Supplementary Methods

General materials. Unlabelled and 5'-end FAM-labelled poly $(AG)_8$ and $(UC)_8$ RNAs were obtained from Integrated DNA Technologies (IDT). MG-002 and eFT226 were custom synthesized by WuXi Chemistry Services (China). eFT226 was also purchased from GLPBIO (Montclair, CA). CR-1-31B was generously provided by Dr. John Porco Jr (Boston University).

eHAP1 cells were obtained from Horizon Discovery and maintained in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin antibiotics (Pen-Strep), and 2 mM L-glutamine at 37°C and 5% CO_2. 293T/17 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% bovine growth serum, 1% Pen-Strep, and 2 mM L-glutamine. Tests for mycoplasma contamination were routinely performed. MDA-MB-231 cells were obtained from ATCC and were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1% Pen-Strep, and 2 mM L-glutamine. 4T1 cells were obtained from ATCC and were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, 1.5g/L Sodium Bicarbonate, 10mM HEPES, 1mM Sodium Pyruvate, 1% Pen-Strep, and 2 mM L-glutamine. BT474 cells were obtained from ATCC and were grown in Roswell Park Memorial Institute (RPMI) medium supplemented with 10% fetal bovine serum, 1% Pen-Strep, and 2 mM L-glutamine. IMR-90 cells were obtained from ATCC and were grown in Eagles Minimum Essential Medium (EMEM) supplemented with 10% bovine growth serum, 1% Pen-Strep, and 2 mM L-glutamine. MRC-5 were obtained from ATCC and were grown in Eagles Minimum Essential Medium (EMEM) supplemented with 10% bovine growth serum, 1% Pen-Strep, and 2 mM L-glutamine. HUVEC cells were obtained from Lonza and propagated in Endothelial Basal Medium (EBM) supplemented with Endothelial Supplemental Mix.

Immunoblotting. Cells were pelleted, washed in PBS and lysed with RIPA buffer (20 mM Tris-HCl [pH 7.6], 100 mM NaCl, 1 mM EDTA,1 mM EGTA, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 20 mM β-glycerophosphate, 10 mM NaF, 1 mM PMSF, 4 μg/mL aprotinin, 2 μg/mL leupeptin, 2 μg/mL pepstatin). Cell lysates were collected after centrifuging the samples at 16000 x g for 10 mins, resolved on a 10% SDS-polyacrylamide gel, and transferred to PVDF membrane (Bio-Rad). Antibodies against eIF4A1 (ab31217) and eIF4A2 (ab31218) were obtained from Abcam, anti-eEF2 (#2332), anti-V5 tag (#13202), anti-PARP (#9542) and anti-c-Myc (#5605) antibodies were from Cell Signaling Technologies. Anti-eIF4A3 (HPA021878) antibody was obtained from Atlas Antibodies. Anti-DDX3X antibody was obtained from Novus Biologicals.

Time-lapse microscopy for cell cycle analysis. Eighty thousand MDA-MB-231 cells were seeded in 12-well plates in DMEM supplemented with 10% FBS and antibiotics. Twenty-four hours later, cells were treated with 2.5 mM thymidine dissolved in media to synchronize them in S phase. Twenty hours later, cells were washed twice with warm PBS and released into fresh media containing either DMSO or 10 nM of CR-1-31B, MG-002, or eFT226. Following drug addition, cells were placed in an incubation chamber of a Zeiss Axiovert 200M microscope to maintain temperature and CO_2 levels. Bright-field imaging was performed. Images were taken every 10 minutes at 10x total magnification for a total of 40 hours. For each condition, 50 cells were followed from the start time of the experiment to when they entered mitosis (minutes to mitotic entry), which roughly represents the time they spent in late S and G2 phase of the cell cycle. A one-way ANOVA with Tukey multiple comparisons test was used to compare the average minutes to mitotic entry for each treatment to the vehicle control.

