SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1

(a) Western blot analysis of p16 (left), p21 (centre) and p53 (right) in MDA-MB-468, MDA-MB-231 and HeLa cells (top panels). Bottom panel: cyclophilin B or GAPDH loading controls. **(b)** Heatmap displaying the Hazard Risk for 10 year relapse free survival with a logrank p value <0.05 for 10 RPs whose knockdown did not alter cancer cell or HMEC proliferation. **(c)** Representative immunofluorescence images of HeLa cells treated with GAPDH siRNA or each of the six RP siRNA pools prioritised for further validation (Cell Mask, red). Size bar 50μm. **(d)** Validation of RP siRNA knockdown in MDA-MB-468 cells by qRT-PCR analysis. Cells were reverse transfected with GAPDH or each of the six RP siRNA pools and RNA was harvested at 76 hours post-transfection. CT values were normalised to HPRT1 and mRNA expression levels are shown relative to the GAPDH negative control. Bars denote mean mRNA expression levels +SD of two independent experiments, each containing two technical repeats. **(e)** Validation of RPS3A and RPS7 siRNA knockdown in MDA-MB-468s cells. Western blot analysis 4 days post-transfection. Loading control, β-tubulin (top panels). Densitometry analysis of RPS3A and RPS7 relative to the GAPDH negative control (lower panels). Bars denote mean density levels +SD of two independent experiments. **(f-h)** Heatmap depicting mean Z-scores and no significant change to cell area or cell number following RP siRNA knockdown for (f) HMECs, (g) HMFs and (h) NFKs. *=p<0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001.

Supplementary Figure 2

(a) Representative immunofluorescence images of MDA-MB-468 cells transfected with GAPDH, RPS3A or RPS7 siRNA (DAPI, blue; Ki67, green). GAPDH siRNA treated wells were fixed/stained at 5 days post-transfection while RPS3A and RPS7 siRNA treated wells were fixed/stained at 8 days post-transfection. Size bar 25µm. **(b)** Bar chart showing mean +SD percentage of Ki67-positive nuclei. **(c-e)** qRT-PCR analysis for SASP factors (c) IL-1α (IL-1A), (d) IL-1β (IL-1B) and (e) IL-6 follow RP knockdown. Cells were reverse transfected with GAPDH or each of the six RP siRNA pools and RNA was harvested at 76 hours post-transfection. CT values were normalised to HPRT1 and mRNA expression levels are shown relative to the GAPDH negative control. Bars denote mean mRNA expression levels +SD of two independent experiments, each containing two technical repeats. **(f)** Representative bright field images of MDA-MB-468 cells untreated or transfected with RPS3A or RPS7 siRNA and stained for SA-β-gal activity at 8 days post-transfection. Untreated cells were seeded at an equivalent density and fixed/stained 24 hours post-seeding. Size bar 20µm. **(g-h)** Representative immunofluorescence images of (g) untreated late passage HMECs or (h) HMECs transfected with siGLO (h, left) or CBX7 siRNA (h, right). (DAPI, blue; p16, green; NCL, red). Size bar 10µm**.** **(j)** Representative immunofluorescence images of MDA-MB-468 cells treated with DMSO only (vehicle control, left) or 10µg/mL actinomycin D (right) (NCL, red). Cells were fixed/stained 8 hours after drug treatment. Size bar 25µm. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$.

Supplementary Figure 3

(a-b) Western blot analysis of p53 (a, left) and p21 (b, left) in MDA-MB-468 cells following reverse transfection with GAPDH, CBX7, RPS3A or RPS7 siRNA. Loading control: β-tubulin. Densitometry analysis of p53 (a, right) and p21 (b, right). Bars denote mean density levels +SD normalised to GAPDH siRNA. **(c)** Representative frequency distribution depicting the p16 nuclear/cytoplasmic ratio at 5 days post-transfection following GAPDH, RPL14, RPL18, RPL34 or RPL35A siRNA knockdown in MDA-MB-468 cells. **(d)** Cells were reverse transfected with GAPDH, CBX7, RPS3A or RPS7 siRNA. Lysates were harvested at 4 days post-transfection and western blot analysis of p107 and p130 performed. Loading control: β-tubulin (representative blots are show, left). Densitometry analysis was performed for p107 and p130 (middle and right, respectively). Bars denote mean density levels +SD normalised to GAPDH siRNA. **(e)** Bar chart depicting mean +SD percentage of p107-positive nuclei following reverse transfection of MDA-MB-468 cells with siRNA targeting GAPDH or each of the four remaining RPs. **(f)** Representative frequency distribution depicting p107 intensity levels within the p107 positive population following GAPDH, RPL14, RPL18, RPL34 or RPL35A siRNA knockdown. **(g)** Bar charts showing MDA-MB-468 mean +SD cell number following reverse transfection with 30, 60 or 90nM GAPDH siRNA (left) or 3nM CBX7 siRNA alone or in combination with 60 or 90nM p16 siRNA (right). Three independent experiments were performed, each containing three technical replicates. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$.

