SUPPLEMENTARY INFORMATION

Expression vectors, transfection and inhibitors

The 6xHis-Ub expressing vector was a kind gift from Dr. Shigeo Sato (Stowers Institute). HA-tagged ubiquitin expression vectors were a kind gift from Dr. Bin Wang (UT MD. Anderson Cancer Center). FLAG-WT-USP22 was generated by cloning of the PCR amplified cDNA into the pcDNA3.1-V5-FLAG vector. The C185S mutant was then generated by using a quick change site-directed mutagenesis kit (Stratagene). FLAG-FBP1 and Myc-FBP1 were created using the same approach, but in the Myc-FBP1 case the pCMX-2xMyc vector was used. 293T cells were transfected with the indicated vectors using lipofectamine 2000 (Invitrogen) and following themanufacturer's instructions.

Immunoprecipitatiopn

Forty-eight hours after the transfection, cells were harvested, washed two times in ice cold PBS and incubated in hypotonic buffer (10mM HEPES pH7.4, 1.5mM $MgCl_2$, 10mM KCl) containing protease inhibitor cocktail (Sigma), 20mM *N*-Ethylmaleimide (Sigma) and 10mM 2-Iodoacetamide (GE Healthcare) and nuclei isolated using a glass homogenizer. Nuclei were then lysed in IP Buffer (10mM Tris-Cl pH7.9, 150mM NaCl, 1mM EDTA, 1mM EGTA, 1% NP-40, 1mg/ml Sodium Deoxycholate) containing protease inhibitor cocktail, 25mM *N*-Ethylmaleimide and 10mM 2-Iodoacetamide. The lysates were centrifuged for 30 min at max speed in a microfuge at 4º C and the clear supernatants were used for immunoprecipitation.

Between 3 and 5 mg of total protein were used per IP reaction. The lysates were pre-cleared with Protein A/G agarose (Millipore) (50 µl slurry per 1 ml lysate) and incubated with aFLAG (M2) (Sigma) or aHA (Roche) beads (30 µl slurry per 1 ml lysate). For the co-immunoprecipitation experiments, the beads were incubated with the lysates on a rocking platform at 4ºC for 4 hours

and in the case of the Ub-proteins precipitation - overnight at 4ºC. Beads were washed 3 times as follows: 1-IP buffer, 2-IP buffer containing 350mM NaCl and 3-IP buffer. FLAG precipitated complexes were eluted with 100μg/ml 3xFLAG peptide (Sigma) in TBS. For precipitation of endogenous FBP1 and USP22 detection in the precipitated fractions, the anti-FBP1 antibody (Santa Cruz - Z37) was cross-linked to protein A agarose using Pierce crosslink IP kit (product # 26147) and following the manufacturer's instructions.

Quantitative RT-PCR

Total RNA from the indicated cells was purified by using a RNeasy Plus mini kit and QIAshredder (Qiagen) (Following the manufacturer's instructions) and used as a template for real-time RT-PCR reaction. PCRs were done using an Applied Biosystems 7500 Fast Real-Time PCR System and power SYBR Green PCR Master Mix (ABI). The mRNA abundance of the monitored genes in depleted cells was normalized to the mRNA abundance of these genes in control cells. β-actin was used as an internal control.

Chromatin immunoprecipitation (ChIP)

ChIP experiments were done according to the Upstate protocol for the ChIP kit (cat#17-259) with modifications as follows:

Sub-confluent HeLa cells were crosslinked for 10 min on RT with gentle shaking by adding formaldehyde to the cultural medium to a final concentration of 1%. Glycine (125mM final concentration) was added to quench the crosslinking process and the cells were incubated for an additional 10 min. The medium was then removed and cells washed 2 times with ice cold PBS containing inhibitors. Cells were scraped in 5 ml of PBS, and dishes washed with additional 5 ml

