S1 File: Supporting Information

Ribosomopathy-like Properties of Murine and Human Cancers

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Materials and methods

Selecting murine ribosomal proteins

The Mouse Genomics Informatics (MGI) website (http://www.informatics.jax.org) was queried for genes of type “protein coding gene” containing the name “ribosomal protein” without the strings “mitochondrial,” “kinase,” or “modification.” Ninety genes were identified, but two (Rpl32l and Rpl6l) were not found in the RNA-seq cufflinks output for the hepatoblastoma analysis. Three additional genes (Rpl10l, Rpl39l, Rpl3l) were excluded from the HB analysis due to insufficient counts, resulting in a list of 85 mouse cytoplasmic ribosomal proteins. A total of 82 cytoplasmic RP genes were included in the HCC analysis, as eight of the 90 total cytoplasmic RP genes possessed insufficient counts in the RNA-seq output.

Accessing TCGA RNA-seq and clinical data

TCGA data was accessed through the University of California Santa Cruz Cancer Browser (https://genome-cancer.ucsc.edu/proj/site/hgHeatmap/), downloading data listed under “gene expression (IlluminaHiSeq)” for each tumor type. Matched normal tissues for each cancer were identified by the following: 1) possessing at least two samples in the downloaded database,
one identified as “primary tumor” and one identified as “solid tissue normal” in the column “sample type”, and 2) presence of RNA-seq data for both samples. A list of 80 human ribosomal proteins was assembled from the University of Miyazaki’s Ribosomal Protein Gene Database (http://ribosome.med.miyazaki-u.ac.jp). When queried, three of these gene transcripts (RPL40, RPS30, and RPS4Y) were not found in the genomic data, so the final list used for the following analysis consisted of 77 RP genes. TCGA expression data, which is stored log-transformed, was base-two exponentiated for all samples.

**TCGA: survival curves**

To determine if RP transcript deregulation correlated with survival, this information was combined with clinical data from TCGA regarding days to death or last follow-up. Tumor samples meeting the following criteria were excluded from the survival analysis: 1) samples without corresponding clinical information, 2) samples with no recorded “days to death” or “days to last follow-up”, or 3) days to death or last follow-up less than or equal to zero. There were 357 tumor samples in the HCC cohort with corresponding clinical information, 25 of which were excluded from the survival analysis on these criteria. The CRC cohort contained 278 tumor samples with clinical information and 4 were excluded. The BC cohort contained 1082 tumors with clinical information, 18 of which were excluded. Survival analysis was not performed for the PC cohort, as the requisite clinical information was available for only 6 patients. Tumor samples were then sorted according to the severity of RP transcript deregulation and placed into the upper and lower quartiles. There were 83 tumors per quartile in the HCC cohort, 69 tumors per quartile in the CRC cohort, and 266 tumors per quartile in the BC cohort. Five-year survival curves were generated comparing the top and bottom quartiles in each cohort, with significance
determined by a Log-rank test P-value < 0.05. Survival differences were significant in HCC (P = 0.0435) and BC (P = 0.0046).

TCGA: mutation analysis

TCGA mutation information was accessed using cBioPortal (http://www.cbioportal.org/), from the “TCGA, Provisional” data for each cancer type. Each data set was queried for RP coding mutations in any tumor sample. A literature search was performed in order to classify these observed mutations into three general categories: mutations in RPs previously implicated in a ribosomopathy, mutations identical to those previously identified in a ribosomopathy, and all other mutations. The literature search included the LOVD Diamond-Blackfan Anemia database (http://dbagenes.unito.it/home.php) as well as PubMed searches of each individual ribosomal protein gene identifier.

Quantification of rRNA processing

Total RNAs were purified using RNeasy columns (Qiagen, Inc. Valencia, CA) and then digested with TURBO-DNA free DNase as recommended by the supplier (Thermo-Fisher, Pittsburgh, PA). RNA concentrations were determined with a Nanodrop ND-1000 instrument (NanoDrop Technologies Inc., Wilmington, DE, USA) and RNA integrity was evaluated with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). RNA integrity number (RIN) values for all samples in the HCC mouse model and HB tumors >9 and those for HB control livers were >7.5.
To assess rRNA processing intermediates, we quantified 18S-ITS1, ITS1-5.8S, 5.8S-ITS2 and ITS2-18S junctions as depicted in Fig 2A using a Power SYBR® Green RNA-to-CT™ 1-Step Kit (Thermo-Fisher) on a StepOnePlus™ Real-Time PCR System (Thermo-Fisher). PCR primers (IDT, Inc., Coralville, IA) were selected from the murine 47S rRNA Gen Bank sequence BK000964.3 and comprised the following sequences:

18S-ITS1 (Fwd): 5’-AAGACGGGCGTAATTGACTATCTCT-3’ (nt 5797)

(54): 5’-GCCGCGGCTCCCTCCACAGTCTC-3’ (nt 5900)

ITS1-5.8S (Fwd): 5’-CCCGTGAGGTTGCTCAGCACCG-3’ (nt 6844)

(54): 5’-CGCAGCTAGCTGCGTTCTTTCTCATCGA-3’ (nt 6934)

5.8S-ITS2 (Fwd): 5’-TTGATCATCGACACTTCGAACGCAC-3’ (nt 6961)

(54): 5’-CGCAGCGGGTGACGCGATTGAT-3’ (nt 7059)

ITS2-28S (Fwd): 5’-CCTGAGACGCGGCTGCCCGGCTCGT-3’ (nt 8012)

(54): 5’-ACCGGGTGCCACGTCTGATCTGA-3’ (nt 8153)

For each set of PCR primers, reactions were performed in triplicate on 5-8 liver or tumor samples. The results of each set of reactions were normalized to total 18S and 28S rRNA content, which were also obtained from triplicate reactions run in parallel. Control primers consisted of the following sets of oligonucleotides:

18S (Fwd): 5’-CTGAGAAACGGCTACCACATC-3’ (nt 4450)

(54): 5’-GCCTCGAAAGAGTCTGTATTG-3’ (nt 4556)

28S (Fwd): 5’-GTAACGCGCGGAGTAACTATG-3’ (nt 11522)

(54): 5’-GACAGTTGGGAATCTCGTTCATC-3’ (nt 11619)
Each reaction (20 µL in a 96-well fast plate) consisted of 10 ng of RNA template, 0.16 µL RT Enzyme Mix, 10 µL the above-described RT-PCR Mix, 3.84 µL nuclease-free water and 1 µL of primer solution containing both forward and reverse primers at a concentration of 100 ng/µL each. Run conditions for RT-PCR reactions consisted of a 48°C hold for 30 minutes to catalyze reverse transcription followed by a 10-minute hold at 95°C. PCR conditions comprised 40 cycles of a 1 min 95°C melting period followed by a 1-minute 65°C annealing and extension period. Each sample was assayed in triplicate with variances seldom exceeding 5%. P-values were determined using Welch’s t-test.