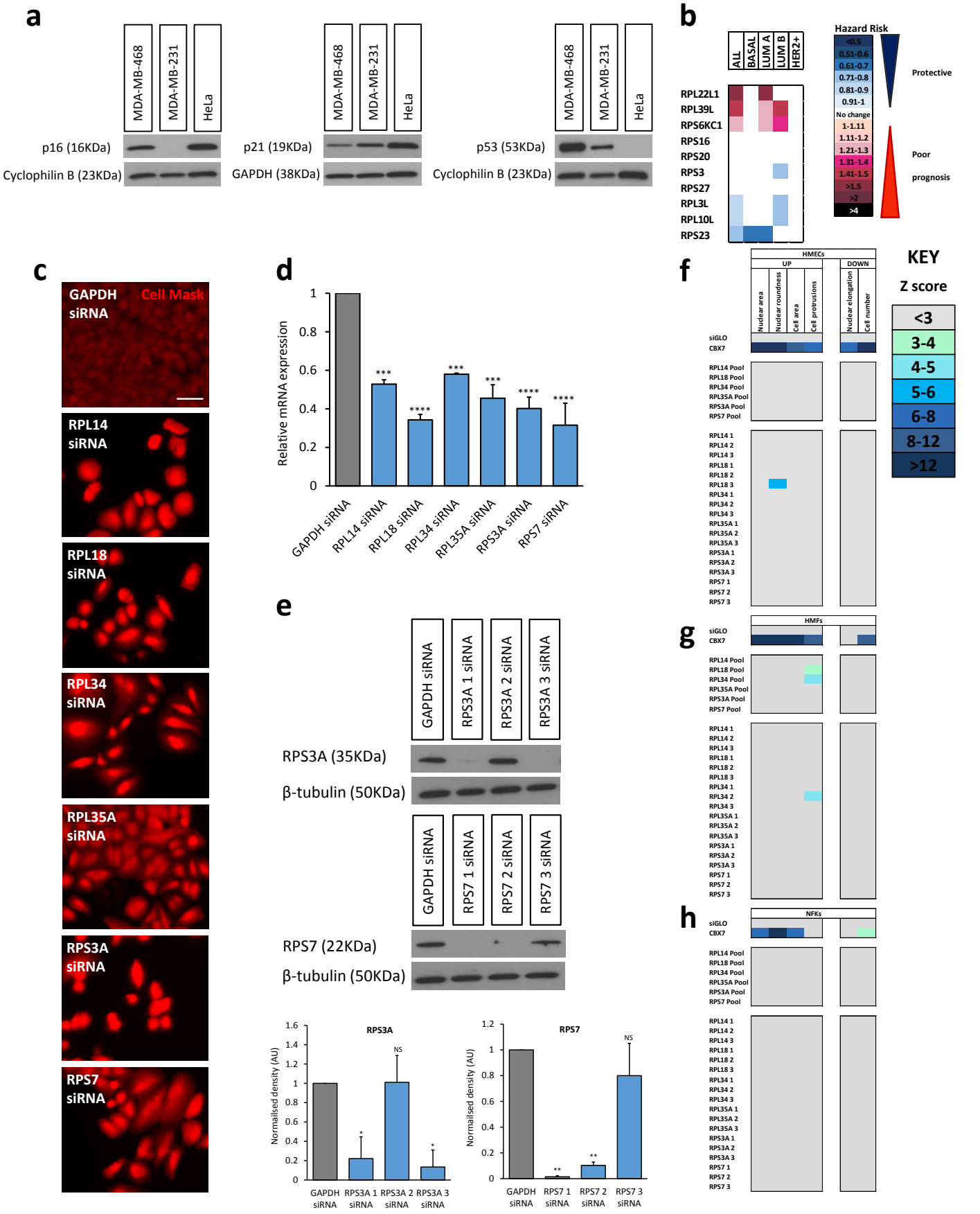
Supplementary Figure 4

(a) Representative immunofluorescence images of MDA-MB-231 cells transfected with GAPDH or CBX7 siRNA (DAPI, blue; Cell Mask, red). Size bar 50µm. **(b)** Bar charts depicting mean cell number (left), cell area (middle), or nuclear area (right) following GAPDH or CBX7 siRNA transfection in MDA-MB-231 cells. **(c)** Western blot analysis of p21 in MDA-MB-231 cells (left). Cells were reverse transfected with GAPDH or CBX7 siRNA and protein was harvested at 5 days post-transfection. Loading control: β-tubulin. Representative frequency distribution of nuclear p21 density in MDA-MB-231 cells following GAPDH or CBX7 siRNA transfection (right). N=1. **(d)** Bar chart depicting mean +SD cell number following CBX7 siRNA knockdown or rescue with 60nM p21 siRNA at 5 days post transfection. Three independent experiments were performed, each containing three technical replicates. **(e)** Representative immunofluorescence images of MDA-MB-231 cells transfected with siRNA targeting each of the four remaining RPs (DAPI, blue; Cell Mask, red). Size bar 50µm. **(f)** Bar chart depicting mean +SD percentage of SYTOX-positive MDA-MB-231 cells at 6 days post-transfection. **(g)** Bar chart depicting mean +SD percentage of Caspase-3-positive MDA-MB-231 cells at 5 days post-transfection. MDA-MB-231 cells were reverse transfected with siRNA targeting GAPDH or each of the four remaining RPs. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$.

