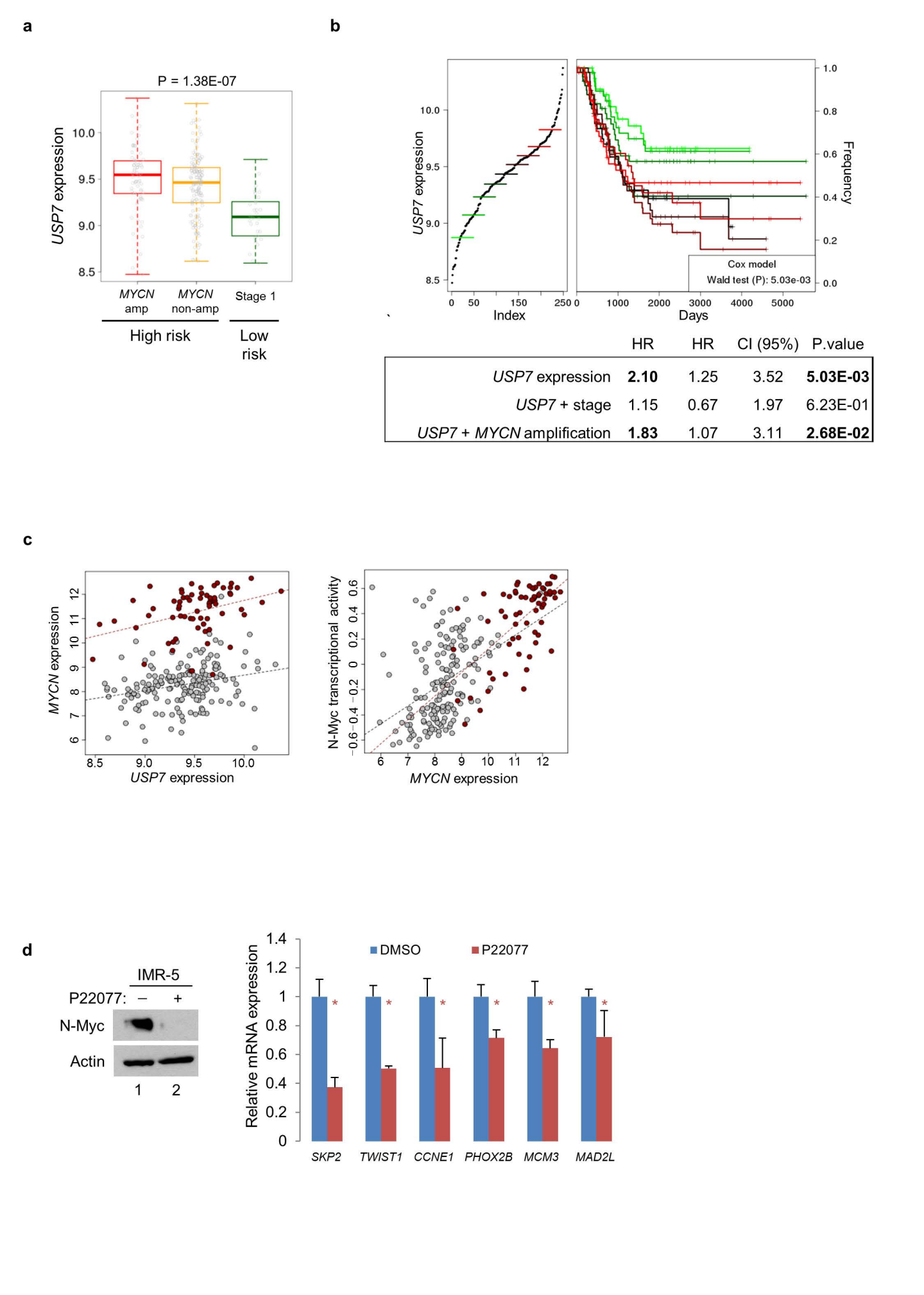


Supplementary Figure 3


a
HAUSP:
Flag-N-Myc:
Ub:
P5091:
Ubiquitinated-N-Myc
250
150
100
75
50
IP:Flag
IB: Ub
HAUSP
Flag-N-Myc
GFP
Input
1 2 3 4 5
b
DMSO
P22077
Relative MYCN mRNA expression
SK-N-DZ
SK-N-BE(2)C
IMR-32
LAN-1
IMR-5
c
DMSO
P5091
Relative MYCN mRNA expression
SK-N-DZ
SK-N-BE(2)C
IMR-32
LAN-1
IMR-5
d
P22077
P5091
HAUSP:
Flag-c-Myc:
Ub:
Ubiquitinated-c-Myc
250
150
100
75
50
IP:Flag
IB: Ub
HAUSP
GFP
Input
1 2 3 4 5
e
HCT116+/+
HCT116−/−
P22077:
HAUSP
c-Myc
Vinculin
1 2 3 4 5
f
Relative MYCN mRNA expression
DMSO
P22077
DMSO
P22077
+MG132
g
DMSO
P22077
BrdU
DAPI
BrdU positive (%):
DMSO: 55%
P22077: 36% *