Cell viability assays and EC_{50} determination. Cell viability assays were performed using sulforhodamine B reagent (SRB). Cells (2000/well) were seeded into 96 well plates and treated with

compound at the indicated concentrations. Cells were then washed with PBS and fixed with 50% TCA for 1 h at 4°C, rinsed with water and air-dried. Fixed cells were stained with 0.5% SRB/1% acetic acid for 1 h, plates were washed with 1% acetic acid four times, and dried. The bound SRB was resuspended in 100 μl 10 mM Tris-HCl [pH 9] and the OD_{550nm} was measured using a microplate reader (Molecular Devices) and relative viability was calculated by normalizing to the DMSO control.

Sensitization assay. Lentivirus (pLX-TRC317) harboring cDNAs to eIF4A1 (TRCN0000491404) and eIF4A3 (TRCN0000471580) were obtained from the Genetic Perturbation Service (GPS) of the Goodman Cancer Institute and McGill Biochemistry Department. The eIF4A2 cDNA was cloned into pLX-TRC317 and DDX3X was cloned into pPRIME-CMV-GFP-recipient. Virus was produced in 293T cells and used to infect parental or $eIF4A1^{F163L}/eIF4A2^{-}$ eHap1 cells. Following puromycin selection (2 μg/μl for 2 days), cells were expanded for experiments.

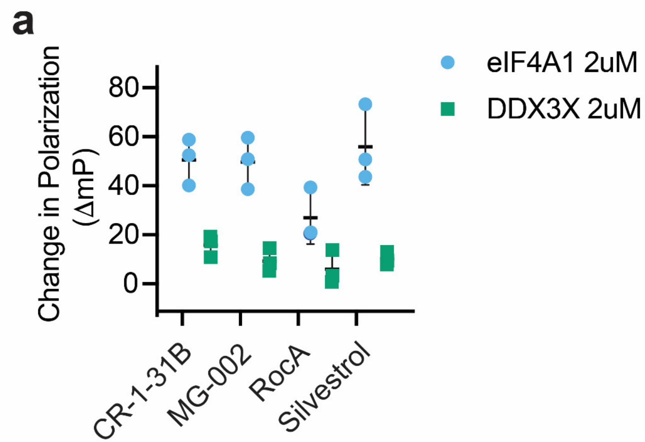
Bioanalytic analysis. Analytical methodology development, plasma protein binding, simulated gastric fluid stability and pharmacokinetic studies were performed by the Platform of Biopharmacy at the Université de Montréal. CBC analyses were performed by the Comparative Medicine and Animal Resources Centre (CMARC) at McGill University.

Table S1. Histopathological Analysis following PO Delivery of MG-002 (0.5 mg/kg) for 6 treatments (M/W/F). Tissues were harvested 12 days after the first injection.

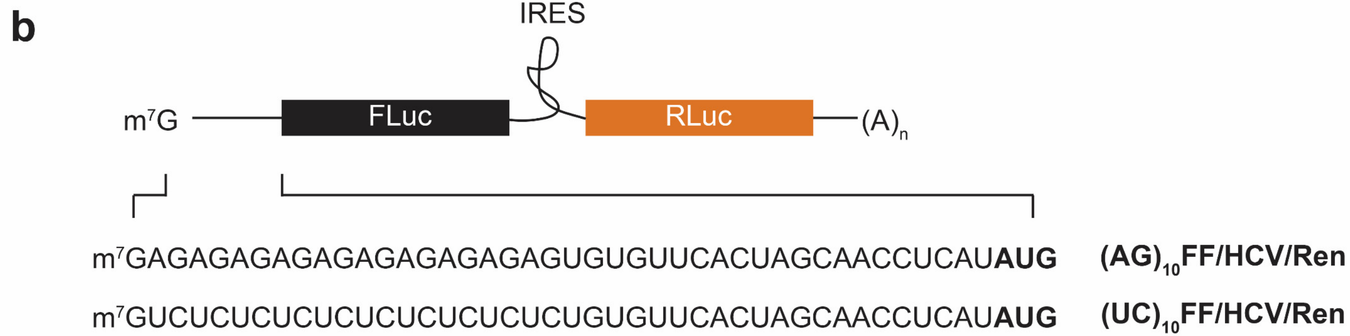
Animal ID	1	2	3	4	5	6	7	8
Treatment	Vehicle	Vehicle	Vehicle	Vehicle	Vehicle	MG-002	MG-002	MG-002
Heart	N	N	N	N	N	N	N	N
Lung	N	N	N	F	N	N	N	N
Lymphocytic inflammation, perivascular, focal				2				
Spleen	F	F	F	F	F	F	F	F
Extramedullary hematopoiesis, diffuse, red pulp	3	1	1	1	1	1	1	1
Kidney	N	N	N	N	N	F	N	N
Renal tubular degeneration and regeneration, focal, unilateral cortex						2		
Bone Marrow	N	N	N	N	N	N	N	N