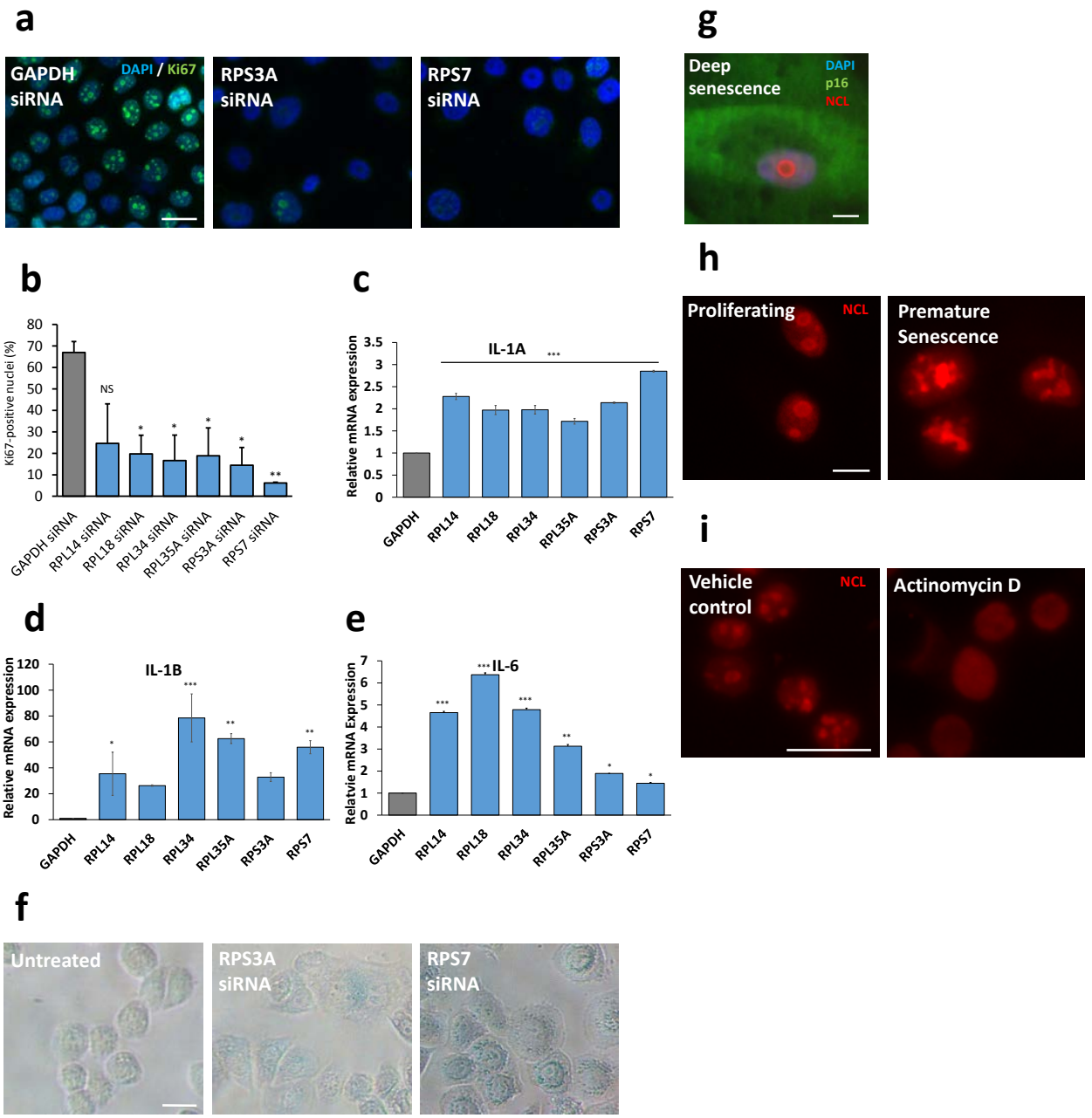
Supplementary Figure 5

(a-d) Western blot and associated densitometry analysis for RPS7 protein levels following RPS3A siRNA knockdown (c-d) and RPS3A protein levels following RPS7 siRNA knockdown (e-f) in MDA-MB-468 cells. **(e-f)** Western blot and associated densitometry analysis for RPS3A and RPS7 following RPS3A and RPS7 siRNA knockdown (siRNA pool) in MDA-MB-231 cells. **(g)** Kaplan Meier 10 year risk free survival plots for median RPS3A expression and lower versus upper tertile RPS7 expression generated by KMPLOT. **(h)** Heatmap displaying the Hazard Risk for 10 year relapse free survival with a logrank p value <0.05 for 10 RPs and p16 for Lung, Gastric and Ovarian cancer. Data generated from KMPLOT for median expression with the exception of RPS7_1 where lower tertile versus upper tertiles is shown (**). **(i)** Scatter plot of RPS3A and RPS7 Log_2-fold gene expression changes for tumour versus normal samples within the METABRIC dataset (N=1980). R^2 value is indicated. **(j-l)** Frequency distribution Log_2-fold expression for (j) RPL18, (k) RPL34, (l) RPL35A in $RPS3A^{LOW}RPS7^{LOW}p16^{LOW}$ (blue) and $RPS3A^{HIGH}RPS7^{HIGH}p16^{HIGH}$ (red) tumours. **(m)** Heatmap for R^2 values for Log_2-fold change gene expression values each of the six RPs versus members of the ribosome for $RPS3A^{LOW}RPS7^{LOW}p16^{LOW}$ tumours (left) and $RPS3A^{HIGH}RPS7^{HIGH}p16^{HIGH}$ tumours (right), all irrespective of PAM50 subtype (RPS, light green; RPL, dark green). (**n-o**) Frequency distribution Log_2-fold expression for (n) Cluster 1 RPL15 and (o) Cluster 3 RPL10 in $RPS3A^{LOW}RPS7^{LOW}p16^{LOW}$ (blue) and $RPS3A^{HIGH}RPS7^{HIGH}p16^{HIGH}$ (red) tumours.