of PBS, and both fractions were pooled in 15 ml conical falcon tubes. Cells were then pelleted by centrifugation at 2,000 rpm for 5 min at 4ºC. Pellets were suspended in SDS lysis buffer (Upstate cat#20-163) at concentration 50 million cells per 1 ml lysis buffer, incubated 10 min on ice and aliquoted in 1.5 ml low retention (siliconized) eppendorf tubes (400 µl each). The cell suspension was then sonicated using a Bioruptor for 2 rounds (12.5 min each) on high power and 30 sec on/30 sec off cycle. The lysates were kept ice cold all the time. Using these conditions, the fragment size of the shared DNA was between 600 and 100 bp. However, sonication conditions should be optimized for each different cell line or experimental set. The sonicated lysates were centrifuged at max speed in a microfuge for 10 min at 4ºC and the clear supernatants were transferred to new falcon tubes. Sonicated DNA fractions were diluted 5 fold with ChIP dilution buffer (Upstate cat#20-153) and pre-cleared with protein A agarose/salmon sperm DNA 50% slurry (70 µl for each 2 ml of lysate) (Upstate cat#16-157C) for 30 min at 4ºC on 360º rotator. After pre-clearing, the agarose was pelleted by brief centrifugation, 50 µl from each fraction was taken and used later as an input. The sonicated fractions were then aliquoted in low retention tubes (~2 ml each) and antibodies added for overnight incubation at 4ºC. Antibodies: αFBP1 (Z37) -15 µl/reaction, αFBP3 - 5 µl/reaction (I-20, x-grade) aFIR - 10µl/ reaction, Normal rabbit IgG (Upstate) 1 µl/reaction.

After the incubation, 60 µl of protein A agarose/salmon sperm DNA 50% slurry was added to each tube and reactions incubated for 2 additional hours at 4ºC on the same rotator. The precipitated complexes were washed according to the protocol using the supplied buffers. DNA was then eluted from the beads and the DNA-protein crosslinks were reversed by adding 20 µl of 5M NaCl (500 µl volume) to each reaction and incubation at 65ºC overnight. DNA then was recovered according to the steps described in the protocol, and precipitated by adding 1.5 µl

GlycoBlue (Ambion) as an inert carrier. The precipitated DNA was dissolved in 30 µl nuclease free water (Ambion) and used for PCR. PCRs were done using an Applied Biosystems 7500 Fast Real-Time PCR System and power SYBR Green PCR Master Mix.

Primers Used

qPCR

Gene	**Forward primer sequence**	**Reverse primer sequence**
p21	TACCCTTGTGCCTCGCTCAG	CGGCGTTTGGAGTGGTAGA
c-myc	TTTCGGGTAGTGGAAAACCA	TTTCGGGTAGTGGAAAACCA
MMP-1	CCCAAAAGCGTGTGACAGTA	CCCAAAAGCGTGTGACAGTA
β-actin	GCCAACCGCGAGAAGATGACC	CTCCTTAATGTCACGCACGATTTC
USP22	GGACAACTGGAAGCAGAACC	TGAAACAGCCGAAGAAGACA
FBP1	ACGCTTTCAAAGATGCACTG	TTTTTGTCCCCCATAACCAT

ChIP Primers

p21 FUSE	CACTGCTGACTTTGTCTCAA	AGGCGGAACAAAGATAGAAC

Protein identification and mass spectrometry

Proteins were identified in the Proteomics Core Facility at the University of Texas Health Science Center at Houston. MSMS analysis was performed on an Applied Biosystems QStar Elite LC/MS/MS mass spectrometer equipped with an LC Packings HPLC for capillary chromatography. The HPLC is coupled to the mass spectrometer by a Nanospray II ESI source for direct analysis of the eluate. For protein identification, a razor was used to cut sequential 1-6 mm sections of the lanes of interest from the gel. The gel piece was cut into $1mm^2$ pieces, destained and then reduced with DTT. After reduction the cysteines were blocked by alkylation with iodoacetamide and subjected to in gel proteolytic digestion with trypsin for 16 hours at $37^{\circ}C$ essentially as described by Simpson (Simpson R.J., 2003). After extraction of the peptides an aliquot is separated by HPLC on a C18 75μ x 10 cm reverse phase capillary column (Vydac 218MS3.07510). The column was developed with a gradient of 2% to 50% acetonitrile in 0.1% formic acid over 30 minutes at a flow rate of 250 nl/min. The nanospray source fitted with a 15 μm tapered fused silica tip (New Objective, Cambridge, MA) was used to elute the peptides directly into the mass spectrometer for MS/MS analysis. The QSTAR was operated in Information Dependent Acquisition mode using a 1 second survey scan followed by two consecutive 3 second product ion scans of 2+, 3+ and 4+ parent ions (m/z 380-1500). Peptides and modifications were identified by Protein Pilot (Applied Biosystems) and verified with MASCOT with an MS and MSMS mass tolerance of 50 ppm and 0.1Da respectively.