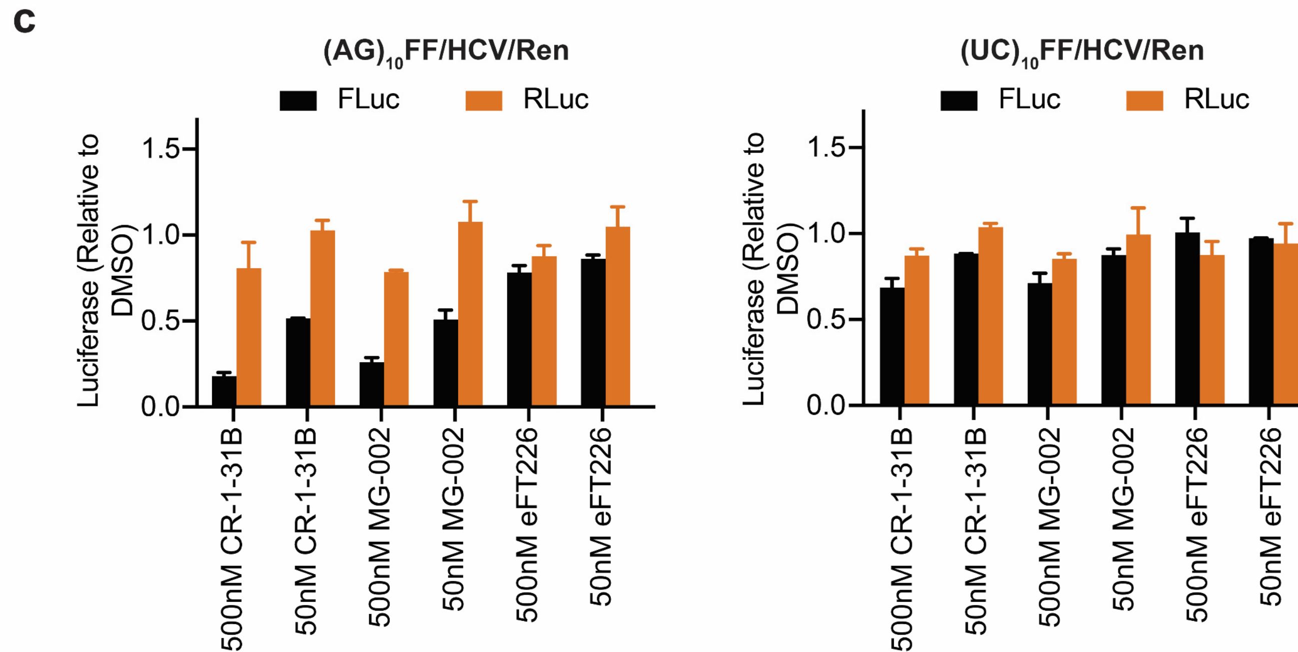
N: Normal
F: Finding

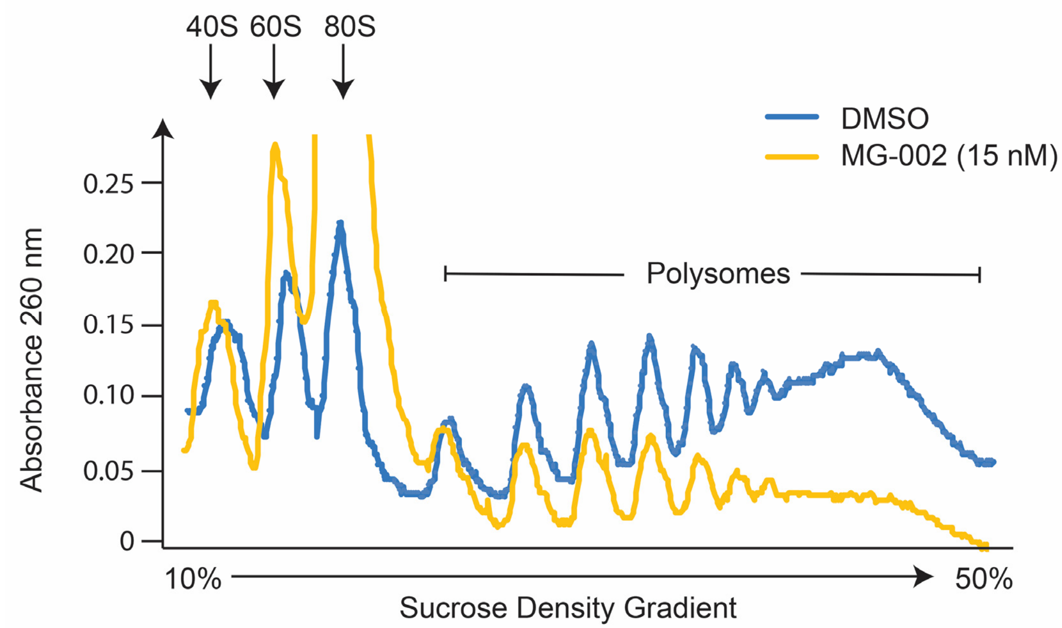
Grade 1 = modest, rare
Grade 2 = mild, infrequent
Grade 3 = moderate, frequent
Grade 4 = severe, diffuse



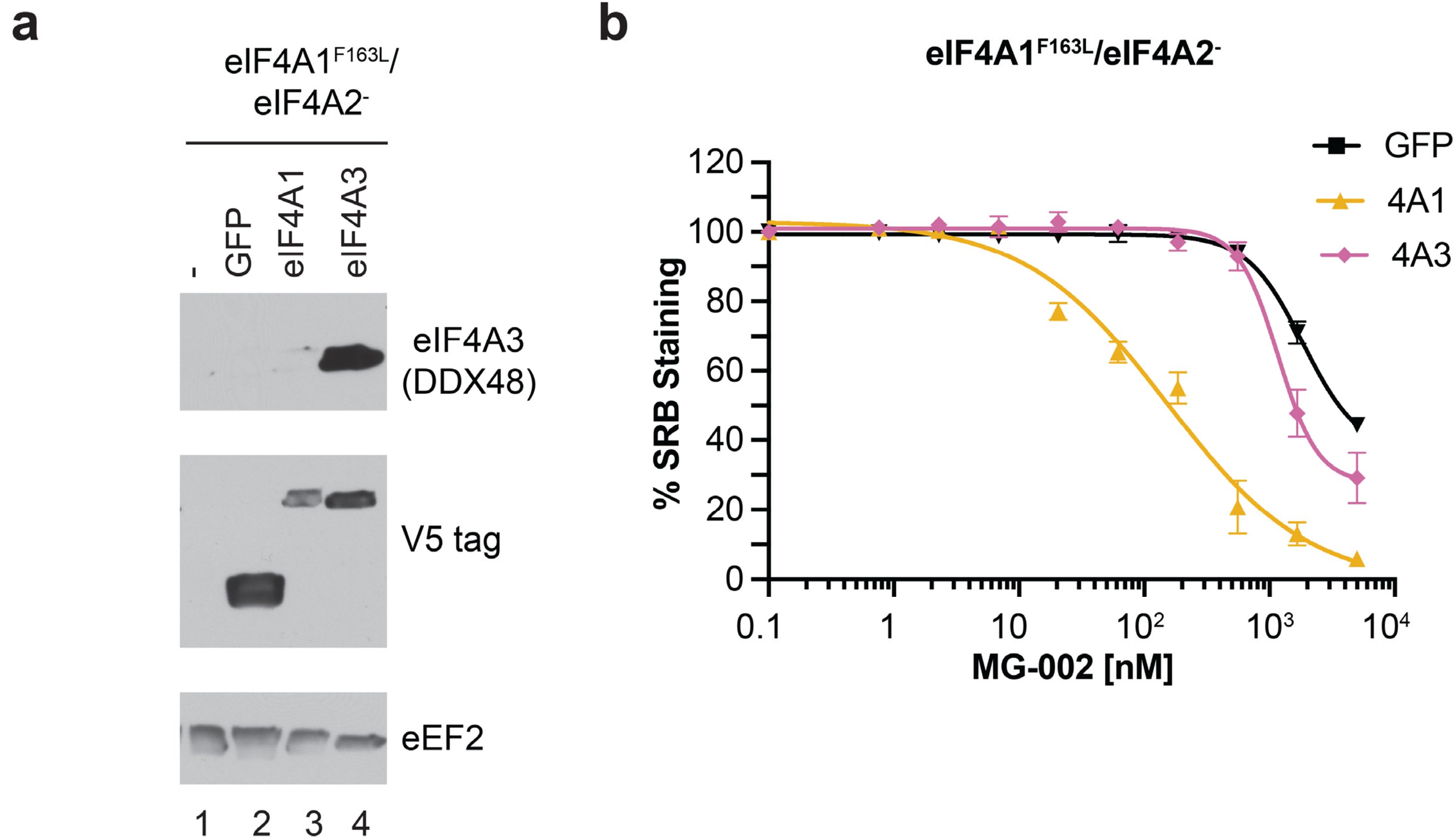
Supplementary Figure 1. MG-002 shows selectivity for mRNAs harboring (AG)-rich 5' leaders. a. Assessing compound-induced clamping of eIF4A1 and DDX3X to FAM-labelled poly$(AG)_8$ RNA. The ΔmP was measured for 2 μM protein and 10 μM compound. The ΔmP obtained relative to DMSO is shown (n=3 ± SD). **b.** Bicistronic mRNA reporters with (AG)- or (UC)- enriched 5' leader regions. **c.** Translational response of the indicated reporter mRNAs used to program Krebs-2 translation extracts (n=3 ± SD).