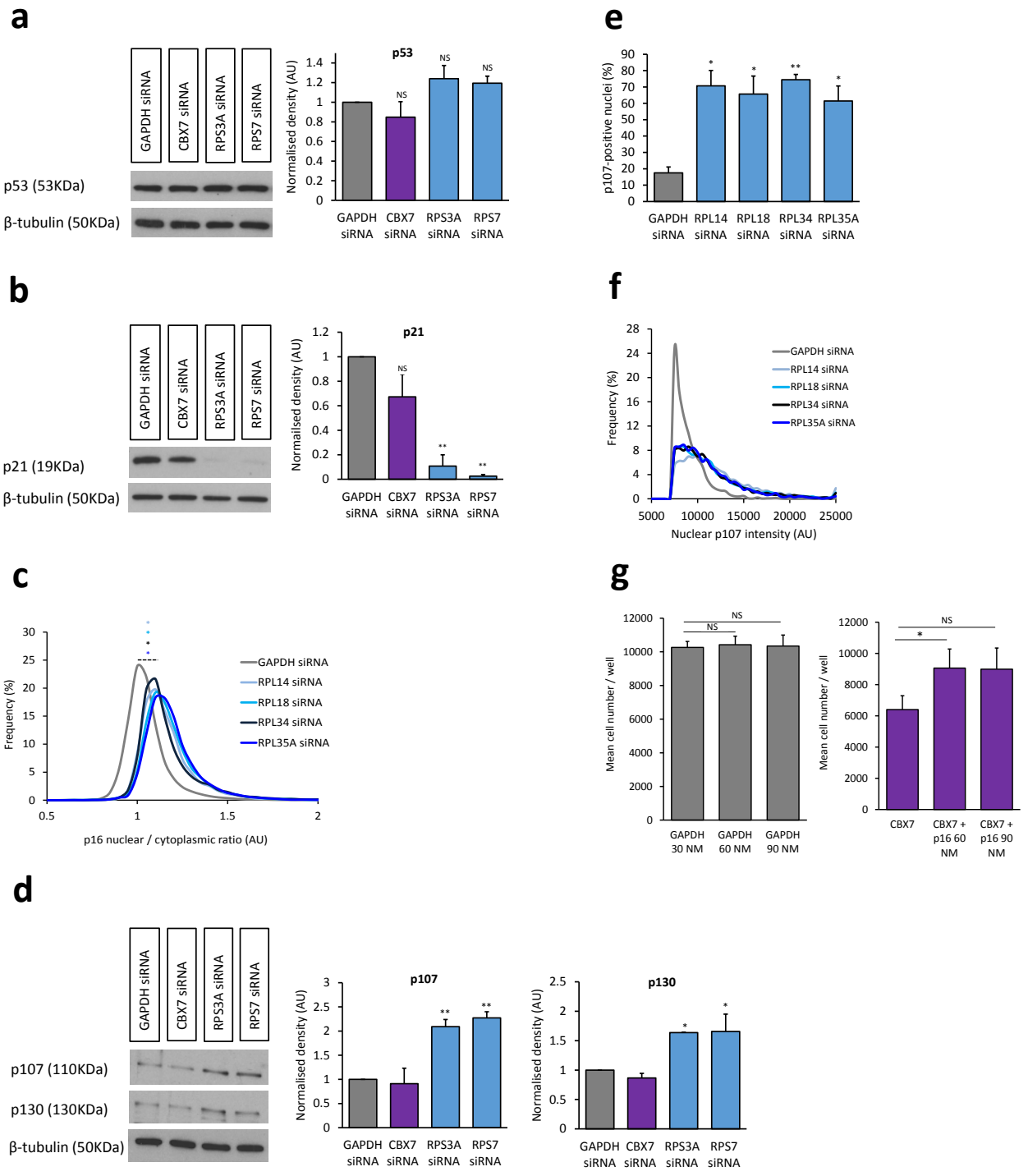
Supplementary Figure 1



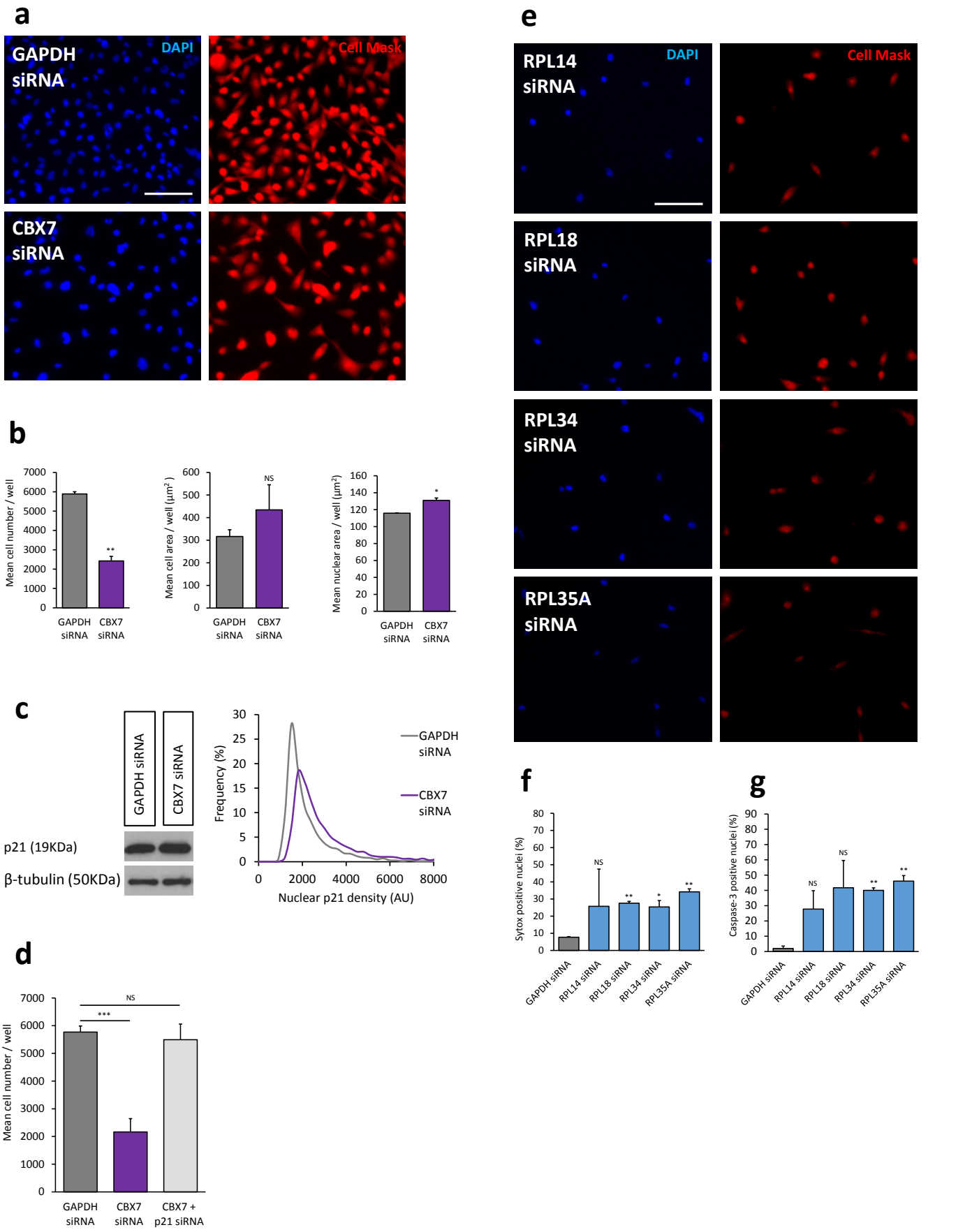
Supplementary Figure 2



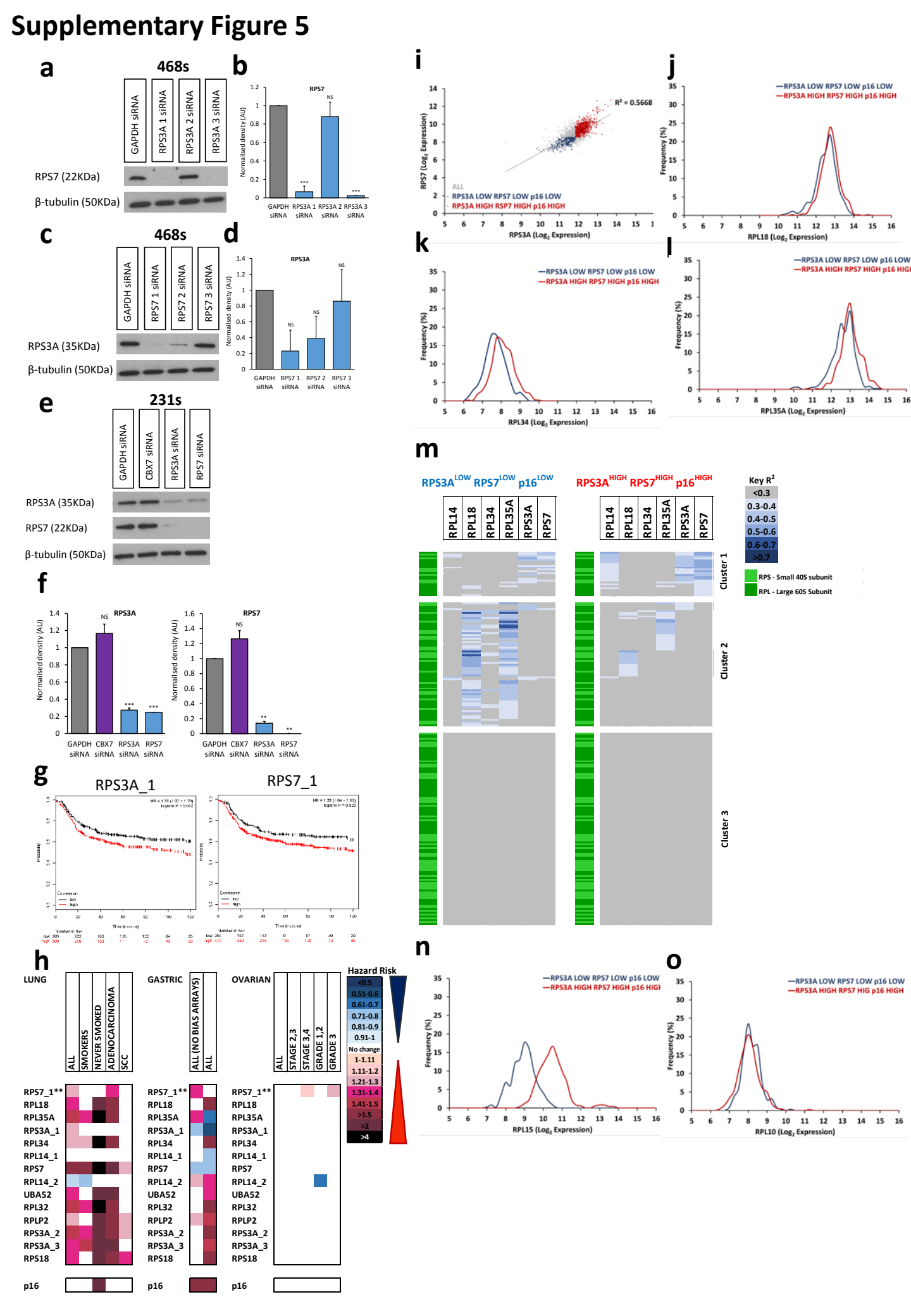
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Table 1. Table of siF

siRNA name	Supplier
RPL14_1 (137435)	Ambion
RPL14_2 (13949)	Ambion
RPL14_3 (13857)	Ambion
RPL18_1 (217192)	Ambion
RPL18_2 (142176)	Ambion
RPL18_3 (9171)	Ambion
RPL34_1 (9365)	Ambion
RPL34_2 (142194)	Ambion
RPL34_3 (9278)	Ambion