**Tissue fractionation immuno-precipitation and immuno-fluorescent staining**

All buffers were supplemented with standard protease and phosphatase inhibitors. Tissue was first washed with ice-cold PBS, minced into small pieces and then homogenized in cytoplasmic extraction buffer (200-250 mg tissue/2 ml of buffer). Cell breakage was monitored under a microscope. Homogenates were centrifuged at 500x g for 5 min at 4°C and the resultant supernatants were used as the cytoplasmic fraction. Pellets were further subjected to membrane extraction buffer followed by centrifugation at 3000x g for 5 min at 4°C to pellet nuclei. Nuclear pellets were divided into two halves. One half was used to solubilize nuclei and release chromatin-bound proteins by extracting with nuclear extraction buffer containing 5 mM CaCl$_2$. The other half was processed to isolate nucleoli as described (http://www.lamondlab.com). For this step, nuclear pellets were re-suspended in a buffer containing 10 mM Hepes, pH 7.9; 0.35 M sucrose and 0.5 mM MgCl$_2$ and sonicated on ice for 6 x 10 sec bursts with 10 sec cooling intervals. Sonicated lysates were layered over a buffer containing 10 mM Hepes, pH 7.9; 0.88 M sucrose and 0.5 mM MgCl$_2$ and centrifuged at 3000x g for 10 min at 4°C. Nucleolar pellets thus
obtained were solubilized in a buffer containing Tris HCl, pH 8.0; 20 mM NaCl; 1 mM EDTA; 0.5% NP40 and 25 mM NaF. Fractions were analyzed by immuno-blotting for GAPDH (cytoplasmic marker), histone H3 (nuclear marker), fibrillarin (nuclear and nucleolar marker). Expression of p53, Mdm2, and p19\textsuperscript{ARF} was assessed by immuno-blotting across each of the fractions following SDS-PAGE.

For immuno-precipitations, freshly isolated cytoplasmic liver and tumor fractions were diluted to a final protein concentration of 3 mg/ml in “IP buffer” containing Tris HCl, pH 8.0; 20 mM NaCl; 1 mM EDTA; 0.5% NP40 and 25 mM NaF supplemented with protease and phosphatase inhibitors and subjected to two rounds of pre-clearing. The first round consisted of rocking 1 ml of lysate with 20 µl Protein G PLUS-agarose beads (Santa Cruz Biotechnology) for 1 h at 4\textdegree{}C followed by centrifugation at 4000 rpm, 4\textdegree{}C for 5 min, to remove agarose beads. The second round of clearing was performed following the addition of 20 µl of isotype specific IgG1-agarose conjugate (LifeSpan BioSciences inc., Seattle, WA) to the pre-cleared lysate for 1 h at 4\textdegree{}C. Upon a brief centrifugation to again remove the agarose-conjugate, the fractions were equally divided. One portion was incubated with 20 µl mouse IgG1-agarose conjugated beads while the other part was incubated with Mdm2 antibody agarose-conjugated beads (Santa Cruz Biotechnology) overnight at 4\textdegree{}C with gentle shaking. Beads were washed four times for 1 h each time at 4\textdegree{}C with IP buffer to remove any unbound protein, followed by re-suspension in SDS sample buffer and denaturation at 95\textdegree{}C for 4 min. Immunoprecipitates were further analyzed by gel electrophoresis and silver staining.

Immunofluorescent staining (Fig. 5C) was performed on liver and tumor frozen sections. Fresh tissues were first fixed in PBS-4% paraformaldehyde for 2-4 followed by an overnight incubation in PBS-40% sucrose at 4\textdegree{}C. The fixed tissues were then embedded in Tissue Plus
O.C.T. Compound (SciGen Scientific, Gardenas, CA), frozen on dry ice and stored at -80°C. Frozen tissues were cryo-sectioned, stained with antibodies against Mdm2 or p53 (Table S1) and counterstained with 4,6-Diamidino-2-phenylindole, dihydrochloride (DAPI) (1 µg/ml) for 5 min. Images were obtained on an Olympus Fluoview FV1000 confocal microscope. The nuclear: cytoplasmic distribution of Mdm2 and p53 were determined and quantified using the Particle Analysis program of Image J software (https://imagej.nih.gov/ij/).

**Mass spectrometry**

Briefly, excised gel bands were washed with HPLC water and de-stained exhaustively with 50% acetonitrile (ACN)/25 mM ammonium bicarbonate. Gel pieces were dehydrated with 100% ACN, reduced with 10 mM dithiothreitol (DTT) at 56°C for 1 hour, followed by alkylation with 55 mM iodoacetamide (35) at room temperature for 45 min in the dark. Gel pieces were then again dehydrated with 100% ACN to remove excess DTT and IAA, and rehydrated with 20 ng/µl trypsin/25 mM ammonium bicarbonate and digested overnight at 37°C. The resultant tryptic peptides were extracted with 70% ACN/5% formic acid, vacuum dried and re-constituted in 18 µl 0.1% formic acid.

Proteolytic peptides were analyzed by a nanoflow reverse-phased liquid chromatography tandem mass spectrometry (LC-MS/MS). They were loaded onto a C18 PicoChip™ column packed with 10.5 cm of Reprosil C18 3 µm 120Å chromatography media with a 75 µm ID column and a 15 µm tip (New Objective, Inc., Woburn, MA) using a Dionex HPLC system (Dionex Ultimate 3000, ThermoFisher Scientific, San Jose, CA) operated with a double-split system (Dr. Steven Gygi from Department of Cell Biology, Harvard Medical School, personal communication) to provide an in-column nano-flow rate (~300 nl/min). Mobile phases used were
0.1% formic acid for A and 0.1% formic acid in acetonitrile for B. Peptides were eluted off the column using a 52 min gradient (2-40% B in 42 min, 40-95% B in 1 min, 95% B for 1 min, 2% B for 8 min) and injected into a linear ion trap MS (LTQ-XL, ThermoFisher Scientific) through electrospray.