Supplementary figure legends

Supplementary figure S1

USP22 regulates the ubiquitination levels of FBP1. (A) USP22 depleted or control 293T cells were co-transfected FLAG-FBP1 and HA-Ub, the ubiquitinated species were purified from nuclear extracts, resolved on NuPAGE and blotted with anti-FLAG (FBP1) antibody. Enriched FLAG-FBP1 ubiquitination was detected upon USP22 depletion. (B) Overexpression of the WT but not catalytically death USP22 reduces the endogenous FBP1 ubiquitination levels. 293T cells were co-transfected with HA-Ub and WT or catalytically inactive (C185S) FLAG-tagged USP22. The ubiqutinated proteins were precipitated with an anti-HA affinity matrix and probed for endogenous FBP1. (C) DNaseI treatment does not impact the ability of ectopically expressed FBP1 do precipitate endogenous USP22. Lysates from FLAG-FBP1 transfected or blank 293T cells were treated with 100 U (final concentration) of DNase I for 30 min at 37°C and subjected to anti-FLAG IP. 3xFLAG peptide eluated fractions were resolved on NuPAGE and probed for USP22.

Supplementary figure S2

USP22 activity does not have significant impact of FBP1 steady levels. FBP1 and p53 levels at different time points after cyclohexamide treatment of UPS22 depleted or control HeLa cells (A) and MCF7 cells (D). (B, E) USP22 levels in the cells used in (A and D). (E) Quantitation of the data in (A).

Supplementary figure S3

Aberrant pulse of c-myc expression in USP22 and FBP1 depleted HeLa and Normal Human Fibroblasts (NHF) cells. Sub-confluent cells stably expressing the indicated shRNAs were cultured in DMEM-H without serum for 4 days. The medium was then replaced with DMEM-H containing 10%FBS, and cells were harvested at the indicated time points. Cell lysates were prepared from HeLa (A) and NHF (C) cells and c-myc protein levels were monitored by western blot. (B) Quantitation of the data in (A). X-ray films were scanned and the images were quantified using ImageQuant software .The highest signal, detected 2 hours after serum addition to control cells was set as 1.

Supplementary figure S4

Cell cycle analyses of MCF7 (A) and H1299 cells (B). Exponentially growing cells were harvested, fixed in 70% ethanol, stained with propodium iodide and subjected to FACS analysis

Supplementary figure S5

Expression levels of *MMP-1* and *p21* in single or simultaneously USP22 and FBP1 depleted MCF7 cells. (A) Total RNA from MCF7 stably expressing the indicated shRNAs was purified and used as a template for qRT-PCR reaction. (B) USP22 and FBP1 silencing efficiency in the indicated cells. The mRNA abundance of the denoted genes was normalized to beta actin.

