SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Ectopic KRas protein is expressed significantly lower than ectopic HRas protein regardless of expression system, related to Figure 1. (A) Lysates isolated from HEK-HT cells stably expressing the indicated FLAG-Ras constructs were diluted where indicated and immunoblotted with αFLAG or αtubulin antibodies. (B, C, D, E) Immunoblot detection with the indicated antibodies of the indicated epitope-tagged Ras isoforms when (B, D, E) transiently transduced into the 293 cell line or (C) stably expressed in the HCT116 cell line. (F) Immunoblot detection of FLAG-tagged Ras isoforms with cysteine to serine mutations at the prenylation site (which abolish protein membrane localization and activity) stably expressed in the HEK-HT cells using αFLAG or αtubulin antibodies. (G) Immunoblot detection of the levels of total and phosphorylated ERK1/2 (ERK1/2, pERK1/2) and total and phosphorylated AKT (AKT, pAKT) downstream of the indicated stably expressed oncogenic FLAG-Ras proteins in serum starved HEK-TtH cells. Tubulin serves as a loading control. One of two experiments. (H) Details of vectors used for expression analysis of the Ras constructs above demonstrating different promoter, 5'UTR, splicing, and polyadenylation characteristics. (I) Alignment of human *HRAS, KRAS* and codon mutants. The cDNA sequences for human *KRAS*, codon-specific substitution mutants, *KRAS*, KRAS-OP* and *HRAS* [1] were aligned using ClustalW [17]. Codon mutants were synthesized by introducing changes in specific rare codons coding for the indicated amino acids. All instances of these codons were altered, except for those in the KRas_IVEPAQNGLFRKop

construct, in which the lysine resides in the hypervariable region of the C-terminal tail were left unchanged. Above is the amino acid sequence encoded by all seven *KRAS* cDNAs. (J) Immunoprecipitation (IP) of the indicated FLAG-tagged Ras proteins after metabolic labeling of HEK-HT cells for 15 minutes. One of two experiments. (K) Relative levels of mRNA encoding ectopically expressed *RAS* isoforms, as determined by quantitative RT-PCR of cDNA cells in (A). Identical primers annealing to vector-specific sequence were used to detect all ectopic *RAS* isoforms. (L) Amino acid identity and codon usage of Ras isoforms of different vertebrate species. The amino acid identity of HRas and KRas for each species is represented in the middle bars, with individual identical amino acids as blue boxes and non-identical amino acids in white. Relative codon usage of *HRAS* versus *KRAS* are represented in the upper and lower bars for each species, respectively; the shade of red represents the relative rarity of the codon in the corresponding genome for each individual amino acid. Grey boxes represent gaps in alignment or instances where the encoded amino acids are not identical. To the right, the codon adaptation index (CAI) relative to the endogenous genome (endogenous) and the human genome (human) were calculated using EMBOSS (cai and codonW programs)[18].

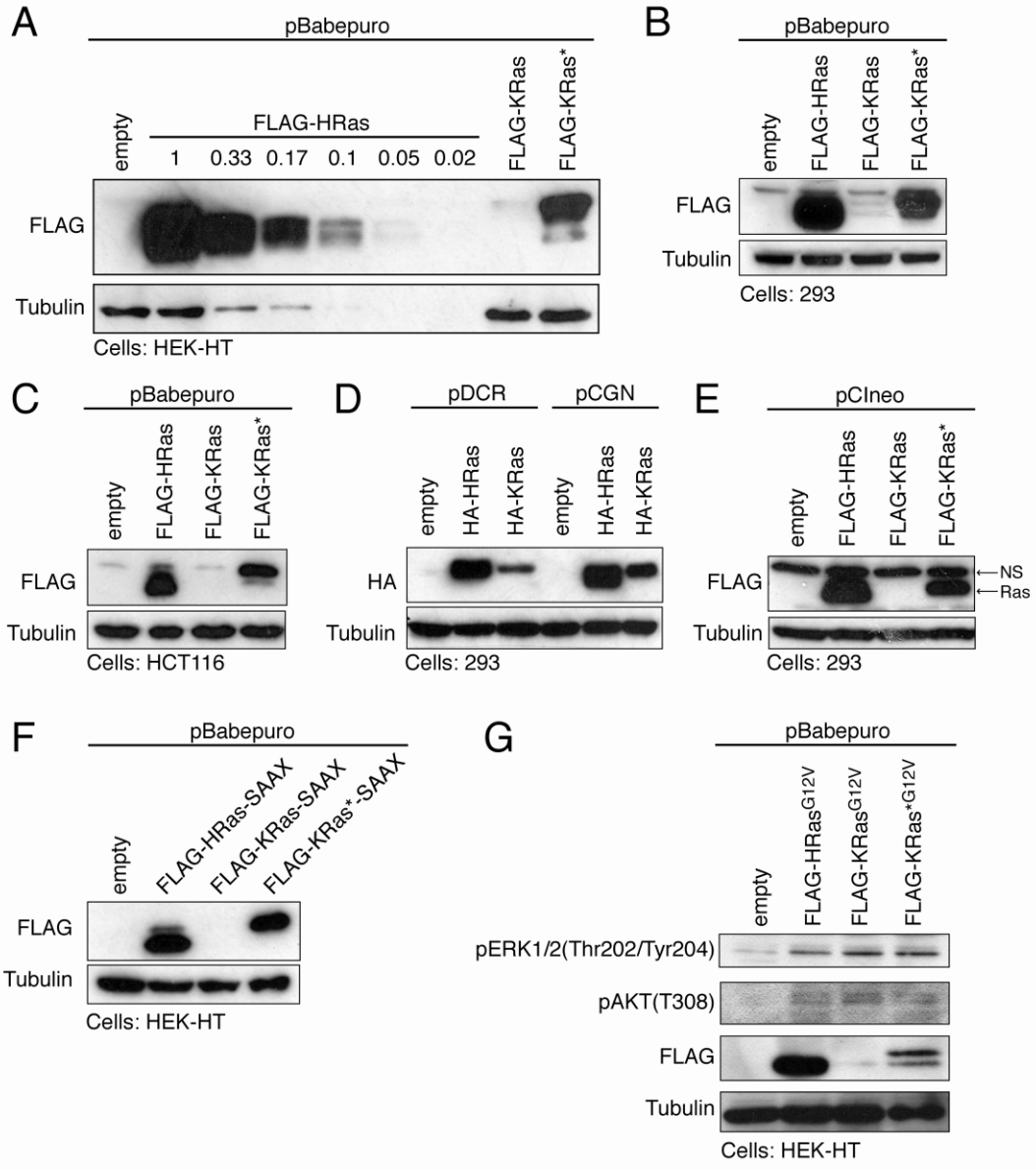
Supplementary Figure 2. Rare codon bias limits translation of endogenous *KRAS* transcripts, related to Figure 2. (A) Immunoblot of lysates isolated from *uaKRAS*^{G13D} knock-in clones relative to limiting dilution analysis of lysates from *opKRAS*^{G13D} knock-in clones with αKRas or αtubulin antibodies. (B) Total *KRAS*