The MS instrument was operated in a date-dependent MS/MS mode in which each full MS spectrum was followed by MS/MS scans of the 5 most abundant molecular ions determined from full MS scan (acquired based on the setting of 1000 signal threshold, 10000 AGC target, 100 ms maximum accumulation time, 2.0 Da isolation width, 30 ms activation time and 35% normalized collision energy). Dynamic exclusion was enabled to minimize redundant selection of peptides previously selected for CID.

MS/MS spectra were searched using MASCOT search engine (Version 2.4.0, Matrix Science Ltd, London, UK) against the UniProt mouse proteome database. The following modifications were used: static modification of cysteine (carboxyamidomethylation, +57.05 Da), variable modification of methionine (oxidation, +15.99 Da). The mass tolerance was set at 1.4 Da for the precursor ions and 0.8 Da for the fragment ions. Peptide identifications were filtered using PeptideProphet™ and ProteinProphet® algorithms (LabKey, Inc. Seattle, WA) with a protein threshold cutoff of 99% and peptide threshold cutoff of 90% implemented in Scaffold™ (Proteome Software, Portland, Oregon, USA).
Figures

Figure A

Relative Percent Expression of RP Transcripts: WT Hepatocytes vs. HBs

No. deregulated = 35

Figure B

Relative Percent Expression of RP Transcripts: KO Hepatocytes vs. HBs

No. deregulated = 41
Figure B

Relative Percent Expression of RP Transcripts: Day 3 vs Liver

No. deregulated = 0

Liver
Day 3

Relative Percent Expression of RP Transcripts: Day 7 vs Liver

No. deregulated = 3

Liver
Day 7

Relative Percent Expression of RP Transcripts: Tumor vs Liver

No. deregulated = 58

Liver
Tumor

Relative Percent Expression of RP Transcripts: Regression Day 3 vs Liver

No. deregulated = 48

Liver
Regression Day 3

Relative Percent Expression of RP Transcripts: Regression Day 7 vs Liver

No. deregulated = 44

Liver
Regression Day 7

Relative Percent Expression of RP Transcripts: Recurrent Tumor vs Liver

No. deregulated = 56

Liver
Recurrent Tumor
Figure C
Figure D

All HCC RP Transcript Variation
N = 373
Figure E

All CRC RP Transcript Variation
N = 288

Percent Deviation from Mean

All CRC Tumors

RP Transcripts
Figure F

All BC RP Transcript Variation
N = 1102
Figure G

All PC RP Transcript Variation

N = 497

Percent Deviation from Mean

All PC Tumors

RP Transcripts
Figure H

Ribosomal biogenesis → Ribosomal stress → \( p19^{ARF} \) → Free RPs → Mdm2 → p53 → Cell cycle arrest/apoptosis
Figure 1

| Group 1 | Group 2 | Group 3 |
|---------|---------|---------|
| MYC: ON | MYC: ON | MYC: ON |
| Liver   | Liver   | Liver   |
| 3D      | 3D      | 3D      |
| 7D      | 7D      | 7D      |
| Tumor   | Tumor   | Tumor   |
| 3R      | 3R      | 3R      |
| 7R      | 7R      | 7R      |
| PT      | PT      | PT      |

- p19<sup>ARF</sup>
- Mdm2
- p53
- GAPDH
Figure J
Figure K
Figure L

1. P62908 (100%), 26,674.5 Da
RS3_MOUSE 40S ribosomal protein S3 OS=Mus musculus GN=Rps3 PE=1 SV=1
8 exclusive unique peptides, 9 exclusive unique spectra, 16 total spectra, 94/243 amino acids (39% coverage)

2. P62242 (100%), 24,206.4 Da
RS8_MOUSE 40S ribosomal protein S8 OS=Mus musculus GN=Rps8 PE=2 SV=2
5 exclusive unique peptides, 5 exclusive unique spectra, 8 total spectra, 60/208 amino acids (29% coverage)

3. P62702 (100%), 29,599.3 Da
RS4_MOUSE 40S ribosomal protein S4, X isoform OS=Mus musculus GN=Rps4a PE=2 SV=2
5 exclusive unique peptides, 7 exclusive unique spectra, 12 total spectra, 66/263 amino acids (25% coverage)

4. P62754 (100%), 28,681.7 Da
RS6_MOUSE 40S ribosomal protein S6 OS=Mus musculus GN=Rps6 PE=1 SV=1
3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 32/249 amino acids (13% coverage)

5. P92351 (100%), 29,885.3 Da
RS3A_MOUSE 40S ribosomal protein S3a OS=Mus musculus GN=Rps3a PE=1 SV=3
3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 34/204 amino acids (17% coverage)

6. P47963 (100%), 24,306.4 Da
RL13_MOUSE 40S ribosomal protein L13 OS=Mus musculus GN=Rpl13 PE=2 SV=3
2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 19/211 amino acids (9% coverage)

7. P62918 (100%), 28,024.8 Da
RL8_MOUSE 40S ribosomal protein L8 OS=Mus musculus GN=Rpl8 PE=2 SV=2
2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 18/257 amino acids (7% coverage)
Figure N

1. P62270 (100%), 17,719.3 Da
RS18_MOUSE 405 ribosomal protein S18 OS=Mus musculus GN=Rps18 PE=1 SV=3
6 exclusive unique peptides, 6 exclusive unique spectra, 7 total spectra, 58/152 amino acids (38% coverage)
M S L V I P E K G Q H L R V N T N I D C R G R I A A A L T A I R C V C R R Y A H V V L R K A D I
D E A A L V R S V S W M Q R R N T R K R D K V K D G E S O S O L A N G L D N L R E D L E R L K K I R A H R G L R F W G L R V R G Q T K T G C R R G R T V G V S K

2. P15097 (100%), 17,805.0 Da
RL12_MOUSE 605 ribosomal protein L12 OS=Mus musculus GN=Rpl12 PE=1 SV=2
4 exclusive unique peptides, 4 exclusive unique spectra, 4 total spectra, 48/165 amino acids (29% coverage)
M P P K F D P E V N K V V Y L R E T C G C E V G A S L A K P K C K L F L S P K K V G D D I A K A T G D W G K L R I T V K L T I G N K O A O I E V E V P S A S A L I L R E K E P P D R K E R N K I R H S N K T F D E I V N I A R O M N H S R A L E S C T I K E K L O T A Q S V G C N V D G R H P H D
I D D I N S G A V E C P A S