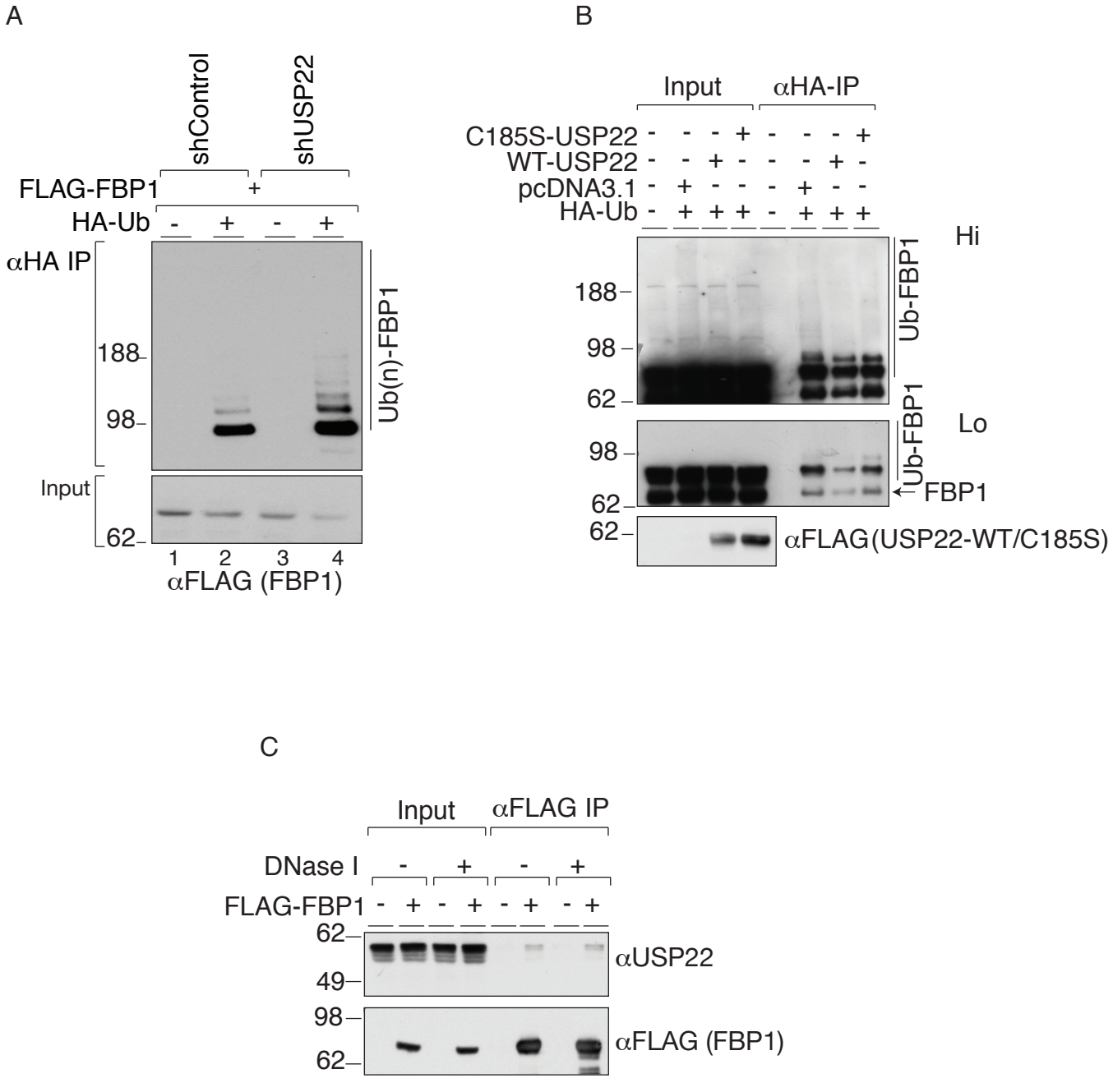
Supplementary table 1

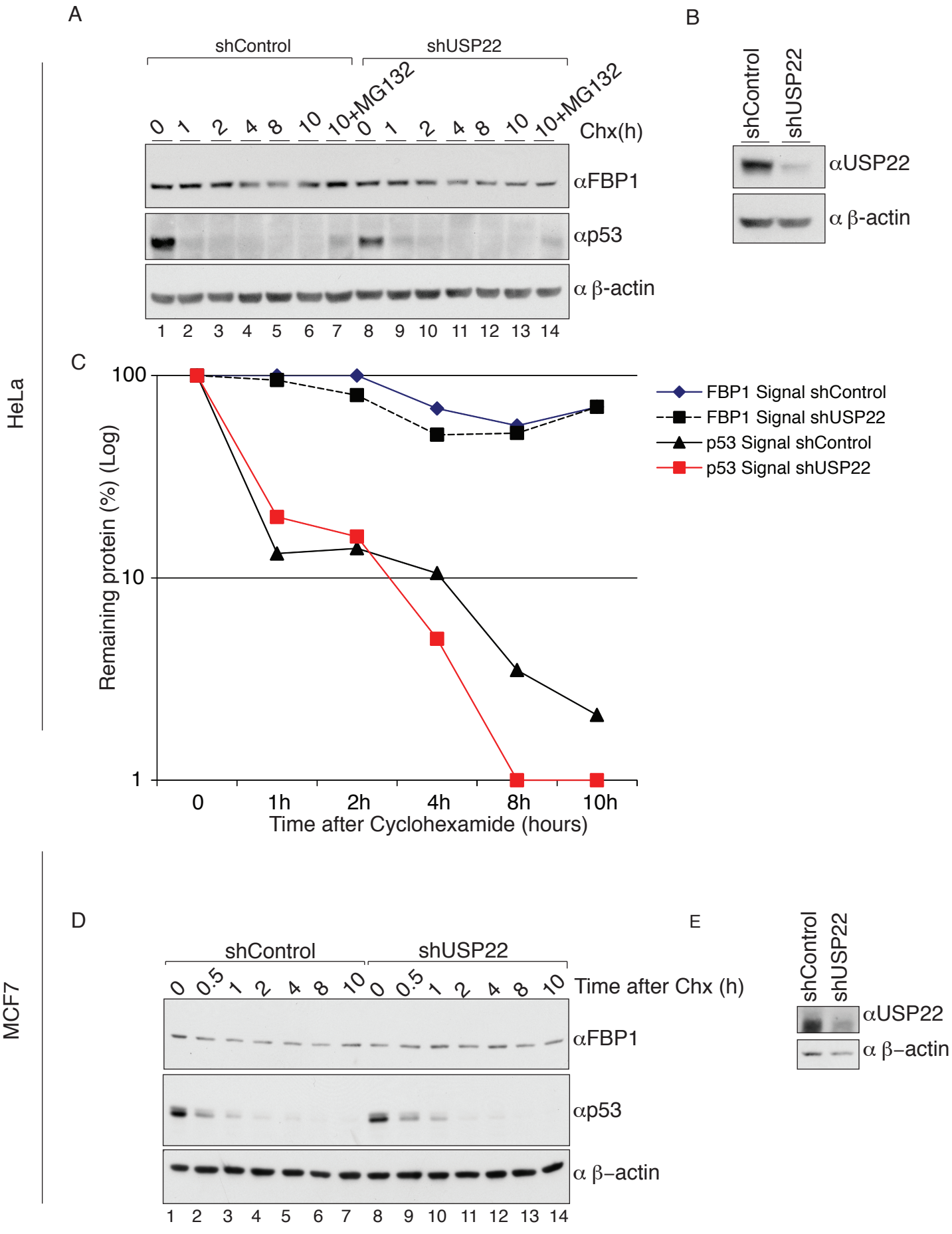
Peptides identified in the 6xHis-Ubiquitin precipitated fractions obtained from shControl and