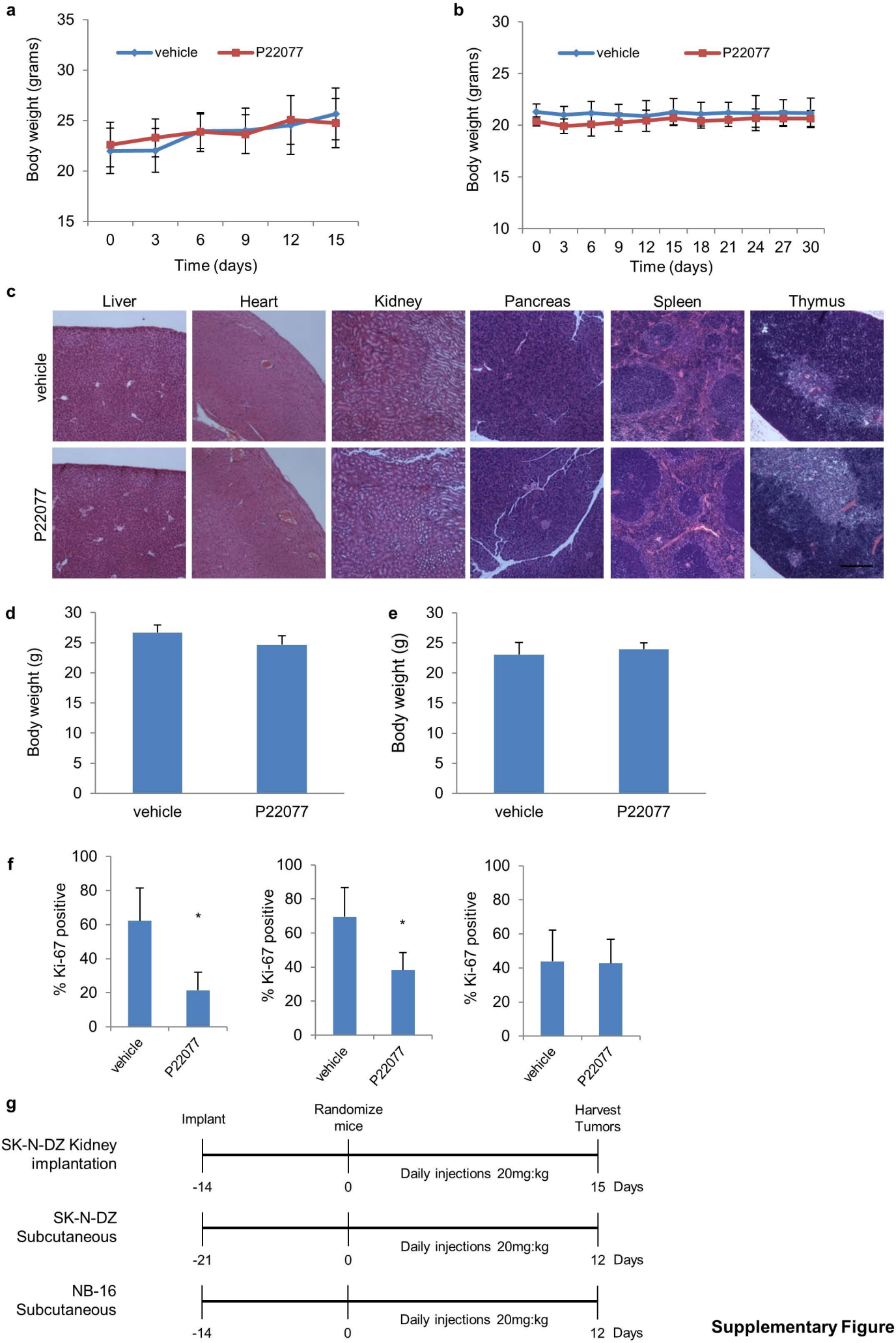


Supplementary Figure 4



	HR	HR	CI (95%)	P.value
USP7 expression	**2.10**	1.25	3.52	**5.03E-03**
USP7 + stage	1.15	0.67	1.97	6.23E-01
USP7 + *MYCN* amplification	**1.83**	1.07	3.11	**2.68E-02**