3. E9Q132 (100%), 15,347.5 Da
E9Q132 MOUSE 605 ribosomal protein L24 OS=Mus musculus GN=Rpl24 PE=2 SV=1
4 exclusive unique peptides, 5 exclusive unique spectra, 5 total spectra, 30/133 amino acids (23% coverage)
M V K E L C S F S G Y S V Y P G C R K Y A R T D G K V E O F L N A C E S A I F L S K R N P R O I N
W T V V L R K H S K G O D S E F O K R T R A V E V F O E T A T G A S L A D I M A R H N Q K P E V
R K A Q E R Q I A R A A K R A K A K Q A S K T A M A A A A K V Y

4. Q0CW4 (100%), 20,253.2 Da
RL11_MOUSE 605 ribosomal protein L11 OS=Mus musculus GN=Rpl11 PE=1 SV=4
3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 39/178 amino acids (22% coverage)
W Q O D O C E K N P M K E R I R K L C L N I C E V C E G D B L T A A K V I F E O L G O T P V F S K R A Y T V E S S F G I R N E K I A V M C T V R G A A K E I L E K G L K V R E Y E R R N N S F D T G N F C G C I O E H I D L G I K D P S I G I Y L C D F Y V V L G R P G F S I A D K K R T G C I G A H R I S K E E A M R W F Q Q Y D G I I L P G K

5. P62301 (100%), 17,223.2 Da
RS13_MOUSE 405 ribosomal protein S13 OS=Mus musculus GN=Rpl13 PE=2 SV=2
3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 30/151 amino acids (20% coverage)
M C R H M A P P C G L S O S A L P Y R E R S V P T W L K L T S D D V K E O I Y K L A K G L T P S Q I G D W G K L R I T V K L T I G N K O A O I E V E V P S A S A L I L R E K E P P D R K E R N K I R H S N K T F D E I V N I A R O M N H S R A L E S C T I K E K L O T A Q S V G C N V D G R H P H D
H L E R N R K D D K A K F R E L I E T S K M H R L A R Y Y K T K R Y L P P N W K Y E S T A S A L V A

6. P62911 (100%), 15,860.4 Da
RL32_MOUSE 605 ribosomal protein L32 OS=Mus musculus GN=Rpl32 PE=2 SV=2
2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 27/135 amino acids (20% coverage)
M A A L R P I V K P K I V K E R T K K F I R H O S D R Y V K I K R N W R K P R G I D N R V B R R F K G O I L M P N G Y C O S N R K T K H M L P S G T R K F L V H V N K E L E V L L M C N K S Y C A E I A H V N S S N K A A I V E R A A Q L A I R V T P N A R L R E E N E

7. B1AAR3 (100%), 12,217.1 Da
B1AAR3_MOUSE 605 ribosomal protein L26 (Fragment) OS=Mus musculus GN=Rpl26 PE=2 SV=1
3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 18/103 amino acids (17% coverage)
M K F N P F F V T V D R S K N K R H N F A P S H I R K I K S S P L S K E L R D K Y V N V S M P I R K O D E V E Q V V K H Y K G Q D Q G I K V Y Q V Y R K K V Y K Y I E R V R E K A G N T T V V H G I H P S K

8. P14115 (100%), 16,605.4 Da
RL37A_MOUSE 605 ribosomal protein L27a OS=Mus musculus GN=Rpl27a PE=2 SV=5
2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 23/148 amino acids (16% coverage)
M P S L B R E T K R L R H V S H Q H C R I C K K H R K P G C R N A G C M H M H R I N D K H Y H P G V C E V C M H R Y L H C S M O S G O D V N M U L T E D V T R N A A V N R N E K I F I D V V Y S V Y K L G V K G E L P Q K P S P V I V K A K F F S R A A E R I K G V G G A C V L V A

9. P62852 (100%), 13,743.0 Da
RS25_MOUSE 405 ribosomal protein S25 OS=Mus musculus GN=Rps25 PE=2 SV=1
2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 18/125 amino acids (14% coverage)
M P P E D D K K K K D A C K S A D D K D K D P V N K S C C K C A K K K W S Y K C K V R D X E E N N L V L E D R A T Y D K L K C K E V P Y K L I T P R V S E R L K I R G S L R A A A Q E L L S K C L I K L V S K H R A Q V I Y T R N T K G D A P A A G E D A

10. P63323 (100%), 18,016.3 Da
RS10_MOUSE 405 ribosomal protein S10 OS=Mus musculus GN=Rps10 PE=1 SV=1
2 exclusive unique peptides, 2 exclusive unique spectra, 2 total spectra, 23/165 amino acids (14% coverage)
M P P K N K R Y N R H L R P C G V M U A K R D V H M P K H P E L A D E N V P N L V V W E A M O D S E K S B G Y V Q E P A W N H F F W Y T L N E G O I Y L D V Y L T L P F E I V P A T R I S S E B P E P T P E P F E L P E F F E L P A F T R G E A D K D Y R K S A V P P C A D K X F A G A C A T T E F E Q G R C G G F R G R G O P P Q

11. P62281 (100%), 18,431.3 Da
RS11_MOUSE 405 ribosomal protein S11 OS=Mus musculus GN=Rps11 PE=2 SV=1
3 exclusive unique peptides, 3 exclusive unique spectra, 3 total spectra, 19/158 amino acids (12% coverage)
M A D I O T E R A V O K O P T I F O N K K R V L L C G T C K E K L P R Y Y K N I G L G F K P T P E A K E H E G T Y D K K C P T F Q N V S I R G R L S G V T K K M K R T I V I R D L Y H Y R K Y N R F K E R K N S V H L S P F C R D V Q I G D I V T V C E C R P L S K T V R F N L V K T K A C T K Q F Q X F
Figure legends

Figure A. Quantification of RP transcript relative expression in murine HBs. (A) Differential abundance of RP transcripts between WT hepatocytes and WT HBs. Data are derived from the RNA-seq results shown in Fig 1A. Actual fractional abundance is shown with the total adding to 100%. Asterisks indicate those transcripts in HBs whose abundance relative to that of the same transcript in hepatocytes differs (q-value <0.05 after FDR-adjustment). Error bars indicate one standard deviation. (B) The same representation as shown in (A) for KO hepatocytes and HBs.

Figure B. Quantification of RP transcript relative expression over the course of HCC induction, regression and recurrence. Data are presented as described in Figure A. Each time point is compared to normal liver results. (Asterisks indicate p<0.05 after FDR-adjustment).

Figure C. Additional immuno-blots of RPs from representative tissue samples over the course of HCC induction, regression and recurrence. Blots were performed as described for Fig 1F. Three additional groups are shown here.