mRNA levels in the clones depicted in Figure S2A, assessed by quantitative RT-PCR using identical primers annealing in the 5' UTR. (C) A255 polysome profiles of *uaKRas*^{G13D} (clone 1) and *uaKRas*^{G13D} (clone 5) HCT116 cells before and after pactamycin treatment. (D) A255 polysome profiles and semiquantitative RT-PCR analysis of the sedimentation of *HRAS* and *KRAS* mRNAs in HEK-HT cells before and after pactamycin treatment. (E) Left: A255 polysome profiles *opKRAS*^{G13D} (clone 4) HCT116 cells before and after pactamycin treatment. Right: Quantitative RT-PCR analysis of *KRAS*^{G13D} knock-in mRNAs from *opKRAS*^{G13D} clone 4 and *uaKRAS*^{G13D} clone 1 with (+) and without (-) pactamycin (pacta) treatment versus fraction number.

Supplementary Figure 3. Rare codon bias affects *RAS*-mediated tumorigenesis, related to Figure 3. (A) Immunoblot of HEK-HT cells expressing FLAG-KRas^{G12V} before (lane 2) and after (lanes 3-6) growth as xenografts using αFLAG or αtubulin antibodies. HEK-HT FLAG-KRas^{G12V} cells formed tumors 3 months following injection into four immunocompromised mice. Cells from the individual tumors were isolated, grown in culture to reduce contaminating stromal cells, and analyzed for KRas protein expression as indicated. (B) Rare codon usage correlates with Ras isoform expression and mutation frequency in mammalian cells. Immunoblot detection of the indicated FLAG-tagged HRas, KRas and NRas proteins expressed in 293 cells. Tubulin serves as a loading control. Also indicated are the percentage of codons encoded by the most rare or most common of redundant codons [7] for each

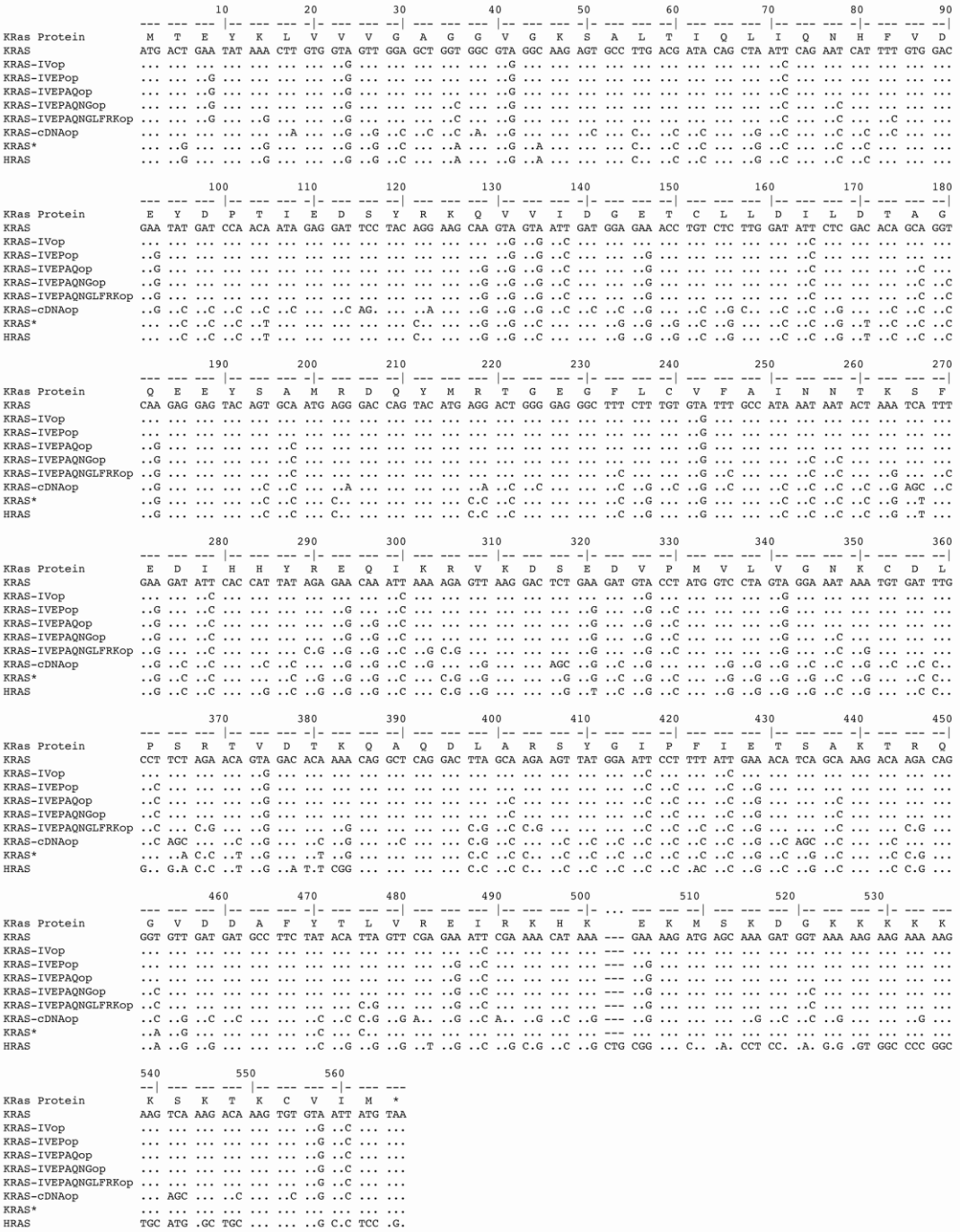
gene (excluding Met and stop codons), and the frequency of oncogenic mutations detected in human cancers [19]. (C) Amino acid identity of human KRas versus NRas and HRas versus NRas is shown in the middle bars. Blue: identical amino acids. White: non-identical amino acids. Relative codon usage of human *KRAS* versus *NRAS* and *HRAS* versus *NRAS* is shown in the upper and lower bars, respectively. Shade of red box: relative rarity of the codon. Grey box: gaps in alignment or non-identical amino acids. Comparison of *KRAS* and *HRAS* is illustrated in Figures 1C and S1L.

