Figure S1:

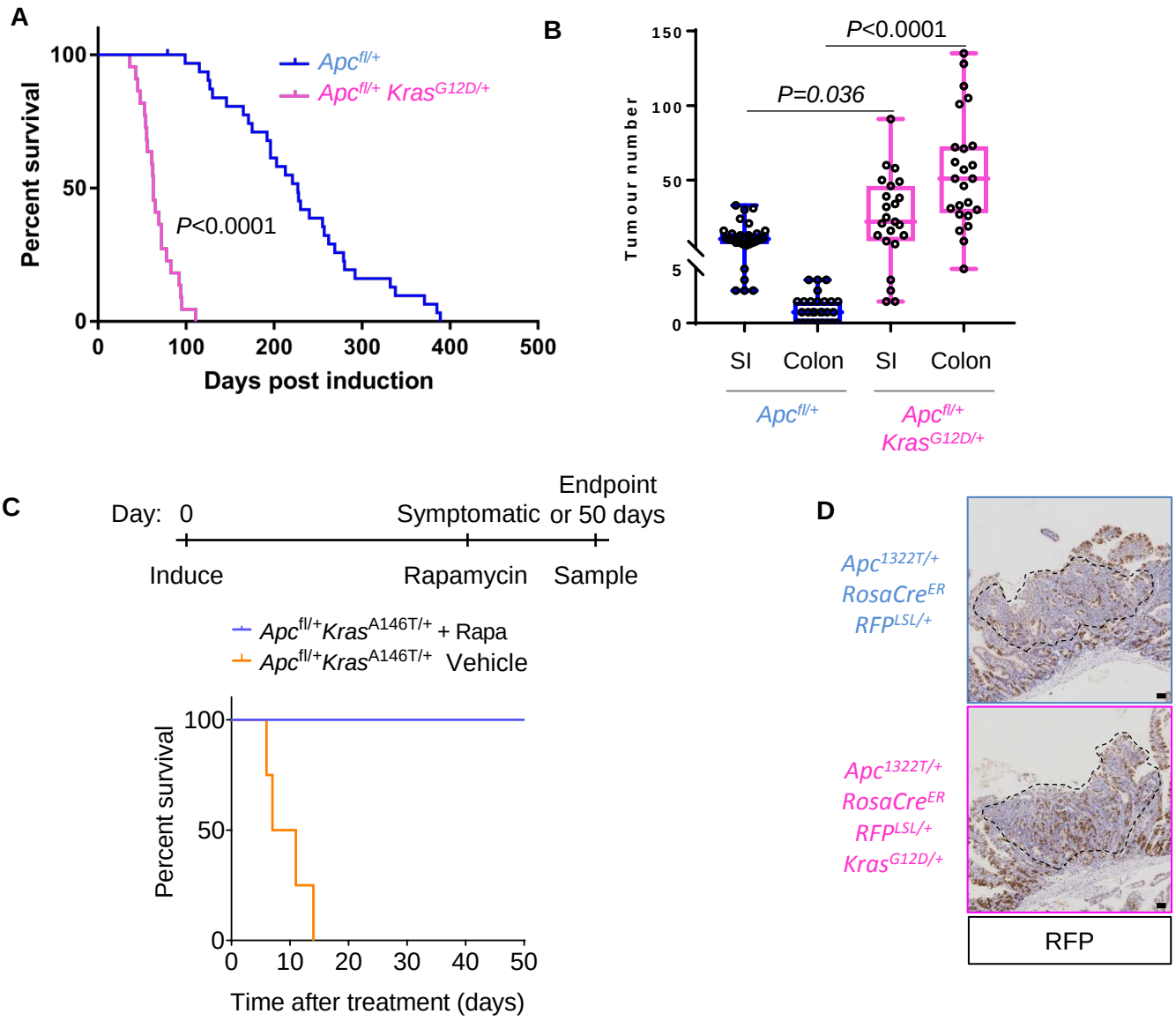


Figure S1: Mutant *Kras* accelerates tumorigenesis in *Apc*-deficient mice

(A) Survival of $Apc^{fl/+}$ and $Apc^{fl/+}$ $Kras^{G12D/+}$ tumor model mice after $Villin^{CreER}$ recombination (n = 31 $Apc^{fl/+}$ and 22 $Apc^{fl/+}$ $Kras^{G12D/+}$). *P* value is from a Log-rank Mantel-Cox test. (B) Intestinal tumor number scored macroscopically at endpoint. Each point represents an individual mouse. *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (C) Experimental schematic showing that $Apc^{fl/+}$ $Kras^{A146T/+}$ tumor model mice were aged until displaying symptoms of intestinal disease then administered rapamycin or vehicle until endpoint or 50 days. Survival plots from these experiments show that rapamycin suppresses tumorigenesis in these genotypes. (D) $Apc^{1322T/+}$ mice exhibiting a tumor burden were treated as in Figure 1C. Staining for the RFP reporter (expressed from the $Rosa^{RFP}$ allele) allowed recombination efficiency to be monitored throughout the intestine, confirming recombination within adenomas. Scale bars, 50µm. See also Figure 1.

Figure S2:

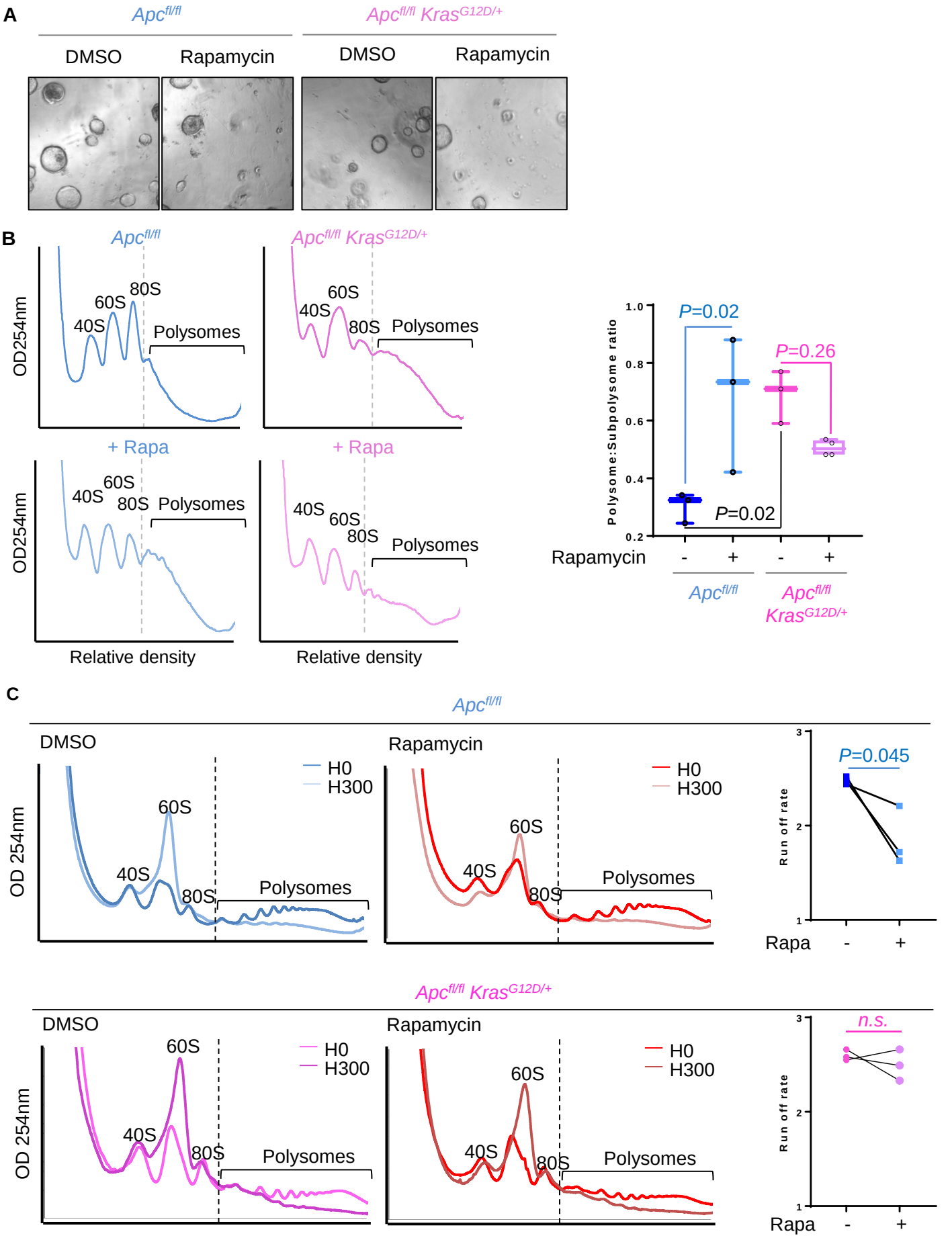

A
Apcfl/fl
Apcfl/fl KrasG12D/+
DMSO
Rapamycin
DMSO
Rapamycin
B
Apcfl/fl
Apcfl/fl KrasG12D/+
OD254nm
40S
60S
80S
Polysomes
+ Rapa
Relative density
Polysome:Subpolysome ratio
P=0.02
P=0.26
P=0.02
Rapamycin
Apcfl/fl
Apcfl/fl KrasG12D/+
C
Apcfl/fl
DMSO
Rapamycin
H0
H300
OD 254nm
Run off rate
P=0.045
Rapa
Apcfl/fl KrasG12D/+
n.s.

Figure S2:

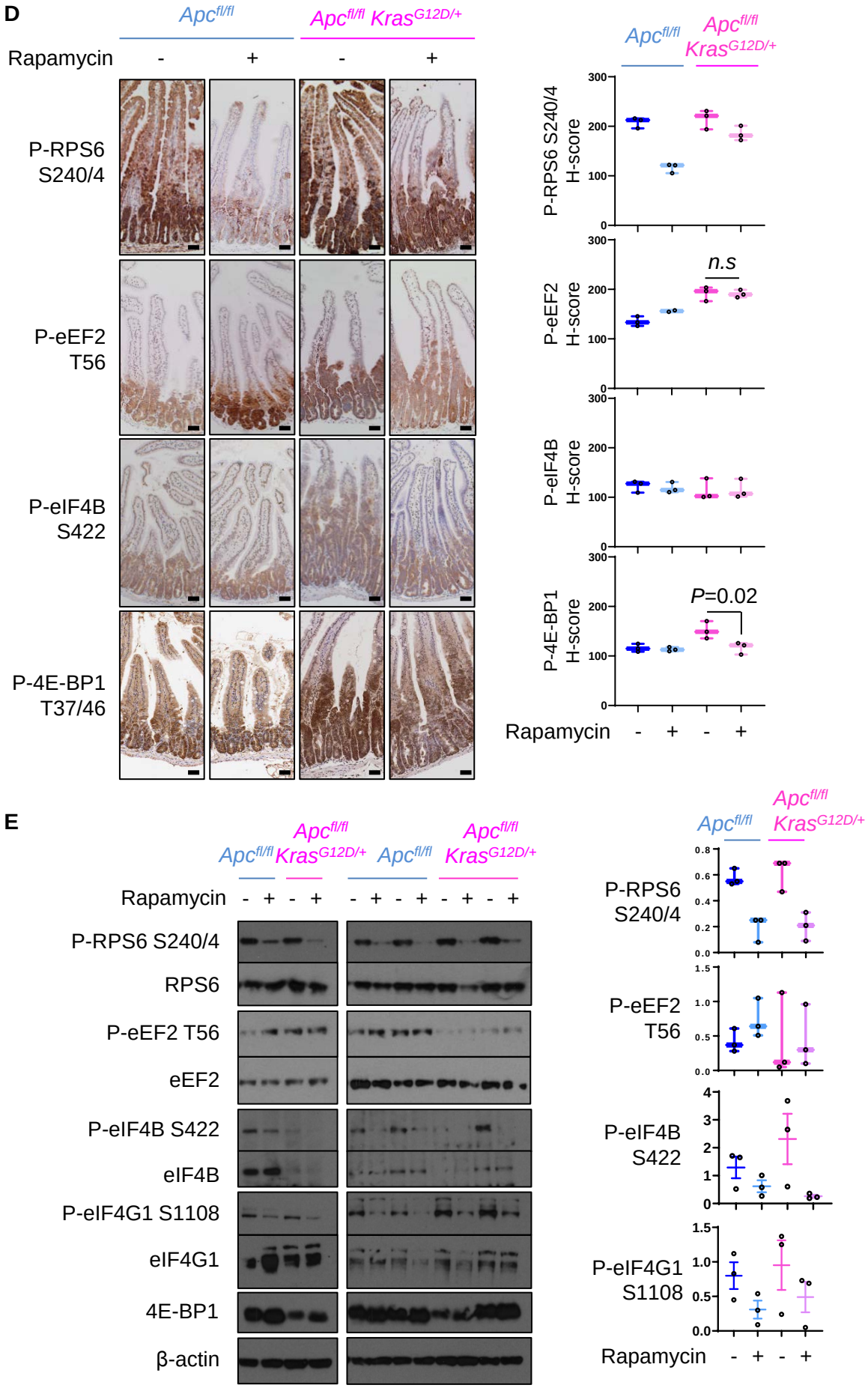

D
Apc fl/fl
Apc fl/fl Kras G12D/+
Rapamycin - + - +
P-RPS6 S240/4
P-eEF2 T56
P-eIF4B S422
P-4E-BP1 T37/46
P-RPS6 S240/4 H-score
P-eEF2 H-score
n.s
P-eIF4B H-score
P-4E-BP1 H-score
P=0.02
Rapamycin - + - +
E
Apc fl/fl
Apc fl/fl Kras G12D/+
Rapamycin - + - + - + - + - + - + - +
P-RPS6 S240/4
RPS6
P-eEF2 T56
eEF2
P-eIF4B S422
eIF4B
P-eIF4G1 S1108
eIF4G1
4E-BP1
β-actin
Rapamycin - + - +

Figure S2: KRAS alters the action of rapamycin on the regulation of translation

(A) Bright-field images of *Apc*$^{fl/fl}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ organoids treated with rapamycin or vehicle for 24 hours. (B) Sucrose density traces of epithelial crypt extracts from *Apc*$^{fl/fl}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ short-term model mice treated with or without rapamycin (10mg/kg). Peaks of ribosomal subunit (40S and 60S), monosomes (80S), and polysomes are indicated. Representative traces are shown next to graph of the averages for each genotype. Each point represents an individual mouse. *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (C) Harringtonine run-off sucrose density gradients from *Apc*$^{fl/fl}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ organoids treated with rapamycin or DMSO (vehicle) for 6hours prior to harvesting. H0 = 0seconds of harringtonine. H300 = 300seconds of harringtonine. Polysome to subpolysome abundance was calculated as denoted by dashed line and run-off rate derived, shown in adjacent graphs. Each pair of linked points represents an organoid line treated with vehicle or rapamycin. *P* values are paired student t tests. (D) Left, IHC staining for P-RPS6 S240/4, P-eEF2 T56, P-eIF4B S422 and P-4EBP1 T37/46, in APC and APC KRAS small intestines treated with and without rapamycin as in (B) Right, H-score quantification of staining from crypt regions of IHCs on the left. (E) Western blotting for total and phosphorylated RPS6, eEF2, eIF4B and eIF4G in *Apc*$^{fl/fl}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ organoids treated with vehicle or rapamycin as in (C). β-actin was used as a sample control. Blots shown are representative of organoids derived from 3 mice. Inset graphs show the quantification of each antibody across the 3 replicates, with each phosphorylated form normalized to total protein. All data are represented as mean ± S.E.M. Scale bars, 50μm. See also Figure 2.

Figure S3:

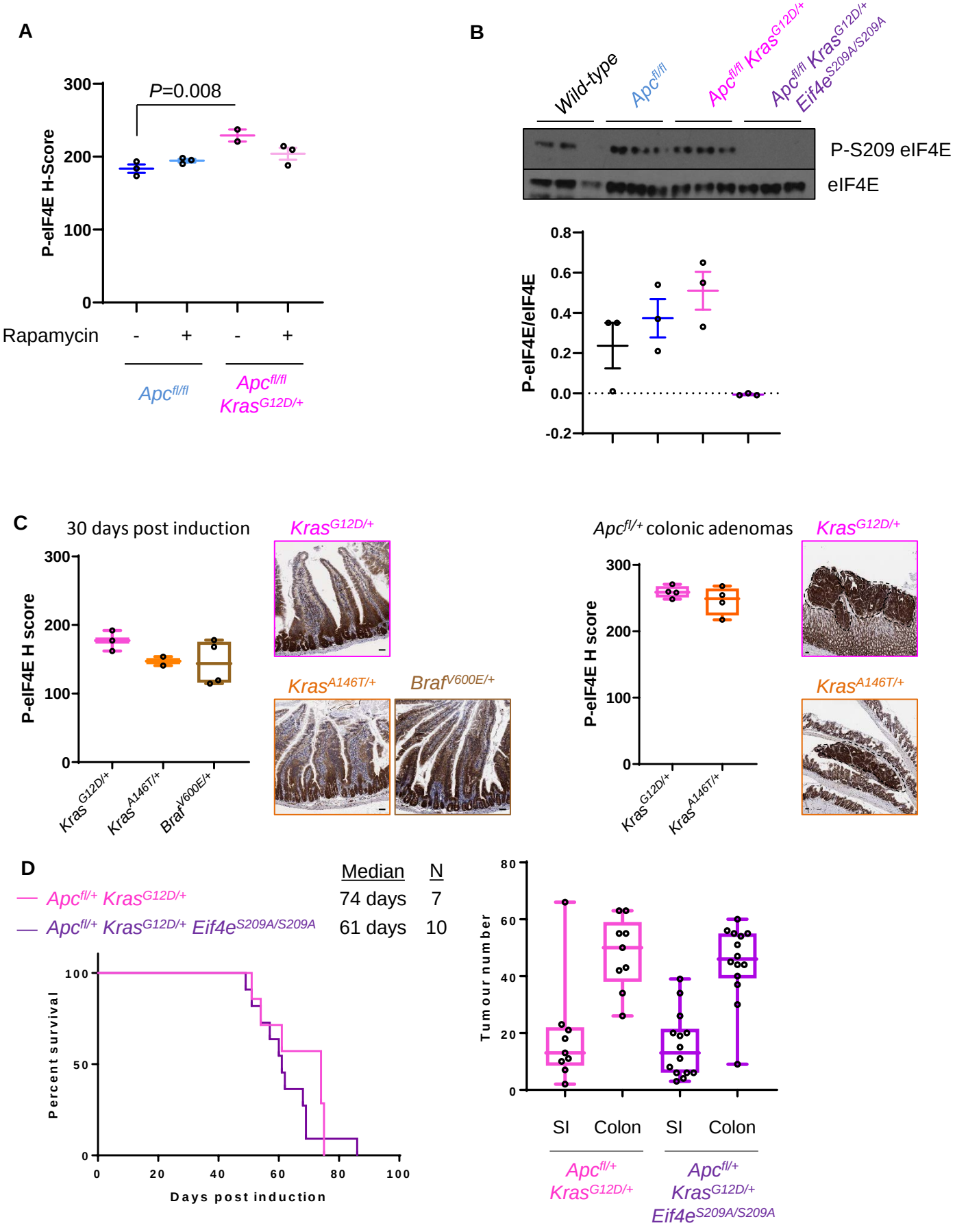

A
P=0.008
P-eIF4E H-Score
300
200
100
0
Rapamycin
-
+
-
+
Apcfl/fl
Apcfl/fl KrasG12D/+
B
Wild-type
Apcfl/fl
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Eif4eS209A/S209A
P-S209 eIF4E
eIF4E
P-eIF4E/eIF4E
0.8
0.6
0.4
0.2
0.0
-0.2
C
30 days post induction
P-eIF4E H score
300
200
100
0
KrasG12D/+
KrasA146T/+
BrafV600E/+
Apcfl/+ colonic adenomas
KrasG12D/+
KrasA146T/+
D
Apcfl/+ KrasG12D/+
Apcfl/+ KrasG12D/+ Eif4eS209A/S209A
Median
N
74 days
7
61 days
10
Percent survival
100
50
0
0
20
40
60
80
100
Days post induction
Tumour number
80
60
40
20
0
SI
Colon
SI
Colon
Apcfl/+ KrasG12D/+
Apcfl/+ KrasG12D/+ Eif4eS209A/S209A

Figure S3:

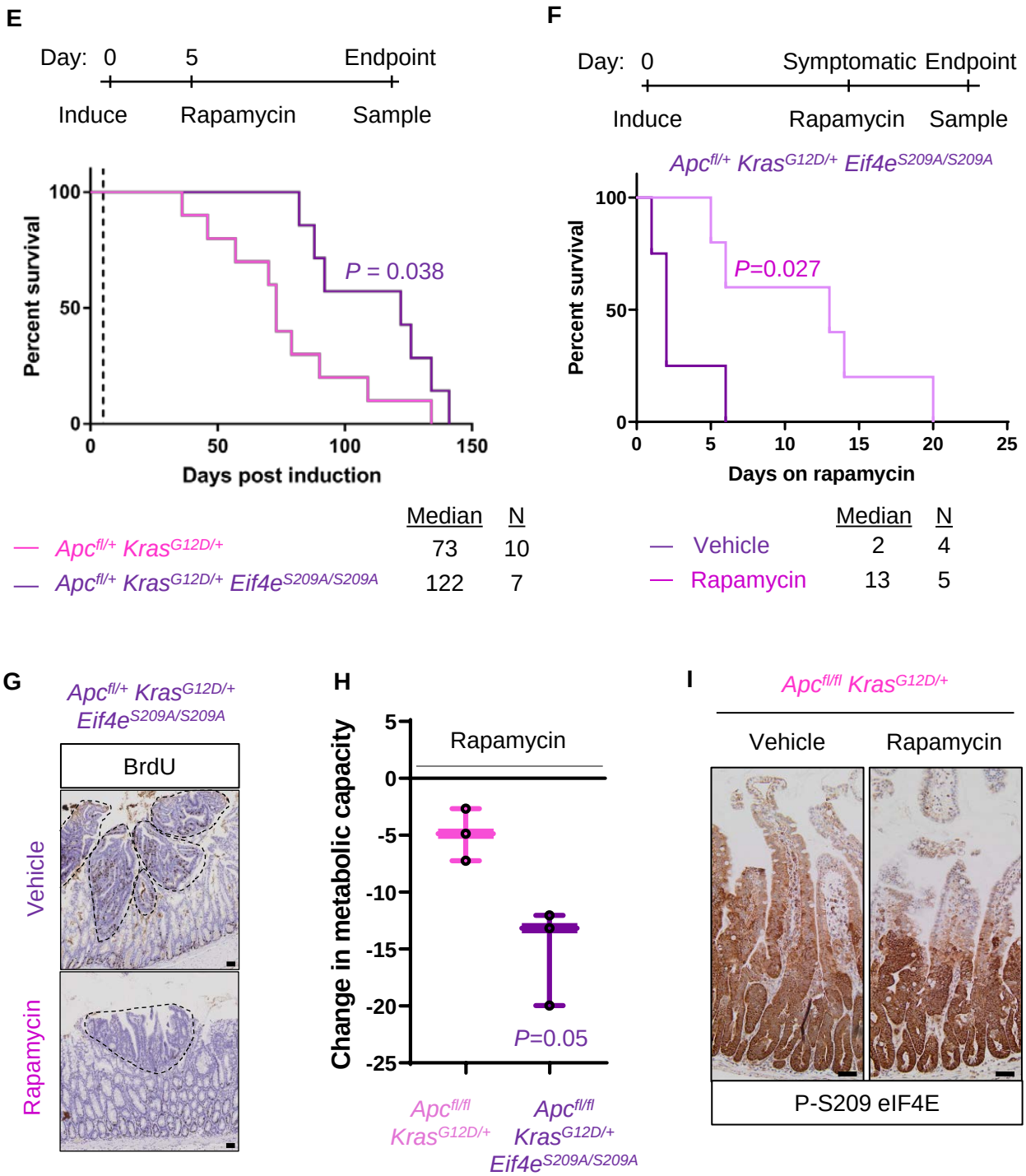

E
Day: 0
5
Endpoint
Induce
Rapamycin
Sample
Percent survival
100
50
0
0
50
100
150
Days post induction
P = 0.038
Median
N
Apcfl/+ KrasG12D/+
73
10
Apcfl/+ KrasG12D/+ Eif4eS209A/S209A
122
7
F
Day: 0
Symptomatic Endpoint
Induce
Rapamycin
Sample
Apcfl/+ KrasG12D/+ Eif4eS209A/S209A
Percent survival
100
50
0
0
5
10
15
20
25
Days on rapamycin
P=0.027
Median
N
Vehicle
2
4
Rapamycin
13
5
G
Apcfl/+ KrasG12D/+
Eif4eS209A/S209A
BrdU
Vehicle
Rapamycin
H
Change in metabolic capacity
5
0
-5
-10
-15
-20
-25
Rapamycin
P=0.05
Apcfl/fl
KrasG12D/+
Apcfl/fl
KrasG12D/+
Eif4eS209A/S209A
I
Apcfl/fl KrasG12D/+
Vehicle
Rapamycin
P-S209 eIF4E

Figure S3: P-eIF4E is increased in APC KRAS small intestinal organoids which respond to rapamycin treatment

(A) H score quantification of P-eIF4E S209 levels in *Apc*$^{fl/fl}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ intestines, treated with or without rapamycin. *P* value is from one-way ANOVA Tukey's multiple comparisons test. (B) Western blotting for P-eIF4E in small intestinal organoids of the indicated genotypes. Each lane represents an independent organoid preparation from each genotype. Graph shows quantified level of P-eIF4E as relative pixel number, standardized to total eIF4E abundance. (C) Right: mice expressing Villin^{CreER} and different *Kras* or *Braf* activating mutations were induced and aged for 30 days. Small intestinal tissue was then stained for P-S209 eIF4E and a H-score calculated. Left: P-eIF4E IHC on colonic adenomas from *Apc*$^{fl/+}$ tumor model mice expressing different *Kras* activating mutations, sampled when exhibiting signs of intestinal tumors. Dashed areas indicate adenomas. Inset images are representative examples of staining. (D) Survival plot of *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ and *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ tumor model mice sampled when showing advanced signs of intestinal tumors. Graph below shows no difference in small intestinal or colonic tumor burden between genotypes. Each point indicates and individual mouse. (E) *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ and *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ tumor model mice, aged with daily rapamycin treatment from 5days post-induction, and sampled at endpoint. *P* value is from a Log-rank Mantel-Cox test. (F) Survival of *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ tumor model mice treated with either rapamycin or vehicle when showing early signs of intestinal tumors and allowed to progress to endpoint. *P* value is from a Log-rank Mantel-Cox test. (G) Representative BrdU staining from *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ tumor model mice treated with either rapamycin or vehicle for 5days when showing early signs of intestinal tumors. Tumors are outlined with a dashed line. (H) *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ organoids were cultured with 1μM rapamycin for 30hours and their change in metabolic capacity relative to vehicle-treated counterparts plotted for 3 independent biological replicates. The same treatment was also carried out in parallel for control *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ organoids. *P* value is a paired student t test. (I) Representative IHC for P-eIF4E in small intestines of APC KRAS mice treated daily with rapamycin or vehicle between induction and sampling. All data are represented as mean ± S.E.M. Scale bars, 50μm. See also Figure 3.

Figure S4:

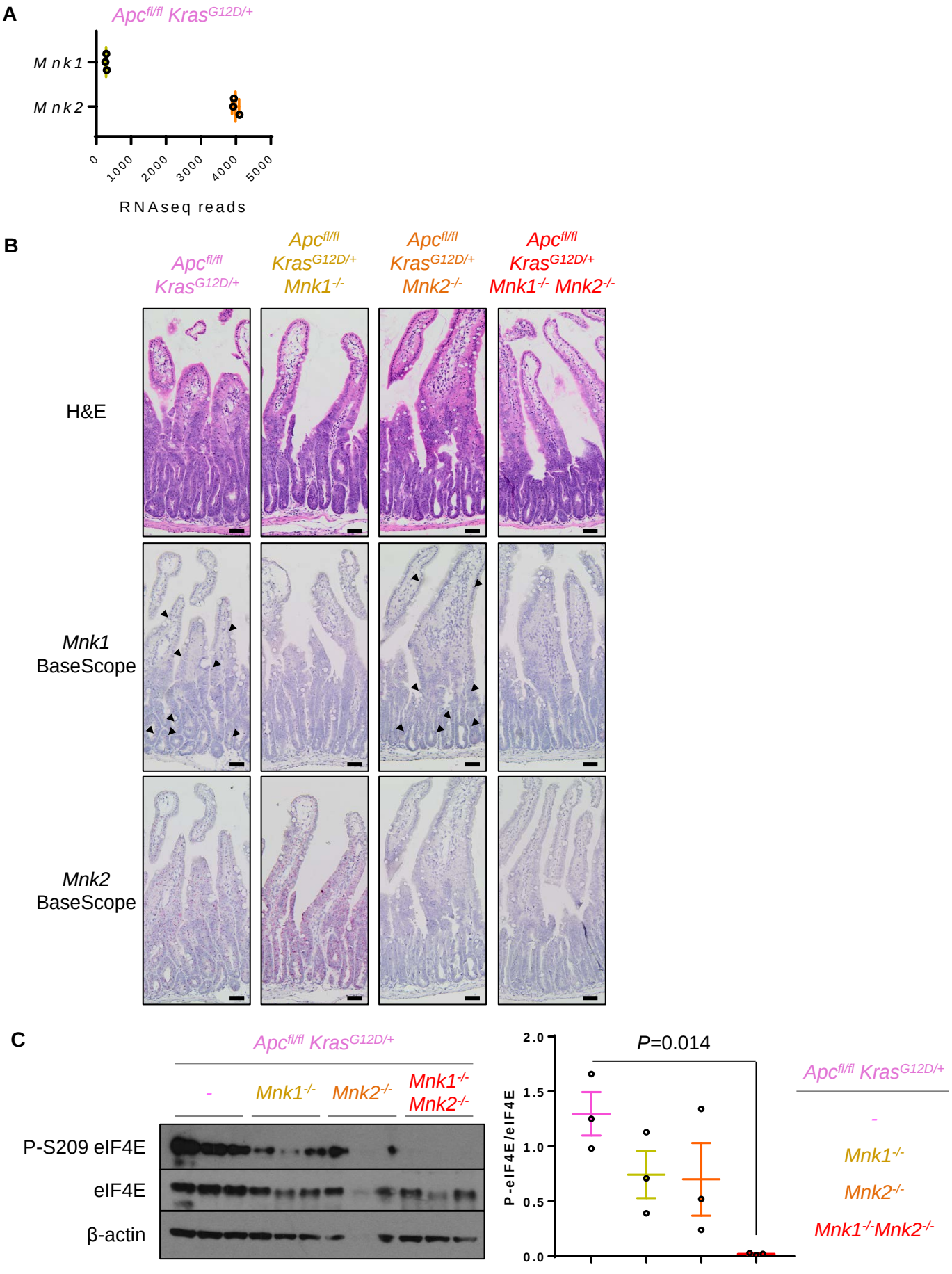

A
Apcfl/fl KrasG12D/+
Mnk1
Mnk2
0
1000
2000
3000
4000
5000
RNAseq reads
B
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Mnk1-/-
Apcfl/fl KrasG12D/+ Mnk2-/-
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
H&E
Mnk1 BaseScope
Mnk2 BaseScope
C
Apcfl/fl KrasG12D/+
-
Mnk1-/-
Mnk2-/-
Mnk1-/- Mnk2-/-
P-S209 eIF4E
eIF4E
β-actin
P=0.014
P-eIF4E/eIF4E
2.0
1.5
1.0
0.5
0.0
Apcfl/fl KrasG12D/+
-
Mnk1-/-
Mnk2-/-
Mnk1-/-Mnk2-/-

Figure S4:

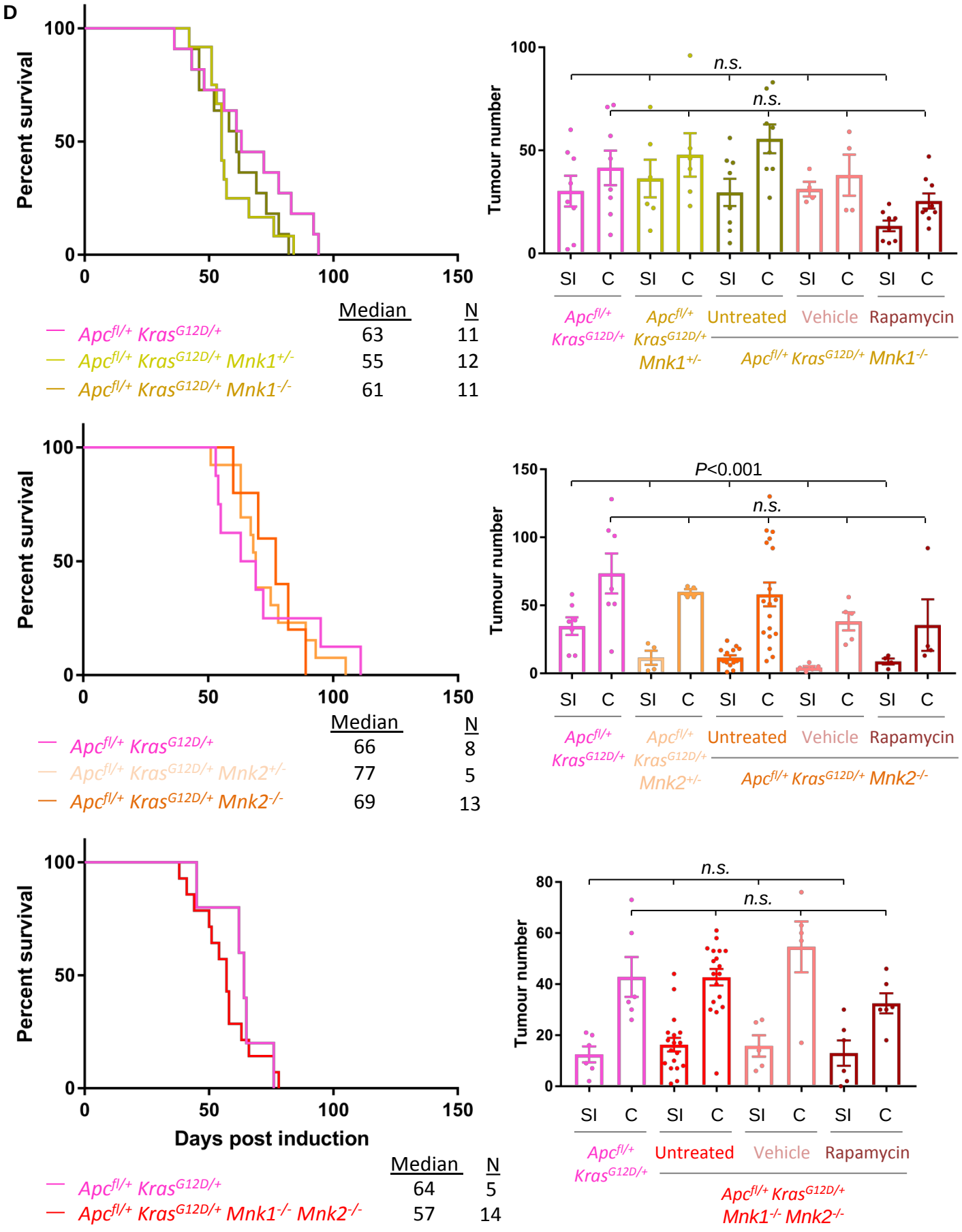

D
Percent survival
100
50
0
0
50
100
150
Median
N
Apcfl/+ KrasG12D/+
63
11
Apcfl/+ KrasG12D/+ Mnk1+/-
55
12
Apcfl/+ KrasG12D/+ Mnk1-/-
61
11
Tumour number
n.s.
n.s.
SI
C
Apcfl/+ KrasG12D/+
Apcfl/+ KrasG12D/+ Mnk1+/-
Untreated
Vehicle
Rapamycin
Apcfl/+ KrasG12D/+ Mnk1-/-
Apcfl/+ KrasG12D/+
66
8
Apcfl/+ KrasG12D/+ Mnk2+/-
77
5
Apcfl/+ KrasG12D/+ Mnk2-/-
69
13
P<0.001
Apcfl/+ KrasG12D/+ Mnk2+/-
Apcfl/+ KrasG12D/+ Mnk2-/-
Days post induction
Apcfl/+ KrasG12D/+
64
5
Apcfl/+ KrasG12D/+ Mnk1-/- Mnk2-/-
57
14
Apcfl/+ KrasG12D/+ Mnk1-/- Mnk2-/-

Figure S4:

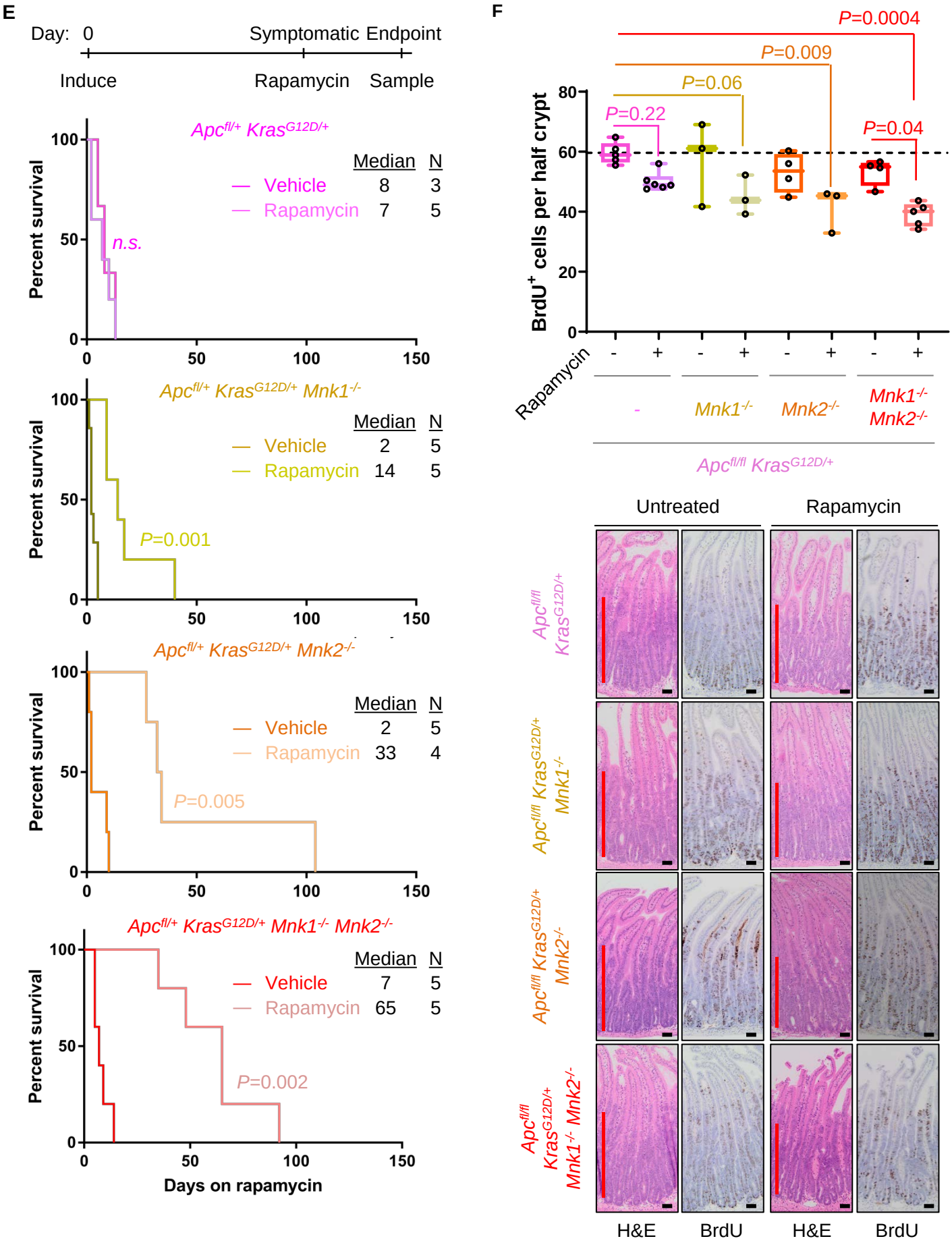

E
Day: 0
Symptomatic Endpoint
Induce
Rapamycin
Sample
Apcfl/+ KrasG12D/+
Median N
Vehicle 8 3
Rapamycin 7 5
n.s.
Apcfl/+ KrasG12D/+ Mnk1-/-
Vehicle 2 5
Rapamycin 14 5
P=0.001
Apcfl/+ KrasG12D/+ Mnk2-/-
Vehicle 2 5
Rapamycin 33 4
P=0.005
Apcfl/+ KrasG12D/+ Mnk1-/- Mnk2-/-
Vehicle 7 5
Rapamycin 65 5
P=0.002
Percent survival
Days on rapamycin
F
P=0.0004
P=0.009
P=0.06
P=0.22
P=0.04
BrdU+ cells per half crypt
Rapamycin
-
Mnk1-/-
Mnk2-/-
Mnk1-/- Mnk2-/-
Apcfl/fl KrasG12D/+
Untreated
Rapamycin
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Mnk1-/-
Apcfl/fl KrasG12D/+ Mnk2-/-
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
H&E
BrdU
H&E
BrdU

Figure S4:

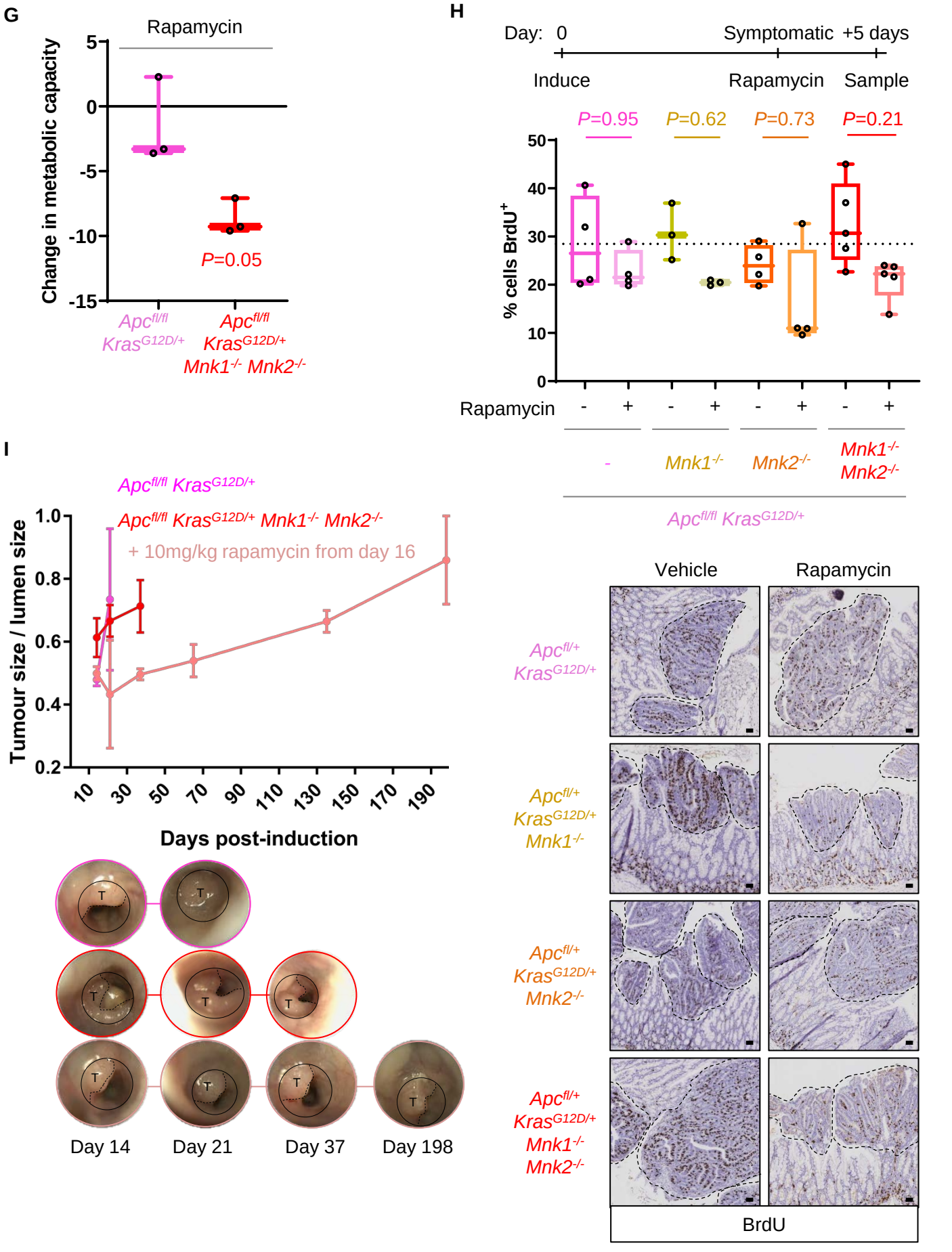

G
Rapamycin
Change in metabolic capacity
5
0
-5
-10
-15
P=0.05
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
H
Day: 0
Symptomatic +5 days
Induce
Rapamycin
Sample
P=0.95
P=0.62
P=0.73
P=0.21
% cells BrdU+
50
40
30
20
10
0
Rapamycin - + - + - + - +
-
Mnk1-/-
Mnk2-/-
Mnk1-/- Mnk2-/-
Apcfl/fl KrasG12D/+
I
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
+ 10mg/kg rapamycin from day 16
Tumour size / lumen size
1.0
0.8
0.6
0.4
0.2
10 30 50 70 90 110 130 150 170 190
Days post-induction
T
Day 14
Day 21
Day 37
Day 198
Vehicle
Rapamycin
Apcfl/+ KrasG12D/+
Apcfl/+ KrasG12D/+ Mnk1-/-
Apcfl/+ KrasG12D/+ Mnk2-/-
Apcfl/+ KrasG12D/+ Mnk1-/- Mnk2-/-
BrdU

Figure S4: The effect of different MNK deletions on P-eIF4E and tumor proliferation

(A) RNA sequencing reads for Mnk1 and Mnk2 from RNA purified from the intestine of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* mice. (B) H&E and BaseScope in situ hybridization for *Mnk1* or *Mnk2* mRNAs in *Apc$^{fl/fl}$ Kras$^{G12D/+}$* short-term model mice with deletion of *Mnk1*, *Mnk2* or both *Mnk1* and *Mnk2*. Arrows indicated cells expressing *Mnk1*.(C) Left, Western blot for P-eIF4E in *Apc$^{fl/fl}$ Kras$^{G12D/+}$* organoids with deletion of *Mnk1*, *Mnk2* or both *Mnks*. β-actin was used as a sample control. Right, graph shows the quantification of P-eIF4E relative to total eIF4E from the 3 biological replicates analyzed. *P* value is from one-way ANOVA Tukey's multiple comparisons test. (D) Survival (left) and tumor number (right, SI = small intestine, C = colon) data from tumor model, vehicle, and rapamycin-treated mice with deletion of *Mnk1* (top), *Mnk2* (middle) or *Mnk1* and *Mnk2* (bottom). *Apc$^{fl/+}$ Kras$^{G12D/+}$* mice were bred within each cohort and are shown in pink. Homozygous *Mnk*-deleted mice in each case were either untreated or treated with vehicle or with rapamycin. Tumor scores show the number of tumors at endpoint in each case. Each point is an individual animal. *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (E) Survival plots of *Apc$^{fl/+}$ Kras$^{G12D/+}$*, *Apc$^{fl/+}$ Kras$^{G12D/+}$ Mnk1$^{-/-}$*, *Apc$^{fl/+}$ Kras$^{G12D/+}$ Mnk2$^{-/-}$* and *Apc$^{fl/+}$ Kras$^{G12D/+}$ Mnk1$^{-/-}$ Mnk2$^{-/-}$* tumor model mice were aged until symptomatic and treated with rapamycin or vehicle until endpoint. Survival is plotted for the number of days on rapamycin or vehicle. Control *Apc$^{fl/+}$ Kras$^{G12D/+}$* and the *Apc$^{fl/+}$ Kras$^{G12D/+}$ Mnk1$^{-/-}$ Mnk2$^{-/-}$* data here is the same as in Figure 4D to allow comparison to the single *Mnk* cohorts. *P* values are from Log-rank Mantel-Cox tests. (F) Top, quantification of BrdU incorporation into small intestinal crypts in *Apc$^{fl/fl}$ Kras$^{G12D/+}$* mice with *Mnk1*, *Mnk2* or *Mnk1* and *Mnk2* deletions. Bottom, H&E staining and BrdU IHC of small intestines from *Apc$^{fl/fl}$ Kras$^{G12D/+}$* short-term model mice with *Mnk* deletions treated with and without rapamycin. Red bar shows extent of proliferative zone. *Apc$^{fl/fl}$ Kras$^{G12D/+}$* and *Apc$^{fl/+}$ Kras$^{G12D/+}$ Mnk1$^{-/-}$ Mnk2$^{-/-}$* data is replotted from Figure 4E. Each point represents the average number of BrdU positive cells from ≥25 half crypts from a single mouse. . *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (G) APC KRAS and APC KRAS MNK1/2 KO small intestinal organoids were treated with rapamycin at 1μM for 30hours and the change in metabolic capacity relative to vehicle treated organoids calculated. *P* values are from paired student t tests. (H) Top, quantification of BrdU incorporation into colonic tumors from tumor model *Apc$^{fl/+}$ Kras$^{G12D/+}$* tumor model mice with *Mnk1*, *Mnk2* or *Mnk1* and *Mnk2* deletions treated with rapamycin or vehicle for 5days prior to sampling. Bottom, representative images of BrdU levels in colonic tumors. Each point represents the average % BrdU positivity from ≥7 adenomas from individual mice. . *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (I) Colon tumor size was calculated as a percentage of visible lumen size in *Apc$^{fl/fl}$ Kras$^{G12D/+}$* and *Apc$^{fl/fl}$ Kras$^{G12D/+}$ Mnk1$^{-/-}$ Mnk2$^{-/-}$* mice following intracolonic tamoxifen induction. Example colonoscopy images are shown below, annotated with 'T' showing the tumor as area within dashed line of the lumen, shown as a circle or oval. All data are represented as mean ± S.E.M. Scale bars, 50μm. See also Figure 4.

Figure S5:

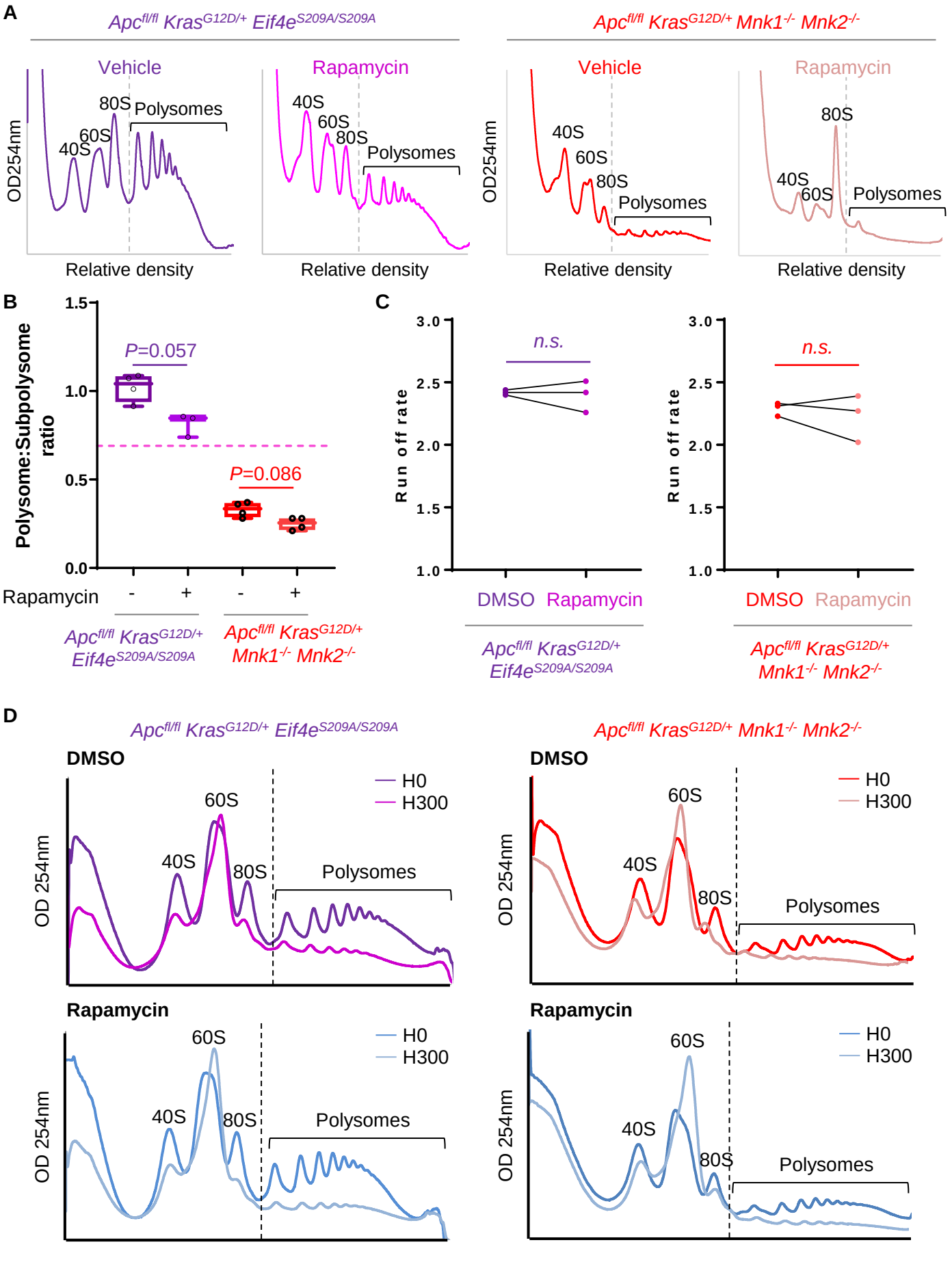
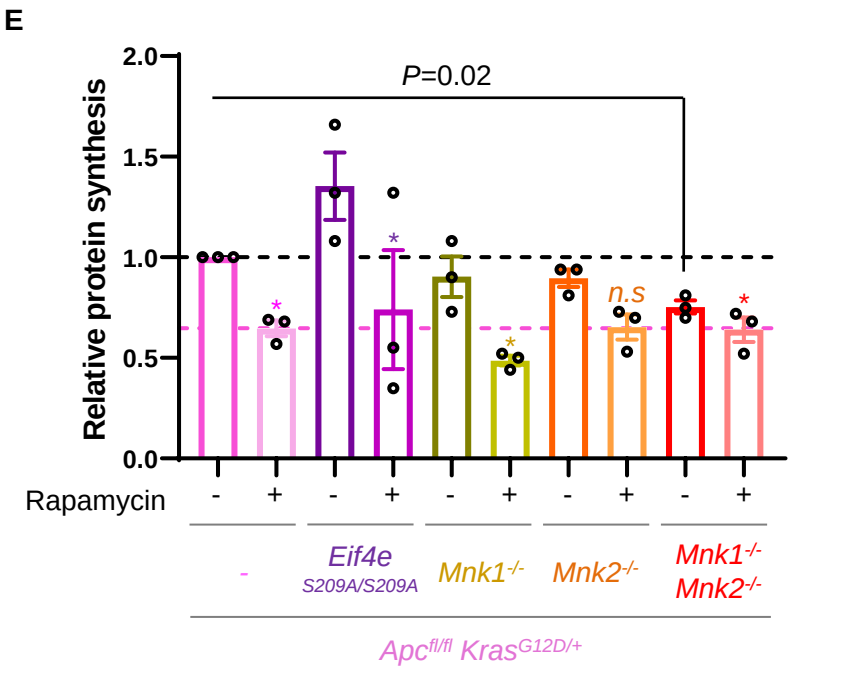