Figure D. 3D Area Plot depicting RP transcript deregulation in human HCCs. The data shown include the matched HCC samples depicted in Fig 3A as well as 323 additional unmatched samples. F-tests were performed comparing variance in relative expression for each RP transcript across the normal matched liver samples and the HCC tumors in order to determine if the variability in RP transcript expression was significantly different across tumors compared
to normal liver. F-tests were significant for 69 of 77 RPs after FDR adjustment. The RP genes Rps26, Rpl9, Rps27, Rps28, and Rpl21, with the lowest expression and greatest variability (without significant F-tests), were excluded from the graph in order to appreciate differences in the other transcripts.

**Figure E. 3D Area Plot depicting RP transcript deregulation in human CRCs.** Data are presented as described in Figure D and include the matched tumor samples shown in Fig 3B. F-tests were significant in 48 of 77 RPs, and the same five transcripts were excluded from the graph.

**Figure F. 3D Area Plot depicting RP transcript deregulation in human breast cancers.** Data are presented as described in Figure D and include the matched tumor samples shown in Fig 3C. F-tests were significant in 71 of 77 RPs, and the same five transcripts were excluded from the graph.

**Figure G. 3D Area Plot depicting RP transcript deregulation in human prostate cancers.** Data are presented as described in Figure D and include the matched tumor samples shown in Fig 3D. F-tests were significant in 23 of 77 RPs, and the same five transcripts were excluded from the graph.

**Figure H. The relationship between ribosomal stress and the p19ARF/Mdm2/ p53 pathway.** The complex and highly regulated process of ribosomal biogenesis [21, 22, 26] can be disrupted
by RP haplo-insufficiency, leading to ribosomal stress [1, 2, 4]. In response, p19<sup>ARF</sup> is induced and inhibits further ribosomal biogenesis by blocking the nucleolar export of 40S and 60S ribosomal subunits [3, 8, 55]. Independently, p19<sup>ARF</sup> can inhibit Mdm2 and thus prevent the latter protein’s binding to and promoting the ubiquitin-mediated degradation of p53 [30]. A subset of free RPs can also bind the p53-recognition domain of Mdm2 and prevent or disrupt the Mdm2-p53 interaction [32]. Additionally, at least one RP, namely RPS26, has recently been shown to interact directly with p53 and supplement its transcriptional activity [33].

**Figure I. Immuno-blotting for p19<sup>ARF</sup>, Mdm2 and p53 in additional HCCs.** Studies were performed as described in Fig 5B.

**Figure J. Mdm2-interacting RPs identified in HCCs.** (A) p53 and Mdm2 co-localize to HCC cytoplasm. A freshly collected HCC tumor was fractionated into cytoplasmic, nuclear and nucleolar compartments. Each fraction was tested for the protein markers localizing to these compartments (GAPDH, histone H3 and fibrillarin, respectively) and in parallel for p53, p19<sup>ARF</sup> and Mdm2. Varying amounts of lysate and exposure times were required to compensate for differential expression of the proteins. (B) Liver and HCC cytoplasmic fractions were immuno-precipitated with control IgG or anti-Mdm2 IgG. Precipitates were resolved by SDS-PAGE and silver stained. Bracketed regions were excised from HCC MDM2-IP lane and subjected to tryptic digestion and mass spectrometry. (C) A Venn diagram showing the comparison of RPs detected in MDM2-IPs between liver, HBs and HCCs. Eight Mdm2-interacting RPs were common with HB while an additional nine were unique to HCCs shown also in Table E and Figures O and P.
**Figure K.** Mdm2-interacting RP peptides identified by in HB cytoplasmic lysates following anti-Mdm2 IP (~24-35 kDa range). HB cytoplasmic extracts were immuno-precipitated with agarose-linked anti-Mdm2 antibody. After release of the Mdm2-interacting proteins and resolution by SDS-PAGE, the silver-stained region of the gel corresponding to the ca. 24-35 kDa region of lane 4 (Fig 5E, red bracket) was excised and subjected to tryptic digestion and mass spectrometric analysis as described in Materials and methods. Peptides identified in the analysis are highlighted in yellow on their corresponding full-length RP. The coverage for each RP ranged from 10-64%. Each of the 12 RPs listed here is also listed in S2 Table.

**Figure L.** Mdm2-interacting RP peptides identified by MS in liver cytoplasmic lysates following anti-Mdm2 IP (~24-35 kDa range). An analysis identical to that described in S3 Table was performed the portion of the gel denoted by the red band depicted in Fig 5E. The coverage for each RP ranged from 7-39%. Each of the 7 RPs listed here is also listed in Table B.

**Figure M.** Mdm2-interacting RP peptides identified by MS in HB cytoplasmic lysates following anti-Mdm2 IP (~14-24 kDa range). Tryptic peptides corresponding to the indicated 17 RPs in the ~14-24 kDa range identified by mass spectrometry lane 4, Fig 5E (blue bracket) and listed in Table D. Each identified peptide is indicated by yellow highlighting and is mapped to its corresponding region in the sequence of the full-length RP. The coverage for each RP ranged from 13-59%.
Figure N. Mdm2-interacting RP peptides identified in liver cytoplasmic lysates following anti-Mdm2 IP (~14-24 kDa range). Tryptic peptides corresponding to the indicated 11 RPs in the ~14-24 kDa range identified by mass spectrometry (Fig 4E, lane 2, blue bracket) and listed in Table D are indicated by yellow highlighting and is mapped to its corresponding region in the sequence of the full-length RP. The coverage for each RP ranged from 12-38%.

Figure O. Mdm2-interacting RPs identified by MS in HCC cytoplasmic lysates following anti-MDM2-IP (~24-35 kDa range). HCC cytoplasmic extracts were immuno-precipitated with agarose-linked anti-Mdm2 antibody. After release of the Mdm2-interacting proteins and resolution by SDS-PAGE, the silver-stained region of the gel corresponding to the ca. 24-35 kDa region of HCC MDM2-IP lane (Figure J, red bracket) was excised and subjected to tryptic digestion and mass spectrometric analysis as described in Materials and methods. Peptides identified in the analysis are highlighted in yellow on their corresponding RP. The coverage for each RP ranged from 7-56%. Each of the 10 RPs listed here is also listed in Table E.