RPL35A_1 (45958)	Ambion
RPL35A_2 (9187)	Ambion
RPL35A_3 (9279)	Ambion
RPS3A_1 (142201)	Ambion
RPS3A_2 (142200)	Ambion
RPS3A_3 (142199)	Ambion
RPS7_1 (142206)	Ambion
RPS7_2 (142207)	Ambion
RPS7_3 (45963)	Ambion
GAPDH	Ambion
p16 (CDKN2A)	QIAGEN
p21 (CDKN1A)	GE
PLK1	Dharmacon
CBX7	Ambion
siGLO (targeting Cyc	Dharmacon

ʁNA target, supplies and sequences.

siRNA target sequence (5’-3’)
CCUUGCACUCAAGUGAGGAtt
GGAAAGCCAAGAUGACAGAtt
GGCAGACAUCAAUACAAAAtt
CCCUGGAUCCUACUCUCUUtt
GCCGAGGCUACAAAAACUAtt
GGCUGUUGGUCAAGUUAUAtt
GGAGCUCUGAUAUAUAUCUtt
GGUUAAAUACUACCAGCACtt
GGCACAAGCACAGAGUCAGtt
GCACACAGCUCUUCUUAAAtt
GGGAGCACACAGCUCUUCUtt
GGUGUUUACGCCCGAGAUGtt
CGAGACAGGUGCUAAAGUUtt
GCUCAUGGAGCUUCAUGGUtt
GCACCUGCUAUGUUCAAUAtt
GCAUGUCGUCUUUAUCGCUtt
CGGGCAAGGAUGUUAAUUUtt
GAUGAACUCGGACCUCAAGtt
GUGGAUAUUGUUGCCAUCAtt
TACCGTAAATGTCCATTTATA
CTACCTTGAAGCTGAAACA, CGCTACCTTGAAGCTGAAA, GCTACCTTGAAGCTGAAAC, GCTGACACTACGCGATTAC
Proprietary pool
GGGTAACACACACCAAGAGT
GAGCCCAGAUCAACCUUUA