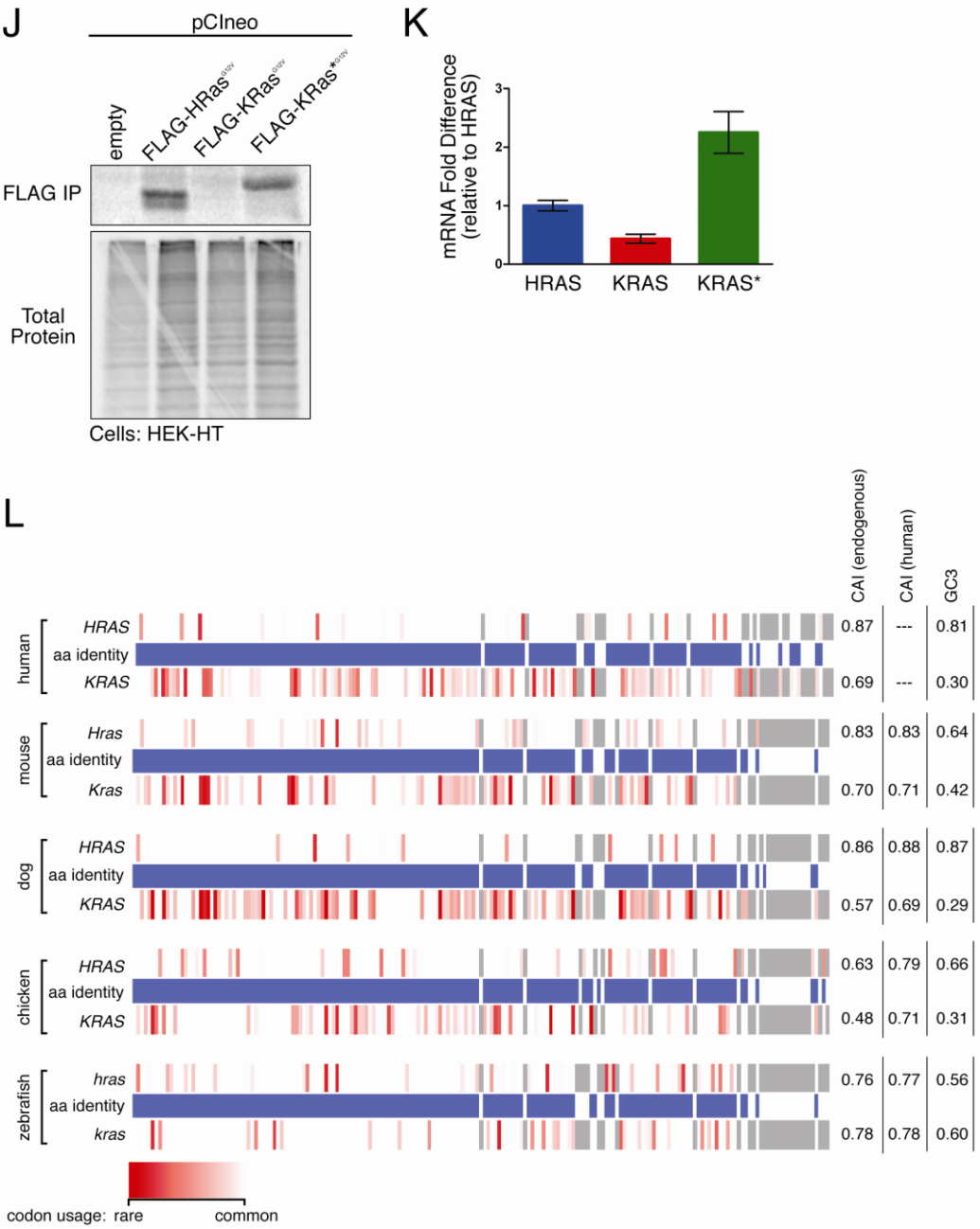
Supplementary Figure 1



H

	pBABE-Puro	pDCR	pCGN	pCIneo
Promoter	MoMuLV 5'LTR	MoMuLV 5'LTR CMV	CMV	CMV
5' UTR			HSV tk	
Intron		C-term rabbit β-globin	C-term rabbit β-globin	N-Term, chimeric intron
PolyA	MoLuLV 3'LTR	rabbit β-globin	rabbit β-globin	SV40