A
Apcfl/fl KrasG12D/+ Eif4eS209A/S209A
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
Vehicle
Rapamycin
OD254nm
40S
60S
80S
Polysomes
Relative density
B
Polysome:Subpolysome ratio
P=0.057
P=0.086
Rapamycin
C
Run off rate
n.s.
DMSO
Rapamycin
D
DMSO
Rapamycin
H0
H300
OD 254nm

Figure S5:


E
Relative protein synthesis
2.0
1.5
1.0
0.5
0.0
P=0.02
n.s
Rapamycin
- + - + - + - + - +
-
Eif4e S209A/S209A
Mnk1-/-
Mnk2-/-
Mnk1-/- Mnk2-/-
Apcfl/fl KrasG12D/+

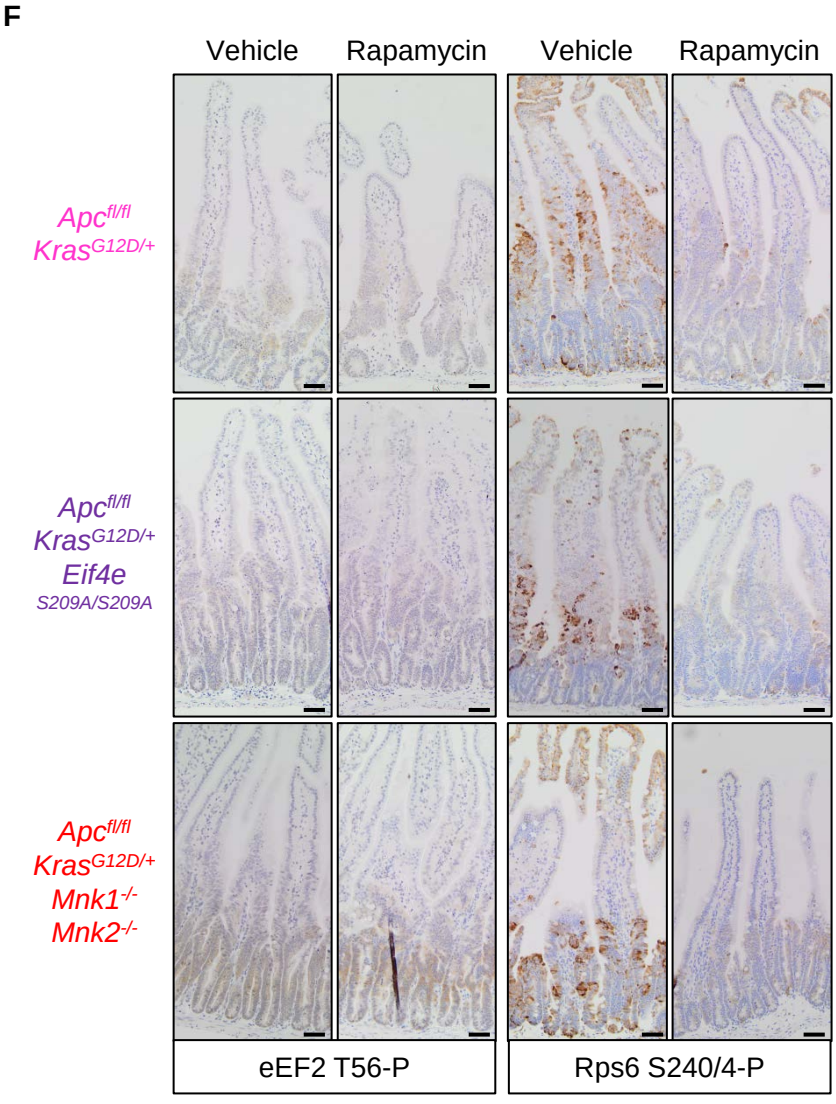

F
Vehicle
Rapamycin
Vehicle
Rapamycin
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Eif4e S209A/S209A
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
eEF2 T56-P
Rps6 S240/4-P

Figure S5:

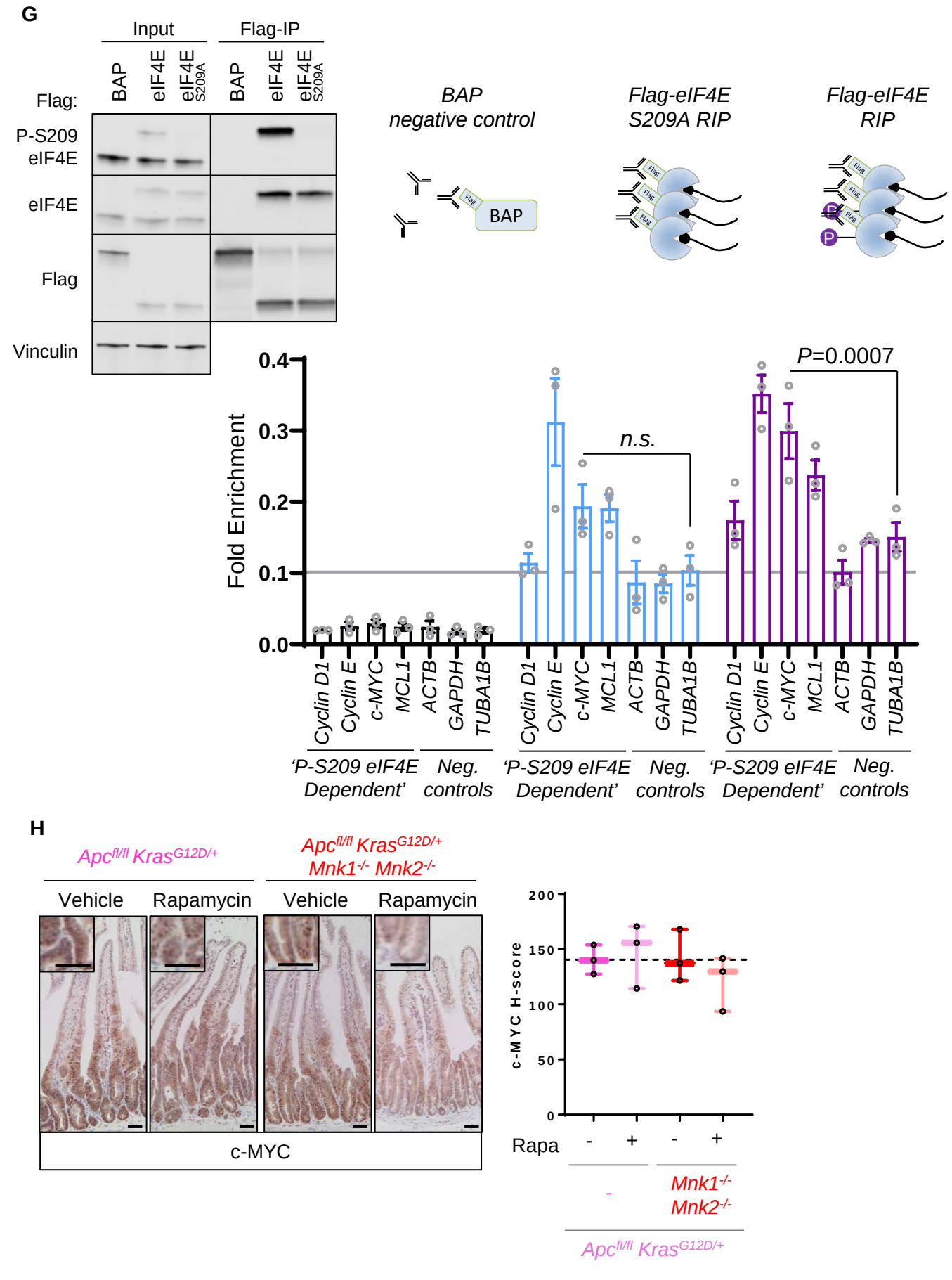

G
Input
Flag-IP
BAP
eIF4E
eIF4E S209A
Flag:
P-S209 eIF4E
eIF4E
Flag
Vinculin
BAP negative control
Flag-eIF4E S209A RIP
Flag-eIF4E RIP
Fold Enrichment
P=0.0007
n.s.
Cyclin D1
Cyclin E
c-MYC
MCL1
ACTB
GAPDH
TUBA1B
'P-S209 eIF4E Dependent'
Neg. controls
H
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Mnk1-/- Mnk2-/-
Vehicle
Rapamycin
c-MYC
c-MYC H-score
Rapa
Mnk1-/- Mnk2-/-
Apcfl/fl KrasG12D/+

Figure S5:

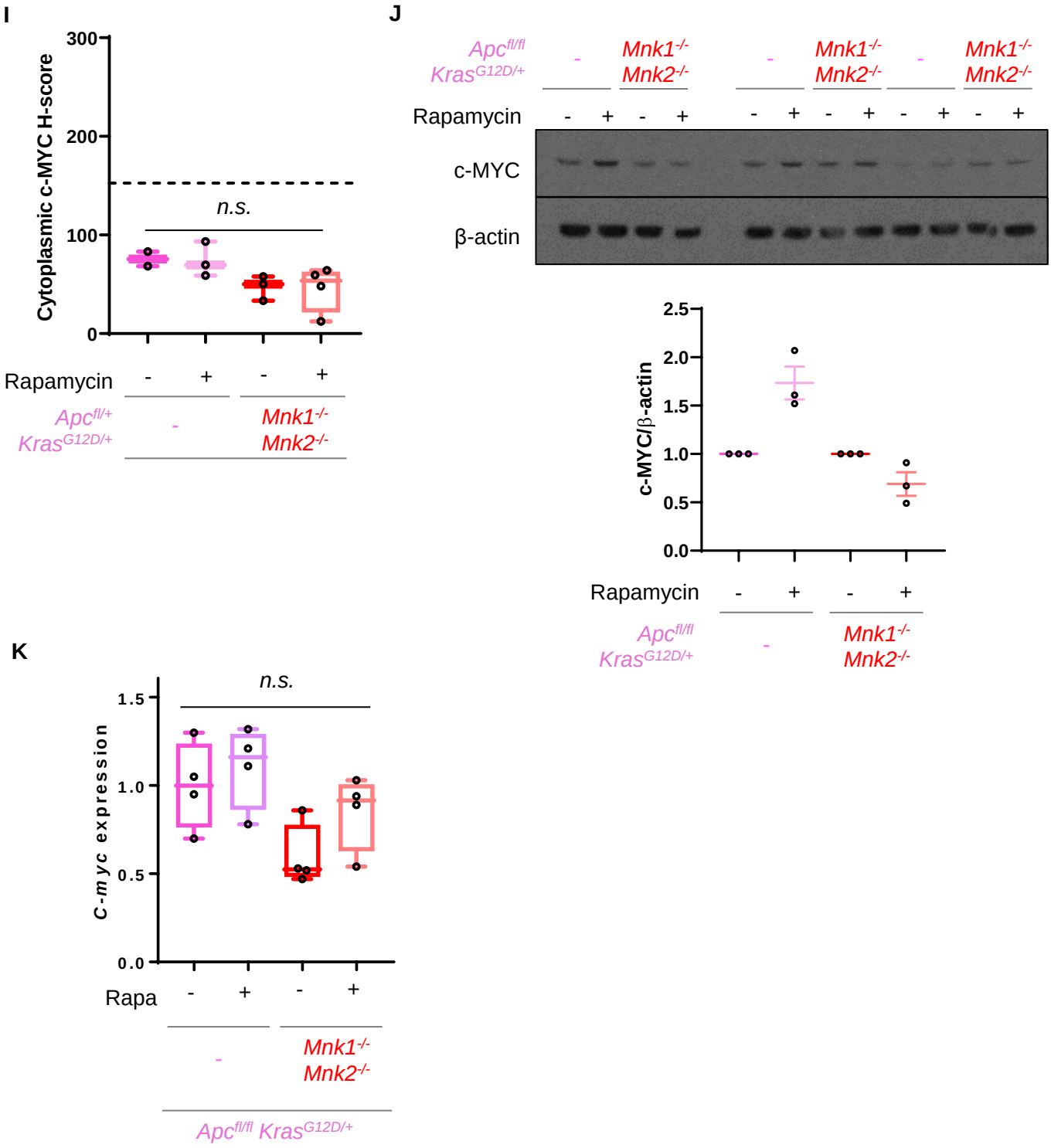

I
Cytoplasmic c-MYC H-score
300
200
100
0
n.s.
Rapamycin - + - +
Apc fl/+ Kras G12D/+
Mnk1 -/- Mnk2 -/-
J
Apc fl/fl Kras G12D/+
Mnk1 -/- Mnk2 -/-
Rapamycin - + - + - + - + - + - +
c-MYC
β-actin
c-MYC/β-actin
2.5
2.0
1.5
1.0
0.5
0.0
Rapamycin - + - +
Apc fl/fl Kras G12D/+
Mnk1 -/- Mnk2 -/-
K
C-myc expression
1.5
1.0
0.5
0.0
n.s.
Rapa - + - +
Mnk1 -/- Mnk2 -/-
Apc fl/fl Kras G12D/+

Figure S5: The effect of eIF4E KI and MNK1/2 KO on protein synthesis and translation signaling

(A) Representative sucrose density traces of epithelial extracts from *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ and *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Mnk1*$^{-/-}$ *Mnk2*$^{-/-}$ short-term model mice, treated with and without rapamycin. (B) Quantification of polysome:subpolysome ratios from (A), with each point representing an individual biological replicate. Pink dashed line shows P:S ratio from vehicle treated *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ short-term mice, depicted in Figure S2A. *P* values were calculated by Mann Whitney tests. (C) Harringtonine run-off sucrose density gradients from *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ and *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Mnk1*$^{-/-}$ *Mnk2*$^{-/-}$ small intestinal organoid cultures treated with rapamycin or DMSO vehicle for 6hours prior to harvesting. Each pair of linked points represents an organoid line with and without treatment. Lack of significance was determined by paired student t test. (D) Raw sucrose density traces quantified for (C). H0 = 0seconds of harringtonine. H300 = 300seconds of harringtonine. (E) Protein synthesis rates measured by ^{35}S methionine incorporation in small intestinal organoids of the indicated genotypes, treated with vehicle or rapamycin for 6hours, relative to control *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ organoids (black dashed line). Pink dashed line shows translation rate in rapamycin-treated APC KRAS. * denotes significance between vehicle and rapamycin treatment for each genotype as measured by student t test ($P<0.05$). Significance between genotypes was determined by Mann Whitney test. (F) IHC for P-eEF2 and P-RPS6 in the small intestine of *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Eif4e*$^{S209A/S209A}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Mnk1*$^{-/-}$ *Mnk2*$^{-/-}$ short-term model mice, with and without rapamycin treatment. (G) HCT116 cells were transfected with Flag-eIF4E, Flag-eIF4E with serine 209 mutated to alanine or Flag-BAP as a negative control. 24hours later lysates were prepared and Flag immunoprecipitated. Western blots confirmed equal expression of Flag-eIF4E (Top left). Top right panel depicts the Flag proteins precipitated in each condition. qPCRs were performed and fold enrichment from RIPs calculated relative to input mRNA (Lower panel). Statistical differences were calculated by 2-way ANOVA with Tukey's comparisons tests. (H) IHC for c-MYC in *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ or *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Mnk1*$^{-/-}$ *Mnk2*$^{-/-}$ short-term mice treated with or without rapamycin. Staining intensity was quantified by a nuclear c-MYC H-score shown in the graph beneath. Each point represents a biological replicate. (I) H-score calculated for cytoplasmic c-MYC expression from the same data presented in Figure 5C. The black dashed line indicates the H-score for nuclear c-MYC in untreated *Apc*$^{fl/+}$ *Kras*$^{G12D/+}$ adenomas. No difference was found by one-way ANOVA analysis. (J) *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ and *Apc*$^{fl/fl}$ *Kras*$^{G12D/+}$ *Mnk1*$^{-/-}$ *Mnk2*$^{-/-}$ organoids were treated with rapamycin for 24hours then protein lysates analyzed for c-MYC expression, using β-actin as a loading control. (K) qPCR for *C-myc* mRNA levels normalized to *Actb* mRNA expression from whole tissue sections taken from mice in parallel to Figure S5G. Lack of significant changes between all samples was determined by Kruskal-Wallis analysis. All data are represented as mean ± S.E.M. Scale bars, 50μm. See also Figure 5.

Figure S6:

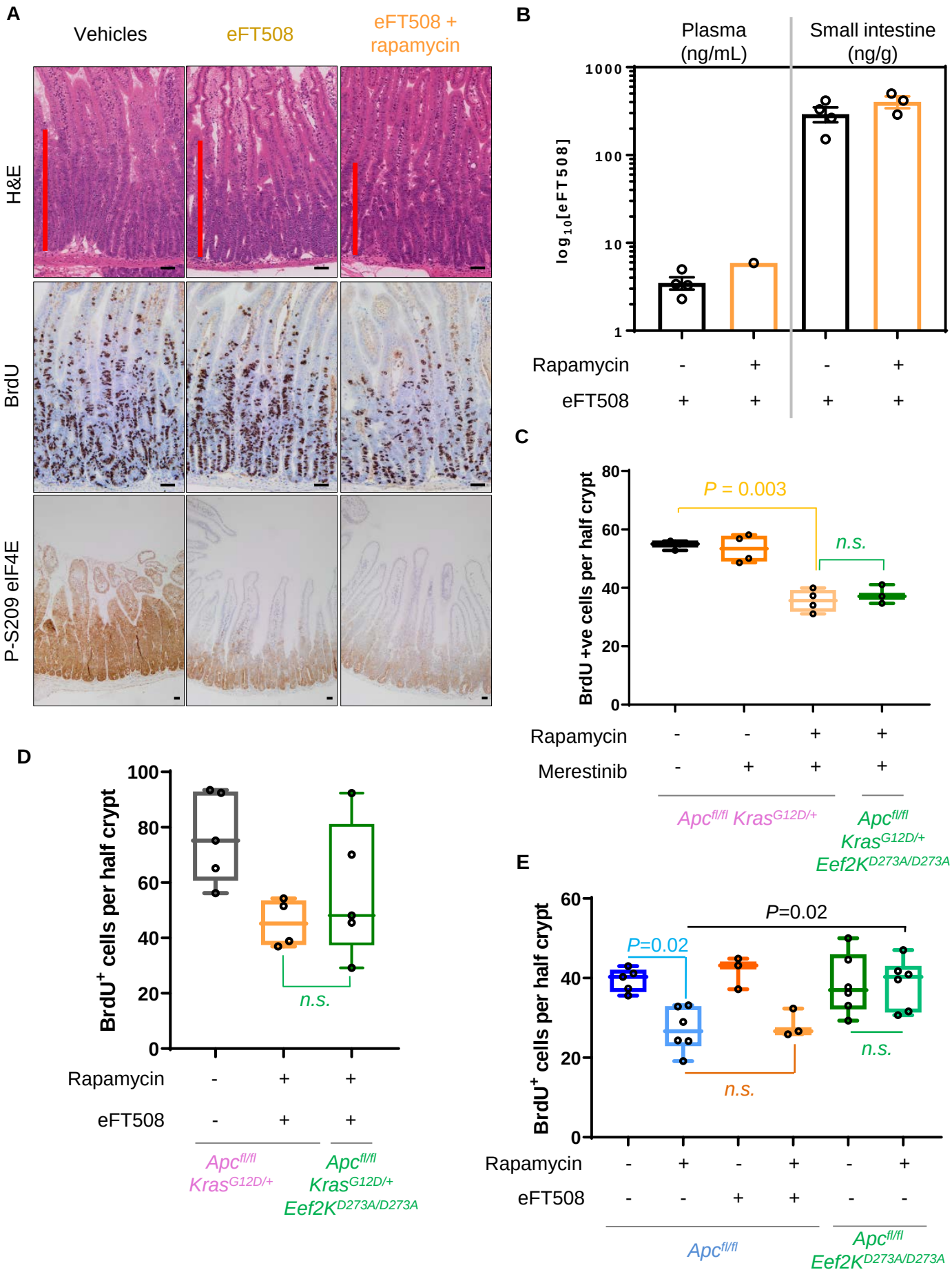
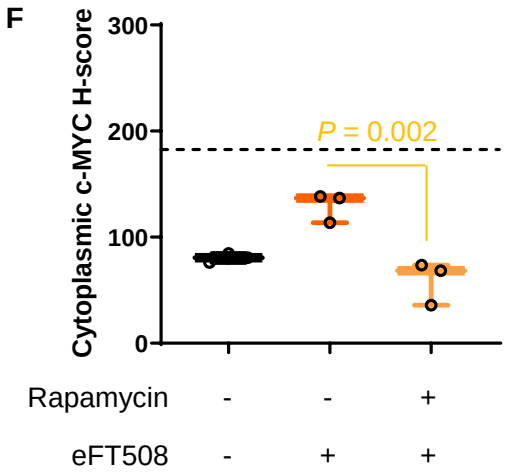
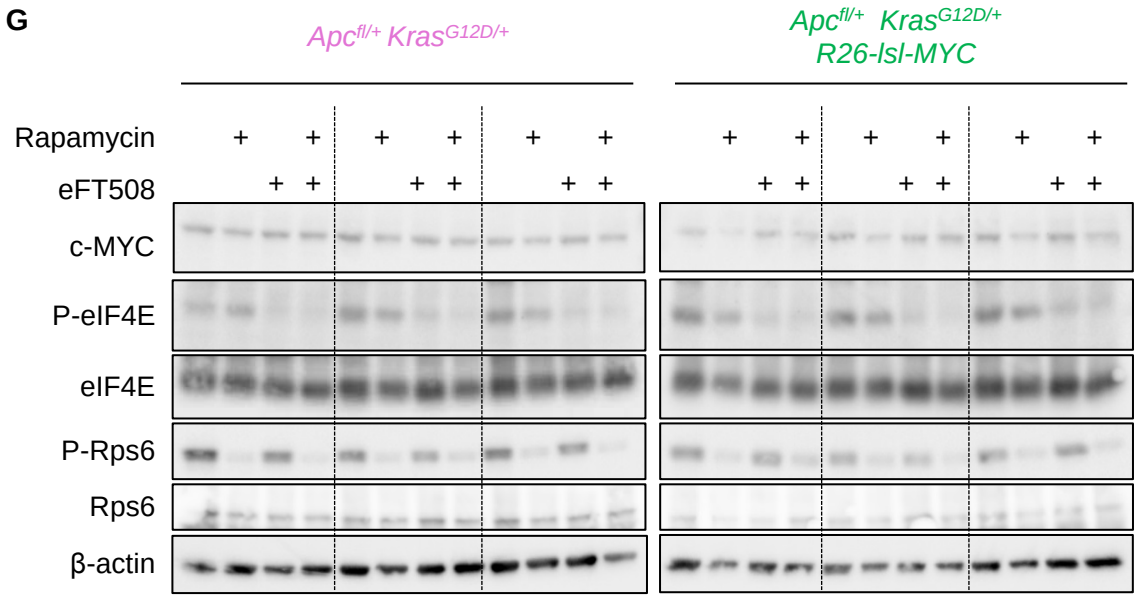