Supplementary Figure 5



Supplementary Figure 6

Signature	Sample Subset	*USP7*^{Exp}-*MYCN*^{Exp}	*MYCN*^{Exp}-N-Myc^{Act}	*USP7*^{Exp}-N-Myc^{Act}	Z-Score	*P* value	Pubmed Id
MYCN functional	***MYCN* Non-Amplified**	**0.26**	**0.40**	**0.55**	**-3.30**	**4.84E-04**	**23091029**
	***MYCN* Amplified**	**0.36**	**0.62**	**0.61**	**-1.89**	**2.96E-02**	
	Combined	**0.30**	**0.71**	**0.55**	**-3.35**	**3.98E-04**	
MYCN Amp/Non-Amp	*MYCN* Non-Amplified	0.26	0.38	0.48	-2.36	9.04E-03	23430699
	MYCN Amplified	0.36	0.67	0.57	-1.59	5.60E-02	
	Combined	0.30	0.72	0.50	-2.63	4.23E-03	
SCHUHMACHER MYC	*MYCN* Non-Amplified	0.26	0.23	0.57	-3.51	2.23E-04	22039435
	MYCN Amplified	0.36	0.70	0.62	-1.95	2.56E-02	
	Combined	0.30	0.66	0.56	-3.61	1.52E-04	
Myc core signature	*MYCN* Non-Amplified	0.26	0.33	0.66	-4.92	4.41E-07	11139609
	MYCN Amplified	0.36	0.64	0.64	-2.22	1.34E-02	
	Combined	0.30	0.61	0.64	-5.06	2.08E-07	

Supplementary Table 1

Supplementary Figure Legends

Supplementary Fig 1. HAUSP regulates and binds N-Myc. (**a**) Western blot of murine brains collected at day E14.5; n = 2. (**b**) Quantitative real-time PCR of *MYCN* mRNA levels from murine embryonic brains of *Hausp*^{FL} (n = 6) or *Hausp*^{FL}nes (n = 4) at E13.5. Error bars represent mean ± SD; n = 3; P value = 0.100 using two-tailed Student's t-test. (**c**) HAUSP expression vector cotransfected with Flag-N-Myc (lane 2), Flag-p53 (lane 3), or empty vector (lane 1) in H1299 cells. Cell lysates were incubated with Flag/M2 beads then subjected to western blot; n = 3. (**d**) HAUSP expression vector cotransfected with HA-N-Myc (lane 2) or empty vector (lane 1) in HEK293T cells. Cell lysates were incubated with HA beads then subjected to western blot; 10% input; n = 3. (**e**) Ponceau S staining of GST and GST-HAUSP used in the direct interaction experiment corresponding to **Fig. 1f**. (**f**) Indicated N-Myc expression vectors cotransfected with HAUSP in HEK293T cells (as in **Fig. 1i**). Cell lysates were incubated with HA beads then subjected to western blot; 10% input; n = 3. (**g**) *In vitro* binding of purified HA-N-Myc 1—464 and HAUSP or purified HA-N-Myc Δ281—464 and HAUSP. Products were incubated with HA beads then subjected to western blot; 1% input; n = 2. (**h**) Western blot analysis of cytoplasmic (c) and nuclear (n) fractionation of HEK293T cells transfected with empty vector, HA-N-Myc (1-464) or HA-N-Myc (Δ281-464) and either an empty vector (—) or wild-type HAUSP (+); n = 2. (**i**) Schematic representation of N-Myc and c-Myc with corresponding sequence homology percentages.