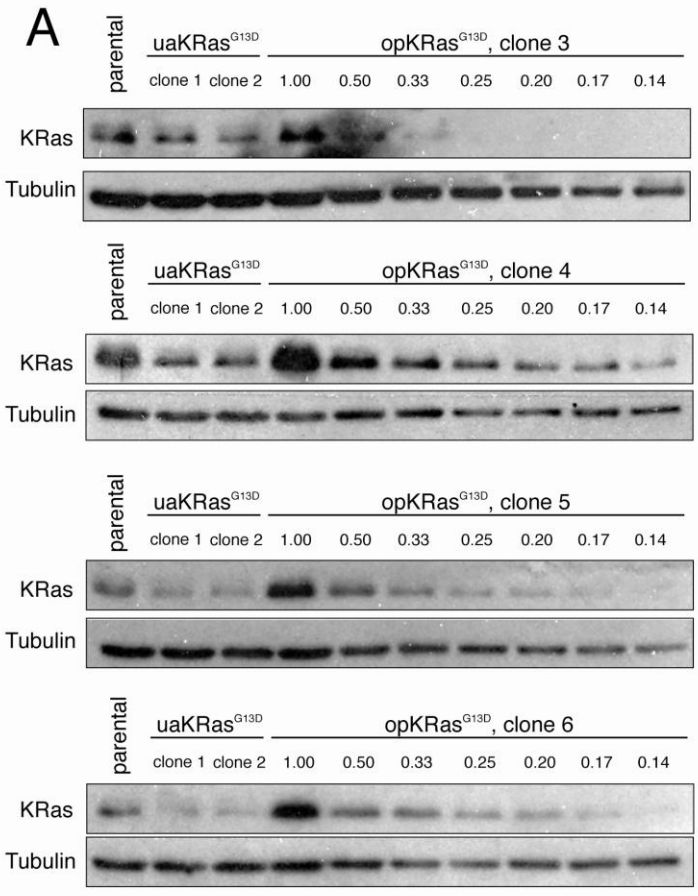


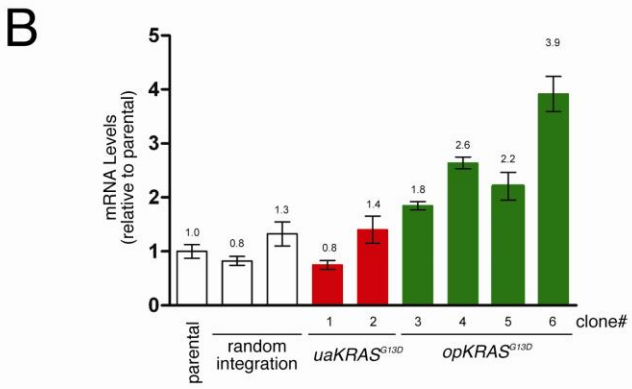

J
pCIneo
empty
FLAG-HRas^wtv
FLAG-KRas^wtv
FLAG-KRas*^wtv
FLAG IP
Total Protein
Cells: HEK-HT
K
mRNA Fold Difference (relative to HRAS)
HRAS
KRAS
KRAS*
L
CAI (endogenous)
CAI (human)
GC3
human
HRAS
aa identity
KRAS
0.87