Figure P. Mdm2-interacting RP peptides identified by MS in HCC cytoplasmic lysates following anti-Mdm2 IP (~14-24 kDa range). Tryptic peptides corresponding to the indicated 24 RPs in the ~14-24 kDa range as identified by mass spectrometry from MDM2-IP lane, Figure J (blue bracket) are listed in Table E. Each identified peptide is indicated by yellow highlighting and is mapped to its corresponding region in the sequence of the full-length RP. The coverage for each RP ranged from 8-39%.
## Tables

### Table A

| Antibody         | Species  | Vendor and catalog No.                           | Dilution used |
|------------------|----------|-------------------------------------------------|---------------|
| RPS10            | Rabbit   | GeneTex (GTX101836)                             | 1:1000        |
| RPS19            | Rabbit   | GeneTex (GTX54725)                              | 1:1000        |
| RPS24            | Rabbit   | GeneTex (GTX47408)                              | 1:1000        |
| RPS26            | Rabbit   | Proteintech (14909-1-AP)                        | 1:1000        |
| RPS27            | Goat     | OriGene (TA302828)                              | 1:1000        |
| RPL5             | Rabbit   | GeneTex (GTX101821)                             | 1:1000        |
| RPL11            | Rabbit   | Abcam (79352)                                   | 1:1000        |
| RPL26            | Rabbit   | Bethyl Laboratories (A300-685A-T)                | 1:1000        |
| RPL30            | Rabbit   | GeneTex (GTX87885)                              | 1:500         |
| BCL-2            | Rabbit   | Cell Signaling (28700)                          | 1:300         |
| GAPDH            | Mouse    | Sigma-Aldrich (G8795)                           | 1:20,000      |
| HRP anti-mouse   | Horse    | Cell Signaling (7076)                           | 1:10,000      |
| HRP anti-rabbit  | Goat     | Cell Signaling (7074)                           | 1:5000        |
| Alexa Fluor 488 anti-rabbit | Goat | Thermo Fisher (A-11008) | 1:1000 |
| AC-Histone H3    | Rabbit   | Santa Cruz (SC8655-R)                           | 1:500         |
| Fibrillarin      | Rabbit   | Cell Signaling (2639)                           | 1:1000        |
| p19 ARF          | Rabbit   | Santa Cruz (SC22784)                            | 1:500         |
| MDM2             | Mouse    | Santa Cruz (SC965)                              | 1:1000        |
| P53              | Mouse    | Calbiochem(OP03)                                | 1:1000        |
| Mdm2 (for immunostain) | Rabbit | Abcam (Ab38618)                               | 1:200         |
| P53 (for immunostain) | Goat | Santa Cruz, (SC6243-G)                       | 1:50          |
| BAX              | Rabbit   | Cell signaling (2772)                           | 1:1000        |
| Caspase-2        | Mouse    | Santa Cruz (SC514472)                           | 1:500         |
| Gene   | Cohorts       | Average Expression Difference vs. Normal Tissues |
|--------|---------------|-----------------------------------------------|
| Rpl36a | HCC, BC, CRC, PC | 131.40%                                       |
| Rpl28  | HCC, BC, PC    | 40.24%                                        |
| Rps21  | HCC, BC, CRC, PC | 33.41%                                       |
| Rpl8   | HCC, BC, CRC, PC | 33.17%                                       |
| Rpl30  | HCC, BC, CRC   | 28.20%                                        |
| Rps2   | HCC, BC, CRC, PC | 27.62%                                       |
| Rpl39  | HCC, CRC, PC   | 26.04%                                        |
| Rpl36  | BC, CRC, PC    | 21.94%                                        |
| Rps19  | HCC, BC, CRC, PC | 21.94%                                       |
| Rpl38  | HCC, BC, CRC, PC | 18.37%                                       |
| Rplp0  | HCC, BC, PC    | 12.76%                                        |
| Rpl23  | HCC, BC, CRC, PC | 11.15%                                       |
| Rps16  | HCC, BC, PC    | 10.97%                                        |
| Rpl23a | HCC, BC, PC    | 10.16%                                        |
| Rps10  | HCC, BC, PC    | 9.02%                                         |
| Rpl37  | HCC, BC, CRC, PC | 8.95%                                       |
| Rps7   | HCC, CRC, PC   | 8.94%                                         |
| Rps24  | BC, CRC, PC    | 7.13%                                         |
| Rpl35a | HCC, CRC, PC   | 4.30%                                         |
| Rps4x  | BC, CRC, PC    | -4.03%                                        |
| Rpl4   | HCC, BC, PC    | -6.45%                                        |
| Rps12  | BC, CRC, PC    | -7.42%                                        |
| Rps6   | HCC, BC, PC    | -9.45%                                        |
| Rpl17  | BC, CRC, PC    | -9.99%                                        |
| Rps14  | BC, CRC, PC    | -11.06%                                       |
| Rps23  | BC, CRC, PC    | -11.19%                                       |
| Rpl10a | BC, CRC, PC    | -11.63%                                       |
| Rps13  | HCC, BC, CRC   | -13.55%                                       |
| Rpl3   | HCC, BC, CRC, PC | -15.68%                                       |
| Rps25  | HCC, BC, CRC   | -16.42%                                       |
| Rpl15  | HCC, BC, CRC, PC | -16.58%                                       |
| Rpl22  | HCC, BC, CRC, PC | -17.31%                                       |
| Rps3a  | HCC, BC, CRC, PC | -17.42%                                       |
| Rpl5   | BC, CRC, PC    | -18.57%                                       |
| Rpl11  | HCC, BC, CRC   | -18.72%                                       |
| Rpl34  | HCC, BC, CRC   | -21.37%                                       |
| Rpl26  | HCC, BC, CRC   | -23.71%                                       |
**Table C**

| HB cytoplasmic extract | Normal liver cytoplasmic extract |
|------------------------|---------------------------------|
| RPL7                   | 31 kDa*                         | ND                              |
| RPL8                   | 28 kDa                          | RPL8 28 kDa                     |
| RPL10A                 | 25 kDa*                         | ND                              |
| RPL13                  | 24 kDa                          | RPL13 24 kDa                    |
| RPL14                  | 24 kDa*                         | ND                              |
| RPS2                   | 31 kDa*                         | ND                              |
| RPS3                   | 27 kDa                          | RPS3 27 kDa                     |
| RPS3A                  | 30 kDa                          | RPS3A 30 kDa                    |
| RPS4X                  | 30 kDa                          | RPS4X 30 kDa                    |
| RPS6                   | 29 kDa                          | RPS6 29 kDa                     |
| RPS8                   | 24 kDa                          | RPS8 24 kDa                     |
| RPSA                   | 33 kDa*                         | ND                              |