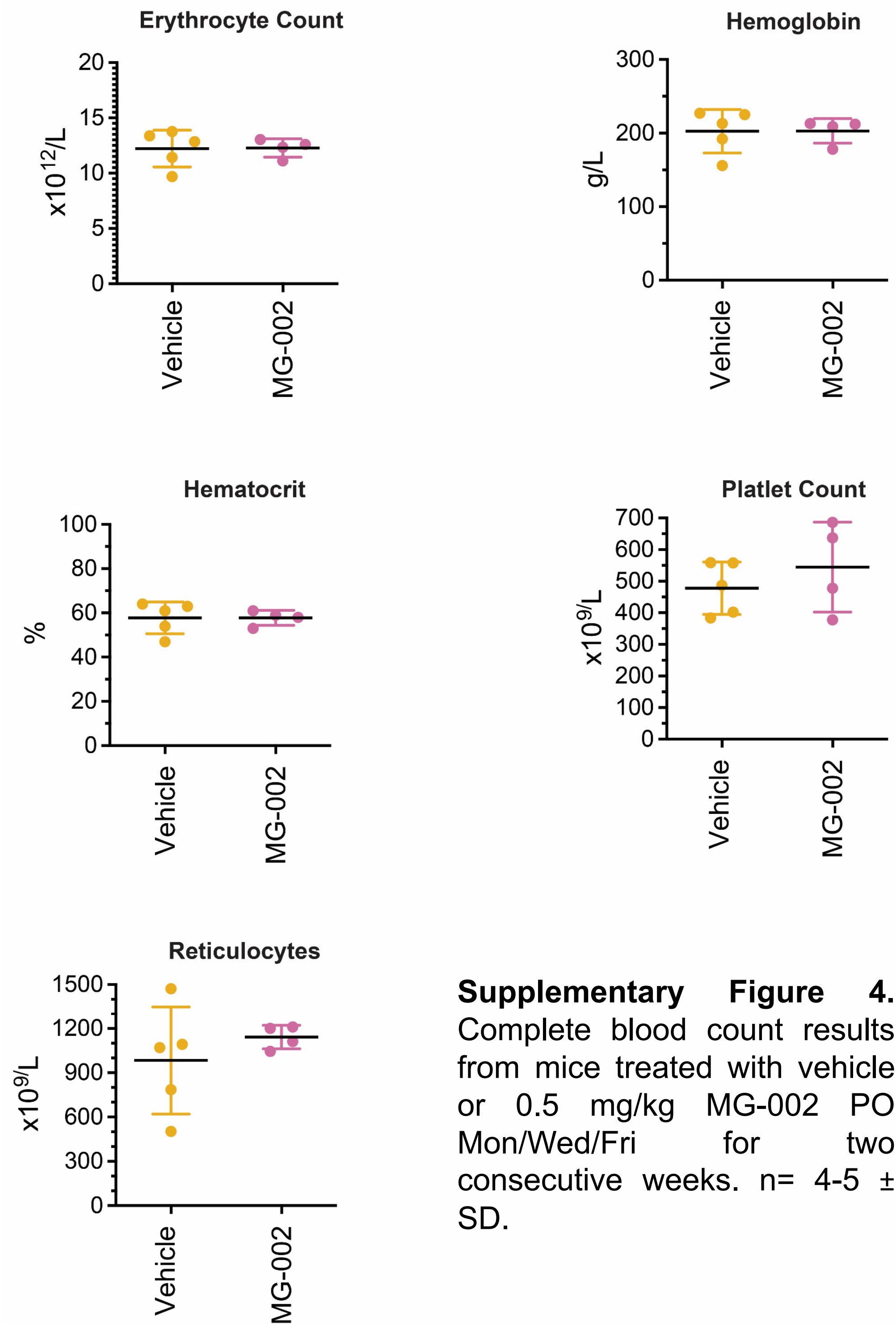




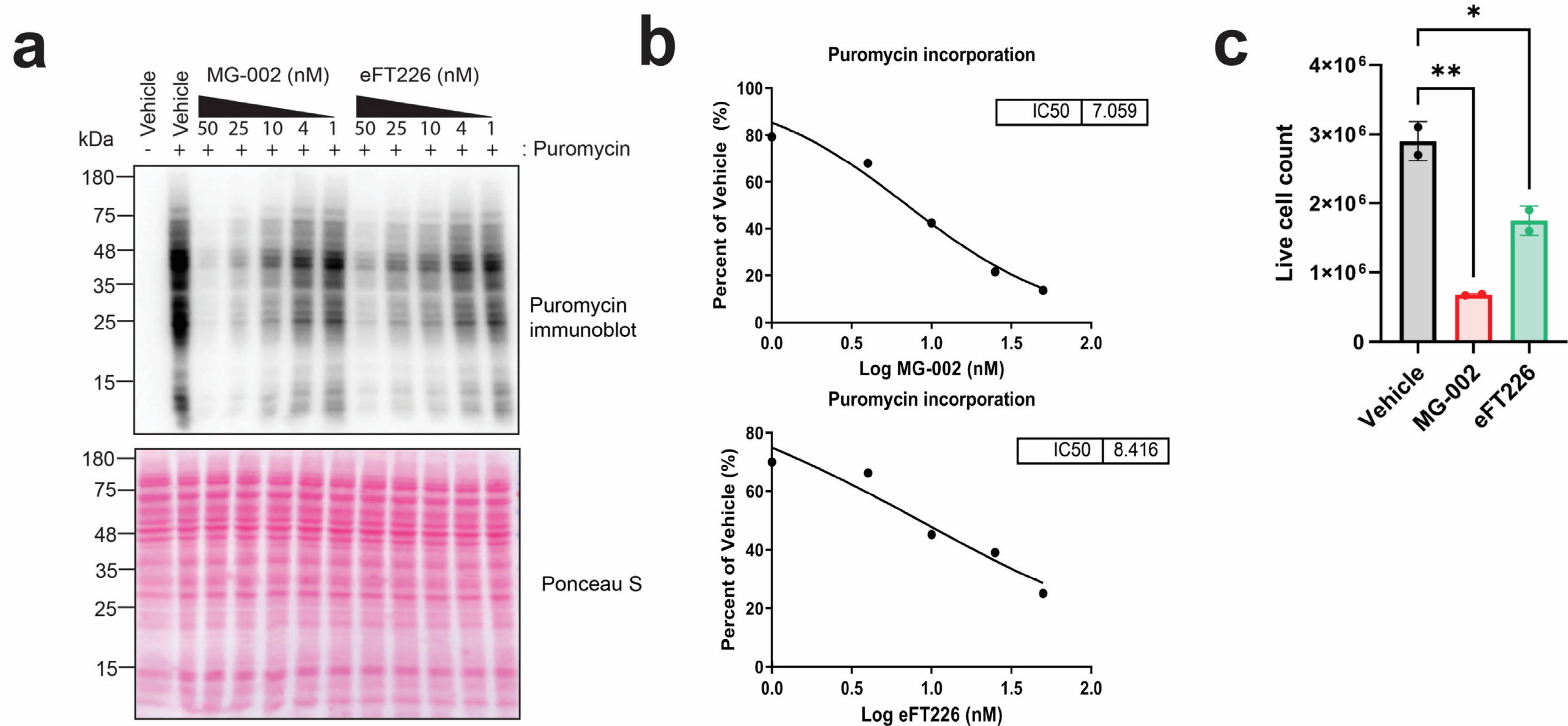
Supplementary Figure 2. Polysome profiles isolated from eHAP1 cells exposed to 15 nM MG-002 for 1 h.