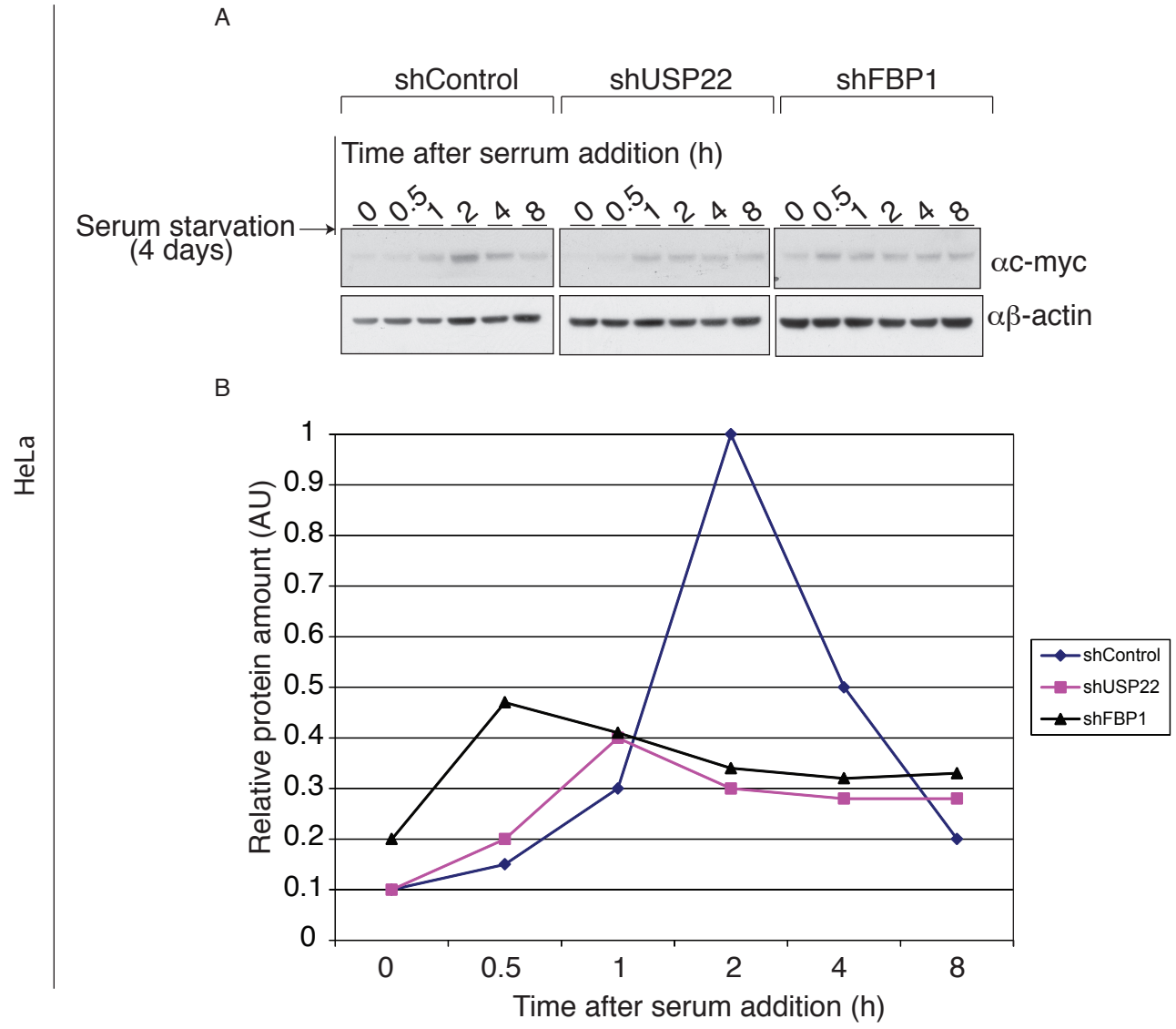
shUSP22 nuclear extracts

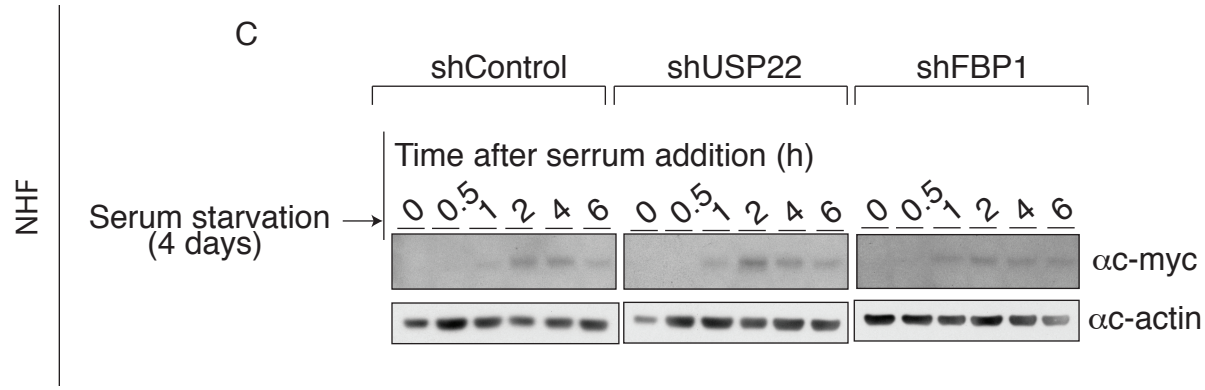
Supplementary references

Simpson, R.J. in “Proteins and Proteomics: A laboratory manual” Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 2003, pp 400-401


A
shControl
shUSP22
FLAG-FBP1
HA-Ub
αHA IP
188
98
Ub(n)-FBP1
Input
62
1 2 3 4
αFLAG (FBP1)
B
Input
αHA-IP
C185S-USP22
WT-USP22
pcDNA3.1
HA-Ub
Hi
Lo
Ub-FBP1
FBP1
αFLAG(USP22-WT/C185S)
C
Input
αFLAG IP
DNase I
FLAG-FBP1
αUSP22
αFLAG (FBP1)