Supplementary Fig 2. HAUSP regulates N-Myc protein and function. (**a**) Western blot analysis of Shep-21N cells transfected with either empty vector or HAUSP; n = 2. (**b**) Quantitative real-time PCR analysis of *MYCN* mRNA levels corresponding to **Fig. 2c**; n = 2. (**c**) Densitometry quantification of N-Myc protein levels calculated using ImageJ software (National Institutes of Health [NIH], Bethesda, MD) and plotted for half-life determination corresponding to **Fig. 2d**. Error bars represent the mean ± SEM of 3 independent experiments. * represents a P value < 0.05 using two-tailed Student's t-test. (**d**) Western blot analysis of SK-N-DZ cells transfected with two-rounds of control RNAi or HAUSP pool. Paired mRNA was extracted and quantitative real-time PCR analysis of *MYCN* mRNA levels are shown; n = 2. (**e**) Quantification of BrdU incorporation of SK-N-DZ cells treated with two rounds of control siRNA, N-Myc siRNA, or HAUSP siRNA. BrdU positive cells were counted and divided by total cells to give % BrdU positive. Scale bar 25 μm; n = 2. * represents a *P* value < 0.01 using two-tailed Student's t-test. (**f**) Quantification of BrdU incorporation of IMR-32 cells treated with two rounds of a control siRNA or HAUSP siRNA. Scale bar 25 μm; n = 2. * represents a *P* value < 0.01 using two-tailed Student's t-test. (**g**) Western blot analysis of SK-N-DZ cells corresponding to **Figs. 2g,h**; n = 3. (**h**) Representative phase-contrast images of SK-N-DZ cells corresponding to **Fig. 2g** at 48 hours. Magnification 10 ×; scale bar 12.5 μm; n = 3. (**i**) Western blot analysis of IMR-32 cells treated with DMSO or Etoposide and SK-N-DZ cells from (**Fig. 2g**); n = 3. (**j**)

Densitometry quantification of colony assay from **Fig. 2h** calculated using ImageJ software (National Institutes of Health [NIH], Bethesda, MD) Error bars represent the mean ± SEM of three independent experiments. * represents a *P* value < 0.01 using a two-tailed Student's t-test.

Supplementary Fig 3. HAUSP does not affect c-Myc protein levels. (**a**) Western blot analysis of H1299 native cells or stably expressing an exogenous N-Myc polypeptide corresponding to **Fig. 2i**; n = 3. (**b)** Western blot analysis of H1299 (lane 1—2) or HCT116$^{-/-}$ (lane 3—4) cells transfected with three-rounds of control RNAi, or a HAUSP; n = 2. (**c**) Western blot analysis of mouse embryonic fibroblasts generated from *Hausp*^{FL} mice either infected with a control adenovirus (Ad-CMV-GFP), or Cre-expressing adenovirus (Ad-CMV-Cre); n = 3. (**d**) Western blot analysis of H1299 cells stably expressing a control sh-GFP, HAUSP-shRNA #1, or HAUSP-shRNA #2; n = 3. (**e**) Cell growth assay of H1299 cells expressing a control sh-GFP, HAUSP-shRNA #1, or HAUSP-shRNA #2. Error bars represent the mean ± SEM of 3 independent experiments. Representative phase-contrast images of H1299 cells at 48 hours. Magnification 10 ×; scale bar 12.5 µm; n = 3. (**f**) Ubiquitin (Ub) and Flag-c-Myc expression vectors were either transfected with empty vector (lane 3) or in combination with HAUSP (lane 4) into HEK293T cells. As negative controls, either ubiquitin was transfected with empty vector (lane 1) or Flag-c-Myc was transfected with empty vector (lane 2). Cell lysates were incubated with Flag/M2 beads then subjected to western blot; n = 2.

Supplementary Fig 4. Effect of HAUSP inhibitors regulating N-Myc. (**a**) Ubiquitin (Ub) and Flag-N-Myc expression vectors were either transfected with empty vector (lane 2) or in combination with HAUSP (lane 3—5) into HEK293T cells. As a negative control, Flag-N-Myc was transfected with empty vector (lane 1). Cells were treated with P5091 for the last 8 hours at 12.5 µm (lane 4) or 25 µm (lane 5). Lanes 1—3 were treated with DMSO as a control. Cell lysates were incubated with Flag/M2 beads then subjected to western blot; n = 3. (**b,c**) Quantitative real-time PCR analysis of *MYCN* mRNA levels from neuroblastoma cell lines treated with P22077 (**b;** corresponding to **Fig. 3b** top panel) or treated with P5091 (**c**; corresponding with **Fig. 3b** bottom panel). Error bars represent mean ± SD; n = 3. (**d**) Ubiquitination assay as performed in (**Supplementary Fig. 4a**) with additional lanes either treated with 20 µm of P22077 (lane 4) or 25 µm of P5091 (lane 5) for the last 8 hours. Lanes 1-3 were treated with DMSO as a control. Cell lysates were incubated with Flag/M2 beads then subjected to western blot; n = 2. (**e**) Western blot analysis of HCT116$^{+/+}$(lane 1—2), or HCT116$^{-/-}$ (lanes 3—5) treated with DMSO (lanes 1 and 3), 10 µm of P22077 (lanes 2 and 5), or 20 µm of P22077 (lane 5) for 8 hours; n = 2. (**f**) Quantitative real-time PCR analysis of *MYCN* mRNA levels corresponding to **Fig. 3d**; n = 2. (**g**) BrdU incorporation of SK-N-DZ cells either treated with DMSO or 10 µm of P22077 for 24 hours. Scale bar 25 µm; n = 3. * represents a *P* value < 0.01 using two-tailed Student's t-test. (**h**) Representative phase-contrast images of neuroblastoma cells treated with DMSO or P22077 corresponding to **Fig. 3d—i** at 48 hours. Magnification 10 ×; scale bar 12.5