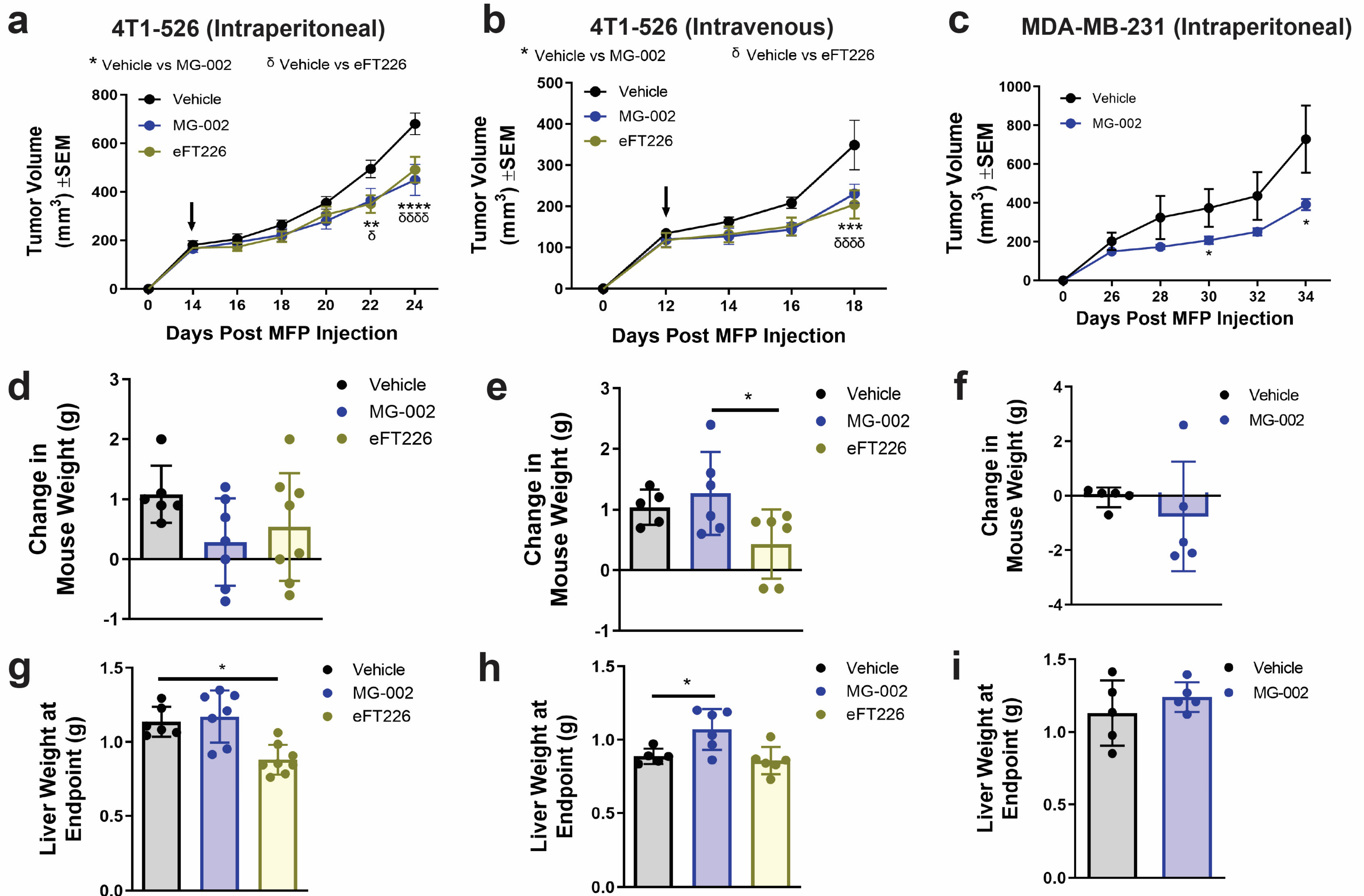
Supplementary Figure 3. a. Western blot of eIF4A1^{F163L}/eIF4A2^{-} eHAP1 cells ectopically expressing GFP, eIF4A1, or eIF4A3. Blots were probed with antibodies targeting the V5 tag, eIF4A3 or eEF2. **b.** Cytotoxicity of eIF4A1^{F163L}/eIF4A2^{-} eHAP1 cells ectopically expressing GFP, eIF4A1, or eIF4A3 following a two-day exposure to the indicated compound concentration of MG-002 (n=3 ± SD).