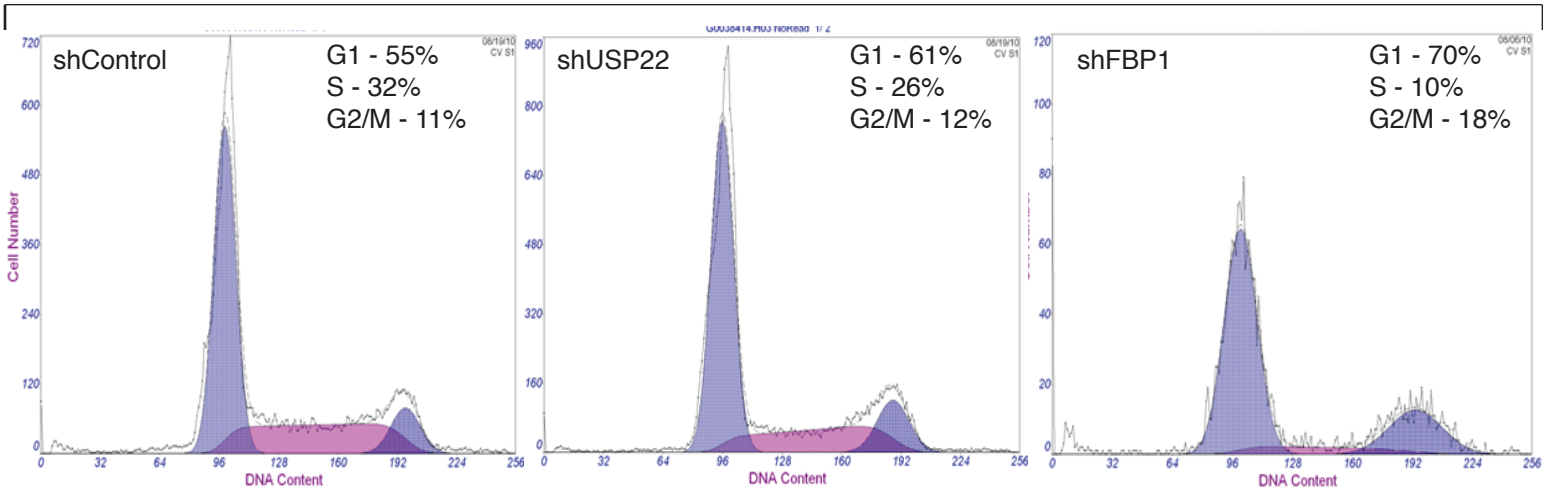

A
shControl
shUSP22
0 1 2 4 8 10 10+MG132 0 1 2 4 8 10 10+MG132
Chx(h)
αFBP1
αp53
α β-actin
1 2 3 4 5 6 7 8 9 10 11 12 13 14
B
shControl
shUSP22
αUSP22
α β-actin
HeLa
C
100
10
1
Remaining protein (%) (Log)
0 1h 2h 4h 8h 10h
Time after Cyclohexamide (hours)
FBP1 Signal shControl
FBP1 Signal shUSP22
p53 Signal shControl
p53 Signal shUSP22
MCF7
D
shControl
shUSP22
0 0.5 1 2 4 8 10 0 0.5 1 2 4 8 10
Time after Chx (h)
αFBP1
αp53
α β–actin
1 2 3 4 5 6 7 8 9 10 11 12 13 14
E
shControl
shUSP22
αUSP22
α β–actin


HeLa
A
shControl
shUSP22
shFBP1
Time after serrum addition (h)
0 0.5 1 2 4 8 0 0.5 1 2 4 8 0 0.5 1 2 4 8
Serum starvation
(4 days)
αc-myc
αβ-actin
B
Relative protein amount (AU)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0 0.5 1 2 4 8
Time after serum addition (h)
shControl
shUSP22
shFBP1


NHF
C
shControl
shUSP22
shFBP1
Time after serrum addition (h)
0 0.5 1 2 4 6 0 0.5 1 2 4 6 0 0.5 1 2 4 6
Serum starvation
(4 days)
αc-myc
αc-actin