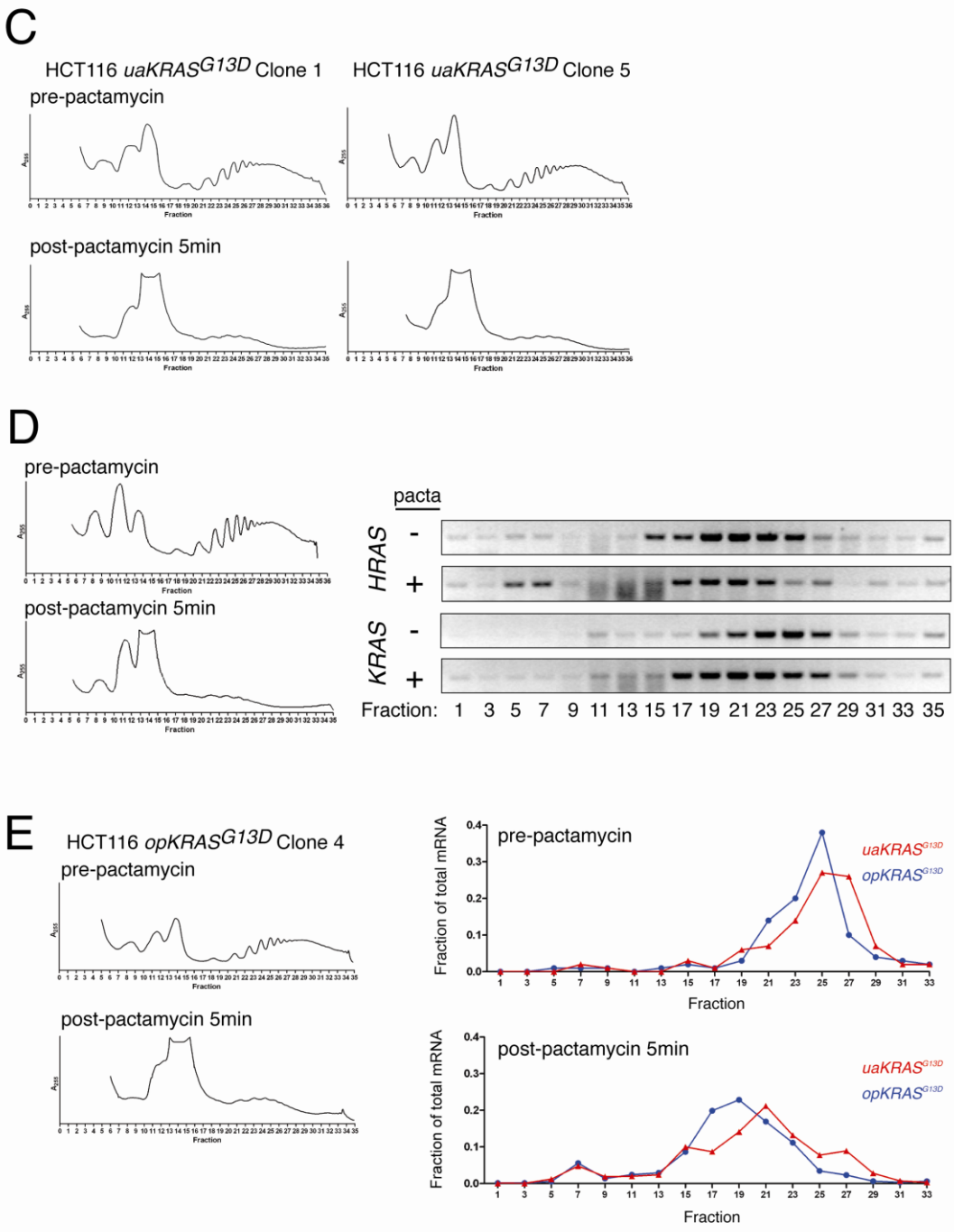
0.81
0.69

0.30
mouse
Hras
aa identity
Kras
0.83
0.83
0.64
0.70
0.71
0.42
dog
HRAS
aa identity
KRAS
0.86
0.88
0.87
0.57
0.69
0.29
chicken
HRAS
aa identity
KRAS
0.63
0.79
0.66
0.48
0.71
0.31
zebrafish
hras
aa identity
kras
0.76
0.77
0.56
0.78
0.78
0.60
codon usage: rare
common

Supplementary Figure 2

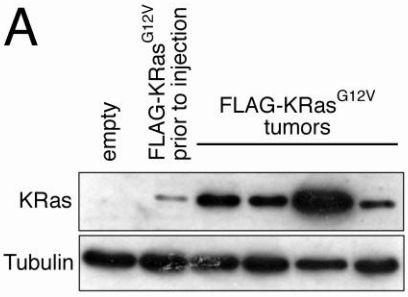




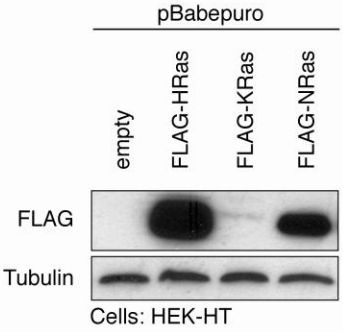

C
HCT116 uaKRASG13D Clone 1
HCT116 uaKRASG13D Clone 5
pre-pactamycin
post-pactamycin 5min
A255
Fraction
D
pre-pactamycin
post-pactamycin 5min
pacta
HRAS
KRAS
-
+
Fraction: 1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35
E
HCT116 opKRASG13D Clone 4
pre-pactamycin
post-pactamycin 5min
Fraction of total mRNA
uaKRASG13D
opKRASG13D
Fraction

Supplementary Figure 3

A

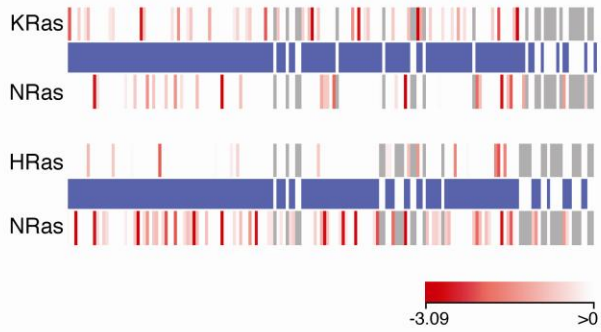


B



% rarest codons	15	40	34
% commonest codons	67	25	37
Mutation incidence in human cancers	3%	22%	8%

C



SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell lines. HEK-HT cells [1], used for all the transient and stable exogenous RAS studies except as indicated in the Supplemental Figures, 293T and IMR90 (ATCC) were maintained at 37˚C in 5% CO_2 in DMEM (Sigma) supplemented with 10% fetal bovine serum (Gibco) and 100U/ml penicillin and streptomycin. HCT116 colon cancer cells (ATCC) were maintained in McCoy's 5A media (Gibco/Invitrogen) with identical supplements. For expression studies, cells were either transduced with retrovirus and selected for stable expression of constructs by antibiotic resistance as described previously [1,2], or transiently transfected with the noted constructs using FUGENE-6 (Roche) according to the manufacturer's instructions.