*Detected only in HB
ND = not detected
Table D

| HB cytoplasmic extract | Normal liver cytoplasmic extract |
|------------------------|----------------------------------|
| RPL11 20 kDa          | RPL11 20 kDa                    |
| RPL12 18 kDa          | RPL12 18 kDa                    |
| RPL13 24 kDa*         | ND                               |
| RPL23A 18 kDa*        | ND                               |
| RPL24 18 kDa          | RPL24 15 kDa                    |
| RPL26 17 kDa          | RPL26 17 kDa                    |
| RPL27A 17 kDa         | RPL27A 17 kDa                   |
| RPL28 16 kDa*         | ND                               |
| RPL32 16 kDa          | RPL32 16 kDa                    |
| RPL35 15 kDa*         | ND                               |
| RPS10 19 kDa          | RPS10 19 kDa                    |
| RPS11 18 kDa          | RPS11 18 kDa                    |
| RPS13 17 kDa          | RPS13 17 kDa                    |
| RPS15 17 kDa*         | ND                               |
| RPS17 16 kDa*         | ND                               |
| RPS24 15 kDa*         | ND                               |
| RPS25 14 kDa          | RPS25 14 kDa                    |
| ND                    | RPS18 18 kDa*                   |

*Detected only in HB
+Detected only in normal liver
ND = not detected
Table E

| HCC-D (~24-35 kDa) | HCC-E (~14-24 kDa) |
|--------------------|--------------------|
| RPL13 | 24 kDa |
| RPS9 | 23 kDa* |
| RPL26 | 12 kDa (FRAGMENT) |
| RPS14 | 16 kDa* |
| RPL12 | 18 kDa |
| RPS13 | 17 kDa |
| RPL31 | 14 kDa* |
| RPS3 | 27 kDa |
| RPL23A | 18 kDa* |
| RPS3A | 30 kDa |
| RPS17 | 16 kDa* |
| RPS4X | 30 kDa |
| RPL11 | 20 kDa |
| RPL13 | 24 kDa |
| RPS11 | 18 kDa |
| RPS8 | 24 kDa |
| RPS25 | 14 kDa* |
| RPS6 | 29 kDa |
| RPS24 | 15 kDa* |
| RPS2 | 31 kDa* |
| RPL18A | 21 kDa* |
| RPL8 | 28 kDa |
| RPL28 | 16 kDa* |
| RPL14 | 24 kDa* |
| RPS5 | 23 kDa* |
| RPL10A | 25 kDa* |
| RPL32 | 16 kDa |
| RPL18 | 15 kDa (FRAGMENT)* |
| RPL24 | 18 kDa |
| RPS16 | 16 kDa* |
| RPS10 | 19 kDa |
| RPS23 | 16 kDa* |
| RPS15 | 17 kDa* |
| RPL17 | 21 kDa* |

*Present only in HCC when compared to normal liver
| Gene  | HCC          | CRC                | BC     | PC          |
|-------|--------------|--------------------|--------|-------------|
| RpsA  | Q261R        |                    |        |             |
| Rps2  | V209A        | Splice region      | F84L   |             |
| Rps3  | Q4R (2)      | A71D, G15R         |        |             |
| Rps3a | D196Tfs*2, V22A |                |        |             |
| Rps4x | Y54C, V207M  | I102M, N224H       |        |             |
| Rps4y1| Y149Sfs*9    |                    |        |             |
| Rps5  | E98K, E3K, V113L, F20L, V11G, T126A |    | R130H   |             |
| Rps6  | L133P, K221Lfs*26 |              | S236C, S139N, K93N, M1? | R232H |
| Rps7  | I60V         |                    |        |             |
| Rps8  | C71W         |                    |        |             |
| Rps9  | R54Afs*8     | R79W, R83H, I32I  | R133P  |             |
| Rps11 | G53Afs*24    |                    |        | R139S, K32T |
| Rps12 | E87K         |                    |        |             |
| Rps13 | P7S          | I37M               |        |             |
| Rps15a| N91K         |                    |        |             |
| Rps16 | F9S, T145A   | L47V               |        |             |
| Rps18 | R79C         | A16T               |        |             |
| Rps20 | V55Sfs*4     | G33D, G78D, N39T, V85L |        |             |
| Rps24 | T38A         |                    |        |             |
| Rps25 | A87G         |                    |        |             |
| Rps27 | D6N          | Splice region      |        |             |
| Rps27a| L56Cfs*16    |                    |        | K83del, Y106C |
| Rps29 | S20F         |                    |        |             |
| Fau   | N132Y, V86del| Y112C              |        |             |
## Table G

| Gene | HCC | CRC | BC | PC |
|------|-----|-----|----|----|
| Rpl3 | R174H, G225D, A359S | K124T, T346A | V338G, V85G | K128del |
| Rpl4 | Splice region | E13K, R97C | D179H | |
| Rpl5 | D59E | K258N | N57Efs*12, A97G | N94D, G156del, A77T |
| Rpl6 | F193C (2), T213I | R22Q | V4G | |
| Rpl7 | R22Q | V14G, E188Q | |
| Rpl7a | R196G, R59H, R89L | I151T, R115Q, R89H, N42D | |
| Rpl8 | I158F | K128del | |
| Rpl9 | R125M | R125M | L176V | |
| Rpl10 | R125M | L103S | |
| Rpl10a | K98Q, R7G | P135Lfs*17, A166V | L138F, E88*, E178K | |
| Rpl11 | R146C, T148A, G86C | F166L | L15F, K8Rfs*26, Y131C, C72R | |
| Rpl12 | | | V42Lfs*15 | R117W |
| Rpl13 | R183C, Q111* | | |
| Rpl13a | | | T132I, N65K, R37G, V203L |
| Rpl15 | | | R189Gfs*18, S187Ffs*29 |
| Rpl18 | L27M | | G118V |
| Rpl18a | R116Gfs*12, Q144L, V62F | R43C | R83H, R95Q, R43Pfs*10, R166C | |
| Rpl19 | R16C | A159V | R107K, E28K | K21del, R151H |
| Rpl21 | I93L | F15L | | |
| Rpl22 | C25* | K89Nfs*3, K15Rfs*5 | K15Rfs*5 (4) |
| Rpl23 | K75Rfs*31 | | E99Q |
| Rpl23a | I76V | | |
| Rpl24 | P133H | R105Q | |
| Rpl26 | R21H | | |
| Rpl27 | R21H | | |
| Rpl27a | K7R | | |
| Rpl28 | V78M | D105N, P53S | K65M | |
| Rpl29 | | | R44C |
| Rpl31 | Splice region, E94V | R85H | | |
| Rpl32 | R27Q | F20L | | |
| Rpl34 | | | A16T |
| Rpl35 | R84Q | K79N | | |
| Rpl35a | Y14C | | V33I |
| Rpl36 | | | I81M |
| Rpl36a | A60Gfs*2 | | R57Q |
| Rpl37 | N13S | | A51G |
| Rpl37a | | | K62dup |
| Rpl39 | | | K62dup |
| Rpl41 | | | |
| Rplp0 | P272Lfs*63 | V121A, F316del | A278G (2), A262T, E299K | |
| Rplp1 | | | 150V |
| Rplp2 | | | E92Q |
| Uba52 | Q62* | M94T, L71S | H104Y, N90Y | |
### Table H