μm; n = 3. (**i**) Western blot analysis of SK-N-DZ and IMR-5 cells treated with 10 μm of P22077 (lanes 2 and 4) or DMSO (lanes 1 and 3) for 8 hours. Arrows indicate total Parp 1 or Cleaved Parp 1; n = 3. (**j**) Western blot analysis of LAN-1 cells treated with DMSO, 2.5 μm of Nutlin-3, or 15 μm of P22077 for 12 hours; n = 3. (**k**) Western blot analysis of indicated neuroblastoma cell extracts (corresponding with **Figs. 3h,i**); n = 2.

Supplementary Fig 5. Analysis of *USP7* expression from human neuroblastomas patients. (**a**) Association between *USP7* (HAUSP) mRNA expression and clinical covariates where *USP7* expression is increased in high risk tumors (both *MYCN*-amplified and *MYCN* non-amplified) and reduced in benign (Stage 1) tumors. Kruskal-Wallis test was used to get the *P* value. (**b**) The sample cohort is separated by window shifting across the rank of *USP7* expression (left box). The median of each sample window is used to obtain the color on a heat map from green (indicating low expression) to red (indicating high expression). A Kaplan-Meier diagram is use to visualize the association with survival; *P* value was obtained using a Wald test after fitting a Cox Hazards Model of *USP7* expression with overall survival. Multivariate analysis of *USP7* expression with clinical co-variates showing that USP7 predicts survival independently of *MYCN* amplification (chart at the bottom); HR, hazard ratio; CI, confidence interval. (**c**) Correlation of *USP7* expression to *MYCN* expression (left panel) or correlation of *MYCN* expression versus N-Myc transcriptional activity (right panel) in neuroblastoma patients. Red dots indicate *MYCN*-amplified while gray dots indicate *MYCN* non-amplified (corresponding to **Fig. 4b** and **Supplemental Table 1**). (**d**) Western blot analysis of IMR-5 cells treated with 15 μm of P22077 or DMSO for 24 hours. Paired mRNA was extracted and quantitative real-time PCR analysis of indicated N-Myc transcriptional target mRNA levels are shown; n = 2. Error bars represent the mean ± SEM of two independent experiments. * represents a *P* value < 0.05 using two-tailed Student's t-test.

Supplementary Fig 6. Analysis of P22077 in different animal models. (**a**) Body weight of mice treated with vehicle or with daily injections of P22077 over the duration of the experiment; n = 8 mice per group corresponding to **Fig. 4c—e**. (**b**) Body weight of wild-type mice treated with daily injections of vehicle or P22077 (20 mg/kg) over the duration of the experiment; n = 5 mice per group. (**c**) Representative H&E staining of indicated tissues in animals treated with vehicle or P22077. Magnification 10 ×; scale bar 25 μm; n = 2 sections per mouse organ. (**d,e**) End-point body weight of mice injected with SK-N-DZ (**d**) and NB-16 (**e**) tumor-bearing mice treated with vehicle or with daily injections of P22077 over the duration of the experiment; n = 5 mice per group corresponding to **Fig. 4f,g** and **4h,i**, respectively**.** Error bars represent the mean ± SEM. (**f**) Quantification of Ki-67 staining represented in **Fig. 4e,g,i**. 5 representative slides were counted for positive cells divided by total cells (SK-N-DZ kidney xenograft, left; SK-N-DZ subcutaneous, middle; NB-16 subcutaneous, right). Error bars represent

the mean ± SEM. * represents a *P* value < 0.05 using two-tailed Student's t-test. (**g**) Schematic setup of the three xenografts used in **Fig 4**.

Supplementary Table 1. Correlation analysis of *USP7* vs N-Myc activity using multiple signatures. Identical analysis as done in **Fig. 4b** and **Supplemental Fig. 5c** using three additional signatures. Bolded signature graphs are shown in the paper. Pubmed ID's located at the right of the table.