Plasmids. All constructs undergoing PCR at any step were sequenced to verify the contents. pBabePuro and pBabeBleo constructs containing *HRAS* and *KRAS* and their oncogenic mutants were previously described [3]. pDCR-KRAS, pDCR-HRAS, pCGN-KRAS, pCGN-HRAS for studies of *RAS* expression in alternative vectors were kind gifts from Adrienne Cox [4, 5]. The *KRAS** plasmid was generated by PCR amplification of the 5' end of *HRAS* cDNA (with primer 5'-AGGCCGAGCTCGGATCCACGCGTCCGCCATGGACTACAAGGACGACGATGACAAG-3' and 5'-CACGCACTAGTGTGTAGAAGGCATC-3') and the 3' end of *KRAS* cDNA (5'-GATGCCTTCTATACACTAGTTCGAG-3' and 5'-gacgcgtcgacTTACATAATTACACACT TTG-3') followed by restriction digest with *Bam*HI/*Spe*I (5' end) and *Spe*I/*Sal*I (3' end) for three way ligation into *Bam*HI/*Sal*I digested pBabepuro. The following mutations were then made with five rounds of site directed mutagenesis to create an N-terminus that exactly matched KRas protein sequence: Q95H (CAG to CAT), D107E (GAT to GAG), A121P and A122S (GCTGCA to CCTTCA), E126D and S127T and R128K (GAATCTCGG to GACACTAAG), Y141F (TAC to TTC), E153D (GAG to GAT). The GST-RAS fusion constructs were generated by PCR amplification of either *HRAS* (5'-GGAATTCCCGGGTCGACTCACGGAATATAAGCTTGTG-3' and 5'-GGTAGGTTGCGGCCGCTCAGGAGAGCACACACTTGCAGCTC-3'), STOP-*HRAS* (5'-GGAATTCCCGGGTCGACTCtgaGAATATAAGCTTGTG-3' and *HRAS* reverse primer), *KRAS* (5'-GGAATTCCCGGGTCGACTCACTGAATATAAACTTGT-3' and 5'-GGTAGGTTGCGGCCGCCTTACATAATTACACACTTTG-3'), STOP-*KRAS* (5'-GGAATTCCCGGGTCGACTCtgaGAATATAAACTTGT-3' and GST-*KRAS* reverse primer), *KRAS** (GST-*HRAS* forward primer and GST-*KRAS* reverse primers), or STOP-*KRAS** (GST-STOP-*HRAS* forward primer and GST-*KRAS* reverse primers), followed by SalI/NotI digestion of the PCR products and ligation into

*Sal*I/*Not*I digested pCIneo-GST [5]. To create the AAV targeting construct, the following components were assembled into BluescriptKS in this order: (*i*) PCR amplified 5' homology arm from HCT116 genomic DNA (5'-ATGGTTTACCCGCGGCCGCGTGCCCGG CTCACTTGCATCTCTTAACAGCTG-3' and 5'-CCAACTACCACtAGTTTATATTCAGTCATTTTCAGCAGGC-3') inserted with *Sac*I and *Spe*I digestion, (*ii*) PCR amplified 3' homology arm from HCT116 genomic DNA (5'-ACGAAGTTATGTCGACAGCTGTGAAATCTAGAACAGG-3' and 5'- GGGAACAAAAGCTGGGTACCGC GGCCGCACTCACTGTAACTTGGGAG-3') inserted with *Sal*I-*Kpn*I digestion, (*iii*) either a $KRAS^{G13D}$ codon optimized cDNA (see Gene Synthesis section, below) or a $KRAS^{G13D}$ cDNA with regular codons with a BGH polyA tail fused via overlap extension PCR, using a *Spe*I-*Bam*HI digest, and (*iv*) a shortened neomycin resistance cassette from pNeDaKO [6] without the zeocin resistance cassette (5'-GAACCAGCTGGGGATCCAACAAAAGCTGGAGCTCCACC-3' and 5'-AGAATCGTCGACCCCCCAGCTGGT TCTTTCCGCCTCAGAAGCCATAG-3') using a *Bam*HI-*Sal*I digest of both vector and insert. After all pieces were assembled and verified by sequencing, the entire cassette was moved into pAAV-MCS (Stratagene) using *Not*I non-directional cloning. Other gene pair cDNA sequences were amplified either by RT-PCR or from a publically available plasmid and cloned into pCMV3.1 via infusion cloning. Vector sequences are available upon request.

Gene synthesis. For optimized constructs with many silent mutations (*KRAS* incremental codon replacement, *KRASop*, *CFL2op*, *ORMDL1op*), a gene synthesis approach was used. An Excel macro was employed to codon optimize each gene by replacing each suboptimal codon with the most commonly used synonymous codon [7] and then to split the resulting construct into 60-mer oligonucleotides that overlapped by 12-15 base pairs. These oligonucleotides were diluted to 500nM and mixed with 10μM flanking forward and reverse primers and PCR was carried out using Platinum Taq (Invitrogen) at a gradient of annealing temperatures. The reaction at an annealing temperature that gave the most robust product of an appropriate size was gel purified and ligated into pBluescriptKS. The resulting constructs were sequenced to verify accuracy.

Protein analysis. For all immunoblots, cells were lysed in 25mM Tris pH 7.4, 150mM NaCl, 5mM EDTA, 1% Triton X-100, 1mM DTT, and 1mM PMSF, and then resolved by SDS page. Membranes were blotted with anti-β-tubulin 1:1000 (Sigma), anti-FLAG 1:1200 (Sigma), anti-KRAS 1:200 (Santa Cruz), anti-GST 1:500 (Santa Cruz), anti-myc 1:5000 (Invitrogen), anti-pERK 1:300 (Cell Signaling #9102) or anti-pAKT 1:200 (Cell Signaling #4056).

Metabolic labeling. A 6cm plate of 80% confluent cells stably expressing the noted *RAS* expression constructs were preincubated with methionine- and cysteine-free RPMI (Sigma) with 15% dialyzed FBS (Invitrogen) for one hour. 200μCi of EXPRESS Protein Labeling Mix (Perkin-Elmer) was added; cells were labeled for 15 minutes, collected and washed with PBS, resuspended in 400μl lysis buffer (see Immunoblot section) and lysed on ice for 30 minutes. Lysates were clarified by centrifugation and aliquots set aside for input lanes. The remaining sample was used for overnight immunoprecipitation with M2 anti-FLAG resin (Sigma). Proteins were separated by SDS-PAGE electrophoresis, and the gel was fixed, dried and exposed to film.