Supplementary Table 2. Table of the antibodies used for immunofluorescence staining and imm

Antibody	Supplier	Dilution for immunoblotting
Mouseαp16 (JC2)	Prof. James Koh , Duke Cancer Institute, USA	1:5,000
Rabbitαp53	Cell Signalling	1:1,000
Rabbitαp21	Cell Signalling	1:1,000
Rabbitα53BP1	Bethyl Laboratories	N/A
RabbitαNucleolin	Santa Cruz Biotechnology	N/A
Mouseαp21 (N-terminus)	Abcam	1:500
RabbitαGAPDH	Abcam	1:5,000
Mouseαβ-Tubulin	EnoGene	1:20,000
RabbitαCyclophilin B	Abcam	1:1,000
RabbitαRPS3A	Abcam	1:10,000
MouseαRPS7	Abcam	1:1,000
GoatαIL-6	R and D Systems	N/A
Rabbitαp107	Santa Cruz Biotechnology	1:500
Rabbitαp130	Santa Cruz Biotechnology	1:500
RabbitαKi67	Novocastra	N/A
RabbitαCaspase-3	Abcam	1:500
RabbitαCaspase-9	Abcam	1:1,000

ıunoblotting together with their corresponding dilution.

Dilution for immunofluorescence staining
1:1,000
1:250
1:250
1:200
1:1,000
N/A
N/A
N/A
N/A
1:500
1:500
1:500
1:500
N/A
1:1,000
1:1,000
N/A

Supplementary Table 3. Table detailing the forward and reverse primer sequences used for qRTP(

Target gene	Forward primer (5'-3')	Reverse primer (5'-3')
HPRT1	ACCAGTCAACAGGGGACATAA	CTTCGTGGGGTCCTTTTCACC
RPL14	GACCTTGCACTCAAGTGAGGA	CTTGTCGGACATACTTCTGGTG
RPL18	ATGTGCGGGTTCAGGAGGTA	CTGGTCGAAAGTGAGGATCTTG
RPL34	TGGTGGTTCCATGTGTGCTAA	GCTTGTGCCTTCAACACTTTC
RPL35A	TTGAAGGTGTTTACGCCCGAG	TGCTTCGGAATTTGGCACGA
RPS3A	GGCAAGAACAAGCGCCTTAC	CAGGTGCTTTCACATCATACCAA
RPS7	GTGAAGCCCAATGGCGAGAA	TGAGGTCCGAGTTCATCTCCA

CR.

Supplementary Table 4. Table detailing the 77 ribosomal transcripts that were profiled using the qPCI

Gene Symbol
RPL10
RPL10A
RPL11
RPL13
RPL13A
RPL14
RPL15
RPL17
RPL18
RPL18A
RPL18AP3
RPL19
RPL21
RPL22
RPL23
RPL23A
RPL24
RPL26
RPL26L1
RPL27
RPL27A
RPL28
RPL29
RPL3
RPL30
RPL31
RPL32
RPL34
RPL35
RPL35A
RPL36
RPL36A
RPL36AL
RPL37
RPL37A
RPL38
RPL39
RPL4
RPL5
RPL6
RPL7
RPL7A
RPL8
RPL9
RPLP0
RPLP2

RPS11
RPS12
RPS13
RPS14
RPS15
RPS15A
RPS16
RPS17
RPS18
RPS19
RPS2
RPS20
RPS21
RPS23
RPS24
RPS25
RPS26
RPS27
RPS27A
RPS27L
RPS28
RPS29
RPS3
RPS3A
RPS4X
RPS5
RPS6
RPS6KA1
RPS7
RPS8
RPS9

R array.