| Gene Symbol | Number of Non-RP-Mutant Tumors with Mutation (Total = 192) | Number of RP-Mutant Tumors with Mutation (Total = 31) | P-value       |
|-------------|-------------------------------------------------------------|--------------------------------------------------------|---------------|
| TTN         | 62                                                          | 22                                                     | $4.55 \times 10^{-15}$ |
| RYR2        | 13                                                          | 17                                                     | $2.14 \times 10^{-4}$  |
| MUC16       | 23                                                          | 15                                                     | $1.59 \times 10^{-7}$  |
| LRP1B       | 23                                                          | 15                                                     | $1.59 \times 10^{-7}$  |
| FAT4        | 25                                                          | 15                                                     | $1.59 \times 10^{-7}$  |
| NEB         | 5                                                           | 14                                                     | $1.14 \times 10^{-6}$  |
| DNAH10      | 8                                                           | 14                                                     | $1.14 \times 10^{-6}$  |
| CSMD2       | 10                                                          | 14                                                     | $1.14 \times 10^{-6}$  |
| LRP2        | 21                                                          | 14                                                     | $1.14 \times 10^{-6}$  |
| SYNE1       | 35                                                          | 14                                                     | $1.14 \times 10^{-6}$  |
| USH2A       | 14                                                          | 13                                                     | $7.18 \times 10^{-6}$  |
| CSMD3       | 15                                                          | 13                                                     | $7.18 \times 10^{-6}$  |
| HMCN1       | 17                                                          | 13                                                     | $7.18 \times 10^{-6}$  |
| PIK3CA      | 32                                                          | 13                                                     | $7.18 \times 10^{-6}$  |
| ATR         | 5                                                           | 12                                                     | $4.02 \times 10^{-7}$  |
| DNAH11      | 6                                                           | 12                                                     | $4.02 \times 10^{-7}$  |
| DOCK2       | 9                                                           | 12                                                     | $4.02 \times 10^{-7}$  |
| RYR3        | 11                                                          | 12                                                     | $4.02 \times 10^{-7}$  |
| ACVR2A      | 13                                                          | 12                                                     | $4.02 \times 10^{-7}$  |
| CSMD1       | 15                                                          | 12                                                     | $4.02 \times 10^{-7}$  |
| FAT2        | 17                                                          | 12                                                     | $4.02 \times 10^{-7}$  |
| DNAH5       | 22                                                          | 12                                                     | $4.02 \times 10^{-7}$  |
| ARAP2       | 2                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| CHD6        | 3                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| PAPPA2      | 3                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| PKHD1L1     | 4                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| ZNF292      | 5                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| KIAA1109    | 5                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| FBN1        | 6                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| LAMA2       | 6                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| MAP1B       | 7                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| DST         | 8                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| DNAH8       | 8                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| ANK3        | 8                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| PCDH15      | 9                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| PCLO        | 9                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| PDZD2       | 9                                                           | 11                                                     | $1.99 \times 10^{-7}$  |
| MYH11       | 10                                                          | 11                                                     | $1.99 \times 10^{-7}$  |
| MACF1       | 11                                                          | 11                                                     | $1.99 \times 10^{-7}$  |
| RYR1        | 13                                                          | 11                                                     | $1.99 \times 10^{-7}$  |
| DMD         | 20                                                          | 11                                                     | $1.99 \times 10^{-7}$  |
Table legends.

Table A. Antibodies utilized in the current study.

Table B. Shared RP transcript deregulation across human cancers. Listed are RP transcripts which showed significantly different relative expression in tumors compared to normal tissues, as well as shared directionality in at least 2 of the 4 cancer cohorts examined. BC = breast cancer, HCC = hepatocellular carcinoma, CRC = colorectal carcinoma, PC = prostate cancer. For each RP transcript, relative percent expression was compared between tumor (all matched and unmatched samples) and average relative expression in normal tissue with a two-sided t-test (P <0.05). All P-values were then adjusted based on a false-discovery rate of 5%. Percent difference in relative expression for a given transcript was then calculated by dividing the difference in average relative expression between HCC and normal tissue by the average relative expression in normal tissue. Transcripts were defined as having shared directionality when differences in relative percent expression were either all increased or decreased relative to normal tissues. Transcripts with significantly different relative percent expression in 2 or more cancers but without shared directionality were excluded.

Table C. Mdm2-interacting RPs identified in HB and normal liver cytoplasm (~24-35 kDa range). Note that all seven of the RPs associated with Mdm2 in normal liver cytoplasmic lysates were also identified in IPs from HB cytoplasmic lysates. See Figure K and Figure L for the identities of all detected peptides. Among the non-RPs identified by mass spectrometry in this analysis were five isoforms of the 14-3-3 family which have been previously identified as Mdm2 partners (56-60).
Table D. Mdm2-interacting RPs identified in HB and normal liver cytoplasmic lysates following anti-Mdm2 IP (~14-24 kDa range; blue bracket in Fig 5E). Note that 10 of 11 proteins identified as Mdm2 binding partners in normal liver were also identified in IPs from HBs, which contained seven additional RPs. See Figure M and Figure N for the exact mapping of each identified peptide to its corresponding RP.

Table E. Mdm2-interacting RPs identified in HCC cytoplasmic lysates following anti-Mdm2 IP. See Figure O and Figure P for the exact mapping of each identified peptide to its corresponding RP.

Table F. Mutations in RP small subunit genes identified in four investigated TCGA cancer cohorts, designated with standard HGVS nomenclature.

Table G. Mutations in RP large subunit genes identified in four investigated TCGA cancer cohorts, designated with standard HGVS nomenclature.

Table H. Mutations associated with ribosomal protein mutations in human colorectal cancers. The listed genes are more frequently co-mutated in tumors possessing a ribosomal protein mutation than would be expected by chance alone. P-values were calculated using cumulative binomial distributions and are significant after correction for false discovery.