Tumorigenesis assays. All studies were done according to protocols approved by the Duke University Institutional Animal Care and Use Committee. For HEK-HT tumorigenesis studies, 1×10^7 cells selected for stable expression of the noted *RAS* constructs were mixed with Matrigel (BD Biosciences) and injected subcutaneously into the flanks of 4 SCID/beige mice per cell line, after which tumor volumes were determined at regular intervals as described previously [8]. For HCT116 tumorigenesis studies, $1x10^6$ cells resuspended in 100μl PBS were injected subcutaneously into the flanks of 6 SCID/beige mice per cell line. Tumors were removed, photographed and weighed when one of the lines reached maximal volume.

RT-PCR. The reverse transcription reaction was done with the Omniscript RT kit (Qiagen) according to the manufacturer's instructions. For direct studies of total *RAS* transcript levels, two micrograms of total RNA, isolated with RNAzolB (Tel-Tech) according to manufacturer's instructions, was used for the reverse transcription with oligo-DT priming. The resulting cDNA was diluted 1:25 and assessed by qPCR using SybrGreen PCR mastermix (BioRad). Measurements were done on the BioRad iCycler iQ (v3.1), with 40 amplification cycles. No RT control cDNA was included to ensure against contaminating transcript. For each sample, qPCR reactions were done in triplicate, and the entire analysis was done twice independently. In terms of primer sequences, for exogenous *RAS* studies the following pBabeMCS specific primers were used: 5'-CTCAATCCTCCCTTTATCCAG-3' and 5'-CTGGTTGCTGACTAATTGAGATGC-3'. For quantitation of total *KRAS* in the HCT116 knock-in clones, the following *KRAS* 5'UTR primers were used: 5'-GAGCGAGCGCGGCGCAGGCACTG-3' and 5'-AGTTTATATTCAGTCATTTTC-3'. Reference primers directed against GAPDH: 5'-GAAGGTGAAGGTCGGAGTCAA-3' and 5'- GCAGAGGGGGCAGAGATGAT-3' were used as controls. For polysome analysis, fractions were collected and the entire fraction of purified RNA was used as a template for cDNA synthesis. The following primers were used to detect each of the indicated transcripts: *HRAS* 5'- AAGCTCAGGACATGGAGGTG-3' and 5'- GGCATTTGGGATGTTCAAGA-3'

semi-quantitative at 22 cycles for HCT116 cells and 26 cycles for HEK-HT cells; *KRAS* endogenous allele only 5'-GTCACATAAATGGATTAATTAC-3' and 5'-CCATGACTAATAGCAGTG-3' semi-quantitative at 23 cycles for HCT116 cells and 26 cycles for HEK-HT cells; *KRAS* knock-in allele only 5'-TATAGGGCGAATTGGAGCTCATGACTGAATATAAACT-3'and 5'- CACAGTCGCCATGGTTACAT -3'.

AAV-mediated homologous recombination. AAV targeting was performed as previously described [9]. Specifically, the targeting vector, pHelper, and pAAV-RC (Stratagene) were introduced into 293 cells with Fugene 6 (Roche) transfection reagent. Cells were scraped into 1ml PBS 48 hours later and virus was harvested with three cycles of freeze-thaw in an ethanol-dry ice bath. The lysates were clarified and 350µl added directly to a 60% confluent 10cm plate of HCT116 cells along with 4ml of McCoy's 5A growth media. After a 3-hour incubation, 8ml of growth media were added and cells incubated for 48 more hours. The cells were then trypsinized and resuspended in 3ml PBS. 30µl of this suspension was then added to 200ml of McCoy's 5A plus 400µg/ml neomycin and cells were seeded in twenty 96-well plates. 10-14 days later colonies were scored; generally about 10-13 colonies appeared per plate. Colonies were transferred and replica plated into two 96-well plates. DNA was isolated 2 days later from one plate using the Wizard SV96 genomic DNA purification system (Promega) according to the manufacturer's instructions. The screening primers used spanned the 3' homology arm to give 1393bp product: 5'-ATCAATTCTCTAGAGCTCGCTGAT-3' and 5'-TTTTTACAATAACATGGAGTCAGCA-3'. Step-down PCR was performed with Platinum Taq (Invitrogen) with 4 cycles each at annealing temperatures of 64˚C, 61˚C, 58˚C, and then 30 cycles at an annealing temperature of 55˚C. Positive clones were then expanded and tested for presence and mRNA expression of the recombined allele via RT-PCR and sequencing of the product obtained with the *KRAS* knock-in detection primers detailed in the above RT-PCR section. Determination of the allele in which knock-in occurred was done by PCR amplification and sequencing of the remaining unaltered *KRAS* allele.

Polysome profiling. A 10cm plate of 60-80% confluent cells was washed with PBS plus 200µM cycloheximide. For indicated conditions, pactamycin was added to 200nM for 5 minutes prior to cycloheximide addition. 1ml of lysis buffer (400mM KOAc, 25mM HEPES pH 7.2, 15mM $Mg(OAc)_2$, 1% NP-40, 0.5% sodium deoxycholate, 1mM DTT, 1mM PMSF, 200µM cycloheximide, and 40U/ml RNAseOut [Invitrogen]) was applied directly to the plate for 10 minutes on ice. Collected lysates were centrifuged for 10 minutes at 12000*g* and the supernatant was carefully layered on top of a 15-50% sucrose gradient. Samples underwent ultracentrifugation for three hours at 35000rpm in a Beckman L8-80M ultracentrifuge

(SW40Ti rotor). The gradients were fractionated using an ISCO gradient fractionator with a continuous absorbance monitor into 34-36 fractions of volume 330µl. For RNA isolation, first 2µl PelletPaint (Novagen) was added to each fraction, and then 2.5 volumes GT buffer (4M guanidine thiocyanate, 25mM sodium citrate pH 7.0, 0.5% N-lauryl sarcosine, 5mM EDTA, and 0.1M beta-mercaptoethanol) was added and samples were rotated for 15 minutes at room temperature. Then, 220µl chloroform was added, samples were shaken vigorously and separated by centrifugation at 12000*g* for 15 minutes. The upper aqueous phase was removed (700µl) and mixed with equal volume isopropanol and 0.1 volumes 3M sodium acetate pH 5.2. After overnight incubation at -20˚C, RNA was pelleted via centrifugation at 13000*g* for 20 minutes. The pellet was washed with 75% ethanol and resuspended in 30µl DEPC treated water.