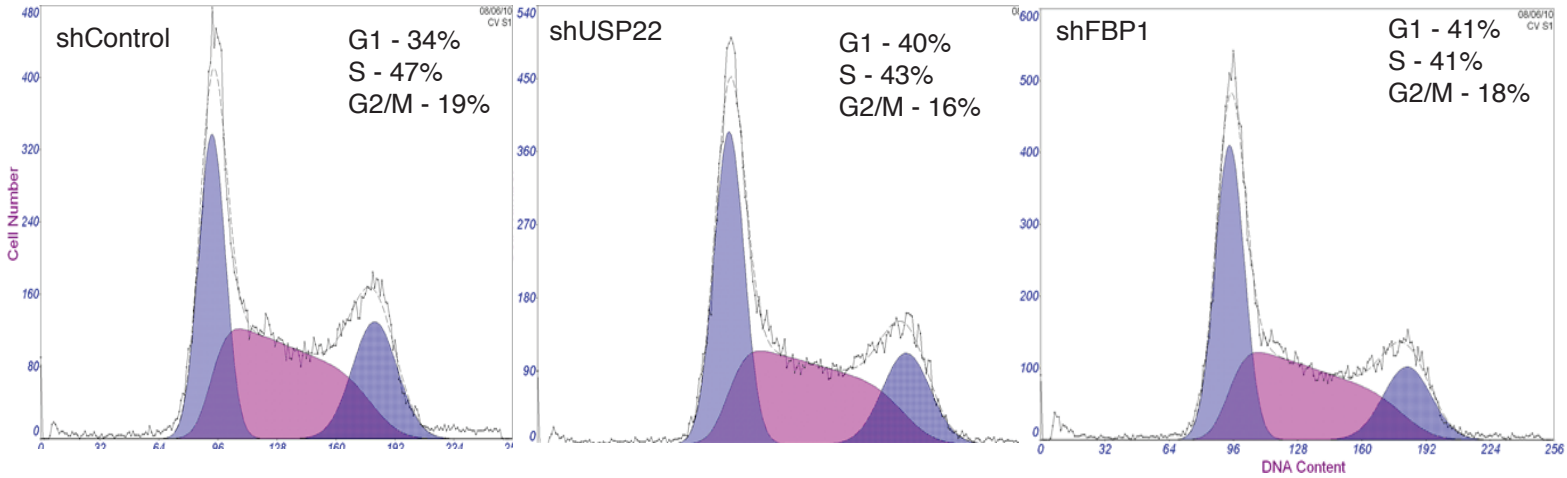
A

MCF7

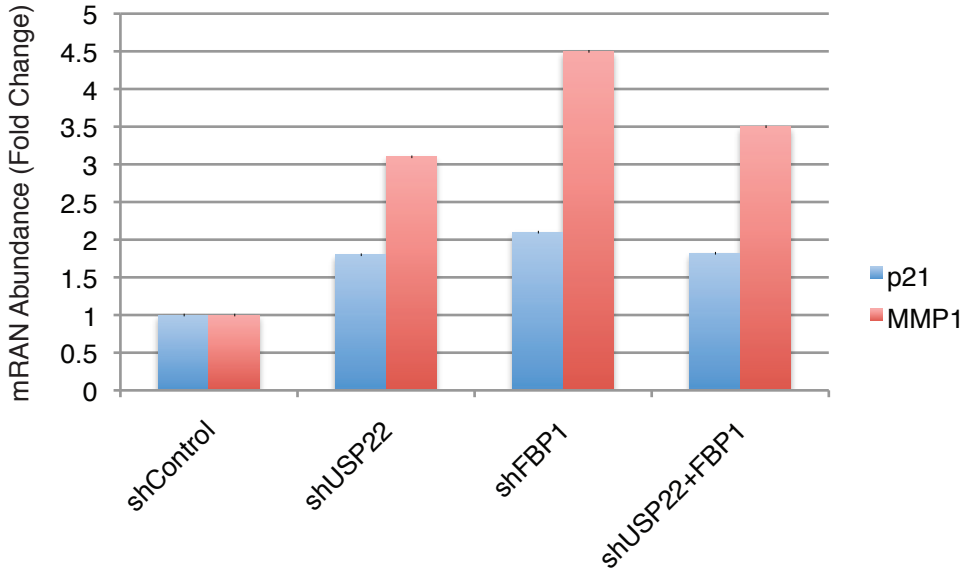


B

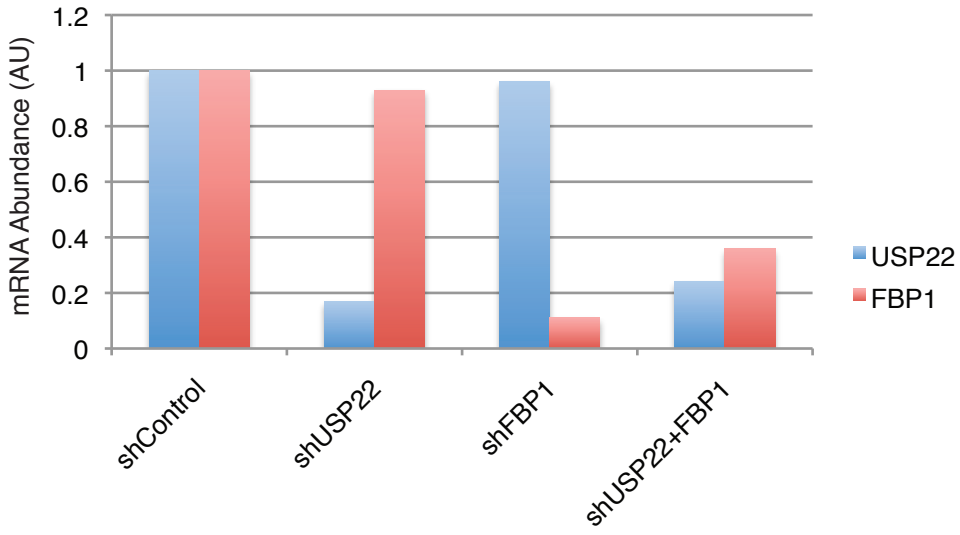
H1299



A


mRAN Abundance (Fold Change)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
shControl
shUSP22
shFBP1
shUSP22+FBP1
p21
MMP1

B


mRNA Abundance (AU)
1.2
1
0.8
0.6
0.4
0.2
0
shControl
shUSP22
shFBP1
shUSP22+FBP1
USP22
FBP1