Supplementary Figure 4. Complete blood count results from mice treated with vehicle or 0.5 mg/kg MG-002 PO Mon/Wed/Fri for two consecutive weeks. n= 4-5 ± SD.



Supplementary Figure 5. a. Puromycin assays assessing *de novo* protein synthesis in 4T1-526 cells treated with increasing concentration of MG-002 (1-50 nM) compared to vehicle control. Puromycin incorporation was visualized by immunoblot analysis using puromycin-specific antibodies and Ponceau S staining verified similar protein content between the various conditions. **b.** Quantification of the data shown in panel **a** to determine the IC_{50} for the ability of MG-002 to inhibit protein synthesis. **c.** Using the IC_{50} concentrations calculated in panel b, MG-002 was tested for its ability to inhibit the growth of 4T1-526 cells after 72 hours in culture, compared to the vehicle control. The live cell count was determined by trypan blue exclusion. The data shown is representative of three independent biological replicates. Technical duplicate mean values across three biological replicates (± SD) are shown and statistical analysis was performed with a One-way ANOVA (Dunnett's multiple comparisons test). *$p<0.05$; **$p<0.01$



Supplementary Figure 6. 4T1-526 mammary tumors were allowed to develop in the mammary fat pads of BALB/c mice and when the tumors reached ~100 mm^3 (arrow), the animals were randomized into three groups: 0.5 mg/kg MG-002, 0.5 mg/kg eFT226, or vehicle control. Compounds were administered either **(a-c)** intraperitoneally (IP) or **(d-f)** intravenously (IV) and mice treated every two days until the experimental endpoint (n = 10-14 tumors/group). (**g-i**) MDA-MB-231 cells were allowed to develop in the mammary fat pads of SCID-Beige mice and when the tumors reached ~100 mm^3 (arrow), the animals were randomized into three groups: 0.5 mg/kg MG-002, 0.5 mg/kg eFT226, or vehicle control, injected intraperitoneally. The data is shown as the average tumor volume ± SEM. At the experimental endpoint, each animal was measured for the **(b, e, h)** average change in mouse weight from the beginning to the end of the study (grams) or their **(c, f, i)** liver weights (grams). The data is representative of 5-7 mice per group. Statistical analysis was performed with a Two-way ANOVA (Tukey's multiple comparisons test). *Vehicle versus MG-002 comparison; $^{\delta}$Vehicle versus eFT226 comparison.