Identification of proteins with high identity exhibiting codon bias. Proteins with high amino acid identity were determined by BLAST [10] alignment of the human proteome (obtained from Ensemble, [11]). BioMart (query http://www.biomart.org/biomart/martview?VIRTUALSCHEMANAME=default&ATTRIBUTES=hsapiens_gene_ensembl.default.feature_page.ensembl_gene_id|hsapiens_gene_ensembl.default.feature_page.ensembl_transcript_id|hsapiens_gene_ensembl.default.feature_page.ensembl_peptide_id|hsapiens_gene_ensembl.default.feature_page.status|hsapiens_gene_ensembl.default.feature_page.transcript_status&FILTERS=hsapiens_gene_ensembl.default.filters.status."KNOWN"|hsapiens_gene_ensembl.default.filters.transcript_status."KNOWN"|hsapiens_gene_ensembl.default.filters.biotype."protein_coding") was used to obtain a reference set of all genes within the Ensembl database (version 62). In order to obtain a set of high confidence sequences, only those genes annotated by Ensembl as producing a known transcript were selected. Protein sequences were downloaded from the Ensembl ftp server (available at ftp://ftp.ensembl.org/pub/release-62/fasta/homo_sapiens/pep); all sequences corresponding to canonical transcripts were formatted into a BLAST database (version 2.2.24) with 20,509 unique entries. The corresponding nucleotide sequences for each protein sequence were obtained using the Ensembl API, and the GC3 percentage (excluding start and end codons) of each associated coding sequence was calculated providing that the sequence contained a start codon, an end codon, and its length was divisible by three (resulting in 20,194 assayed transcripts). Using the same set of sequences, alignments were computed for each pair (using default parameters for protein alignments). Taking the best alignment (based on bit score) per sequence resulted in 20,104 unique alignments (of 4,537,326). Log_2 ratios of GC3 percentages between protein pairs were determined for each available protein alignment and p-values were determined via the pnorm function in the R programming environment [12]. For ontology and KEGG analysis, five lists of genes were generated using the following criteria. To test gene pairs with high amino

acid identity that exhibit biased codon usage, pairs with significantly divergent GC3 ratios were rank ordered by identity, bit score, then by GC3 ratios. To test gene pairs with high amino acid identity that do not exhibit a bias in codon usage, pairs were ordered by identity and bit score. To test gene pairs with low amino acid identity that exhibit a bias in codon usage, pairs were ordered by their GC3, identity (ascending), and bit score (descending). In each case, only those pairs with a bit score greater than a minimum 70 were used, and lists consisted of the top 150 pairs in each set (comprising a total of 300 genes each). In order to assay the effect of GC3 apart from amino acid identity, two lists were constructed representing the top 300 genes ordered by their percent GC3 (both ascending and descending). Associated KEGG pathways [13] and GO terms (using the molecular function subset), were then determined for each list via the software package D.A.V.I.D. [14].

Codon usage heatmaps. Pairwise protein sequence alignments were obtained using the T-COFFEE (version 8.99, using default parameters for protein alignments) software package [15]. Each alignment was used to facilitate the comparison of like amino acids to their associated codons. Codon usage tables were obtained from the Codon Usage Database (available at http://www.kazusa.or.jp/codon/) [16]. The pairwise relationship between each codon was determined according to the formula $\log_2(a/n \cdot b/n)$ where *a* and *b* represent the number of occurrences for the same amino acid, and *n* represents the total number of codons occurring for that amino acid.

IMR90 growth response to oncogenic stress. IMR90 cell growth in the presence of the indicated Ras oncogenes was assayed as previously described with minor modifications [2]. Briefly, IMR90 cells (pd37-39) were transduced with pBabepuro retroviruses encoding the indicated Ras oncogenes and selected for resistance in the presence of 2µg/ml puromycin. 3 days after starting selection, cells were counted and 4,000 cells were plated in quadruplicate in 24-well plates. Cells were maintained in selective media changed every other day; at the indicated time points cells were fixed with 10% neutral buffered formalin for 5 minutes at room temperature and stained with crystal violet. At the completion of the experiment, crystal violet was extracted in 1ml 10% acetic acid per well and quantified by absorbance at 590nm. Values at all time points were normalized to the mean for each cell line at day 0.

Statistics. For xenograft studies, we compared tumor weights and tumor volumes of all mice in each arm on the final day of the experiment using a two sided unpaired t test. For mRNA quantitation in Figure S1K and S2B, fold changes were calculated using the △△Ct method. The △Ct of each sample equals Ct(target) minus Ct(GAPDH control), and fold changes were determined by calculating $2^{\triangle Ct(\text{control average}) -}$

$^{\triangle Ct(sample)}$. A two sided unpaired t test was used to compare mRNA fold changes where indicated. For quantification of the polysome profile, the fraction with the largest amount of message served as the reference baseline and the amount in all other fractions was calculated using the $\triangle\triangle$Ct method. A one-way ANOVA and Bonferroni multiple comparison test were used to compare quantification of IMR90 cells at each time point. All statistical analyses were performed using Graphpad Prism v5 (Graphpad Software).

SUPPLEMENTARY REFERENCES

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