A
Vehicles
eFT508
eFT508 + rapamycin
H&E
BrdU
P-S209 eIF4E
B
Plasma (ng/mL)
Small intestine (ng/g)
log10[eFT508]
1000
100
10
1
Rapamycin - + - +
eFT508 + + + +
C
BrdU +ve cells per half crypt
80
60
40
20
0
P = 0.003
n.s.
Rapamycin - - + +
Merestinib - + + +
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Eef2KD273A/D273A
D
BrdU+ cells per half crypt
100
80
60
40
20
0
n.s.
Rapamycin - + +
eFT508 - + +
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ Eef2KD273A/D273A
E
BrdU+ cells per half crypt
60
40
20
0
P=0.02
P=0.02
n.s.
n.s.
Rapamycin - + - + - +
eFT508 - - + + - -
Apcfl/fl
Apcfl/fl Eef2KD273A/D273A

Figure S6:


F
Cytoplasmic c-MYC H-score
300
200
100
0
P = 0.002
Rapamycin - - +
eFT508 - + +


G
Apc^fl/+ Kras^G12D/+
Apc^fl/+ Kras^G12D/+ R26-lsl-MYC
Rapamycin
eFT508
c-MYC
P-eIF4E
eIF4E
P-Rps6
Rps6
β-actin

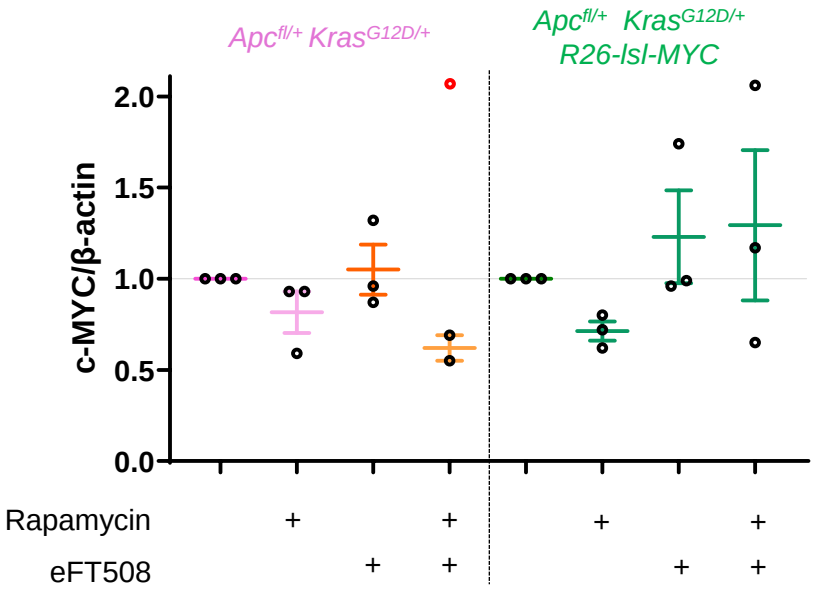
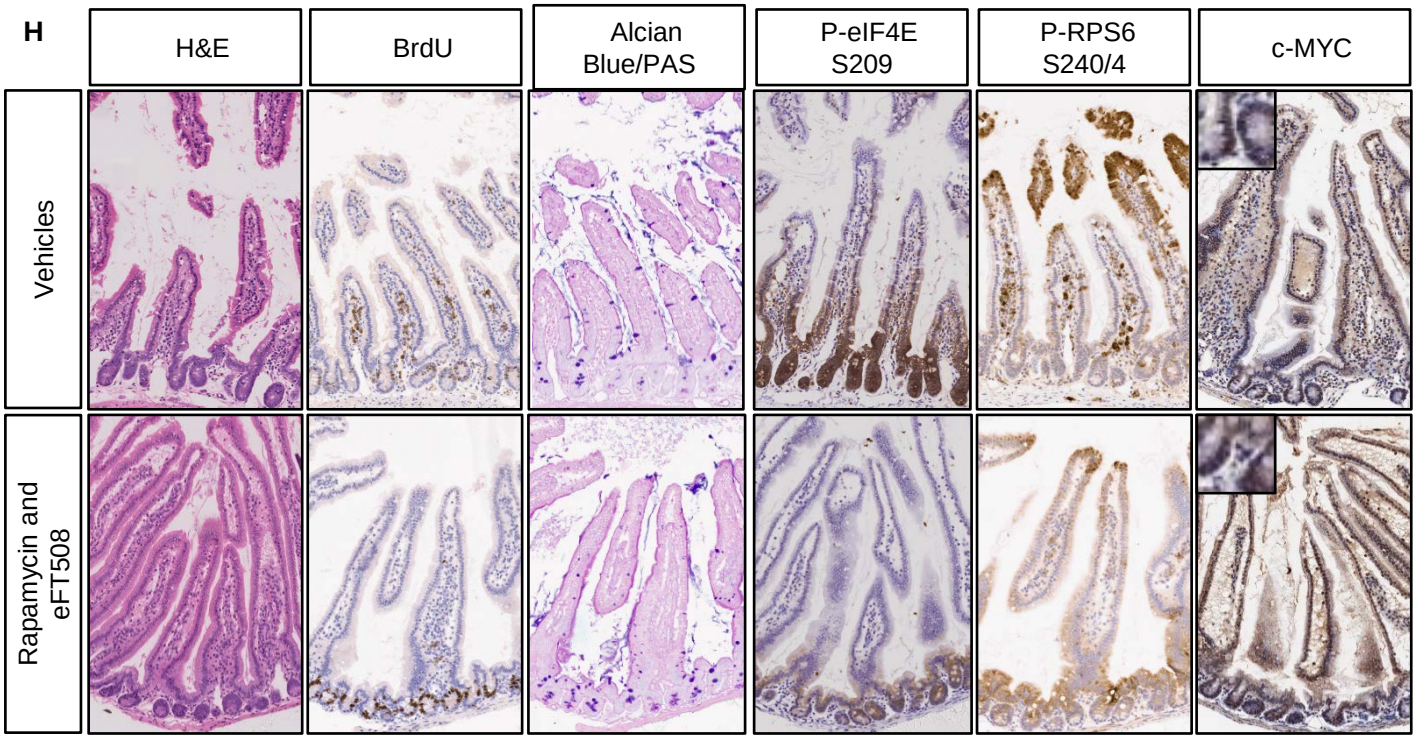
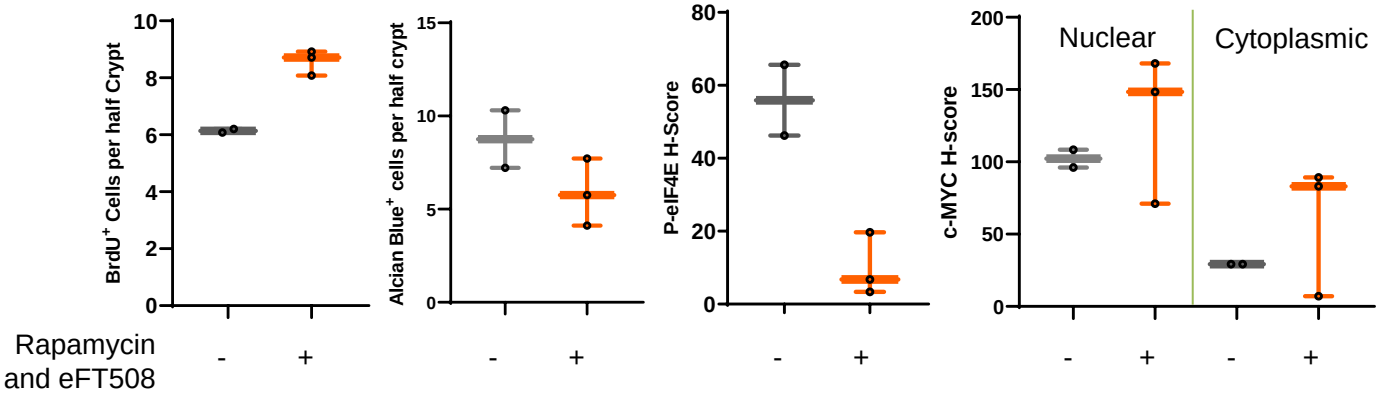
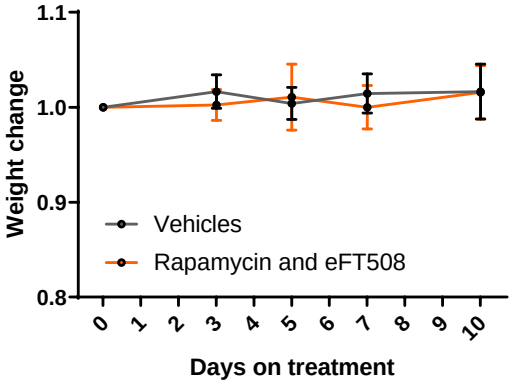

Apc^fl/+ Kras^G12D/+
Apc^fl/+ Kras^G12D/+ R26-lsl-MYC
c-MYC/β-actin
2.0
1.5
1.0
0.5
0.0
Rapamycin
eFT508

Figure S6:







I

Cohort	Median	N
Rapamycin	42	4
eFT508	44	4
Rapamycin and eFT508	129	4

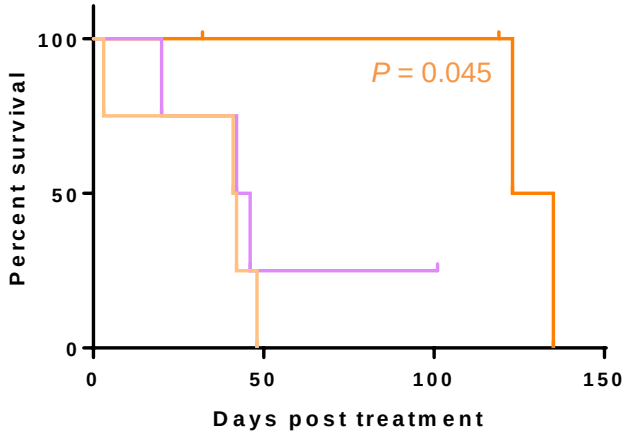


Figure S6:

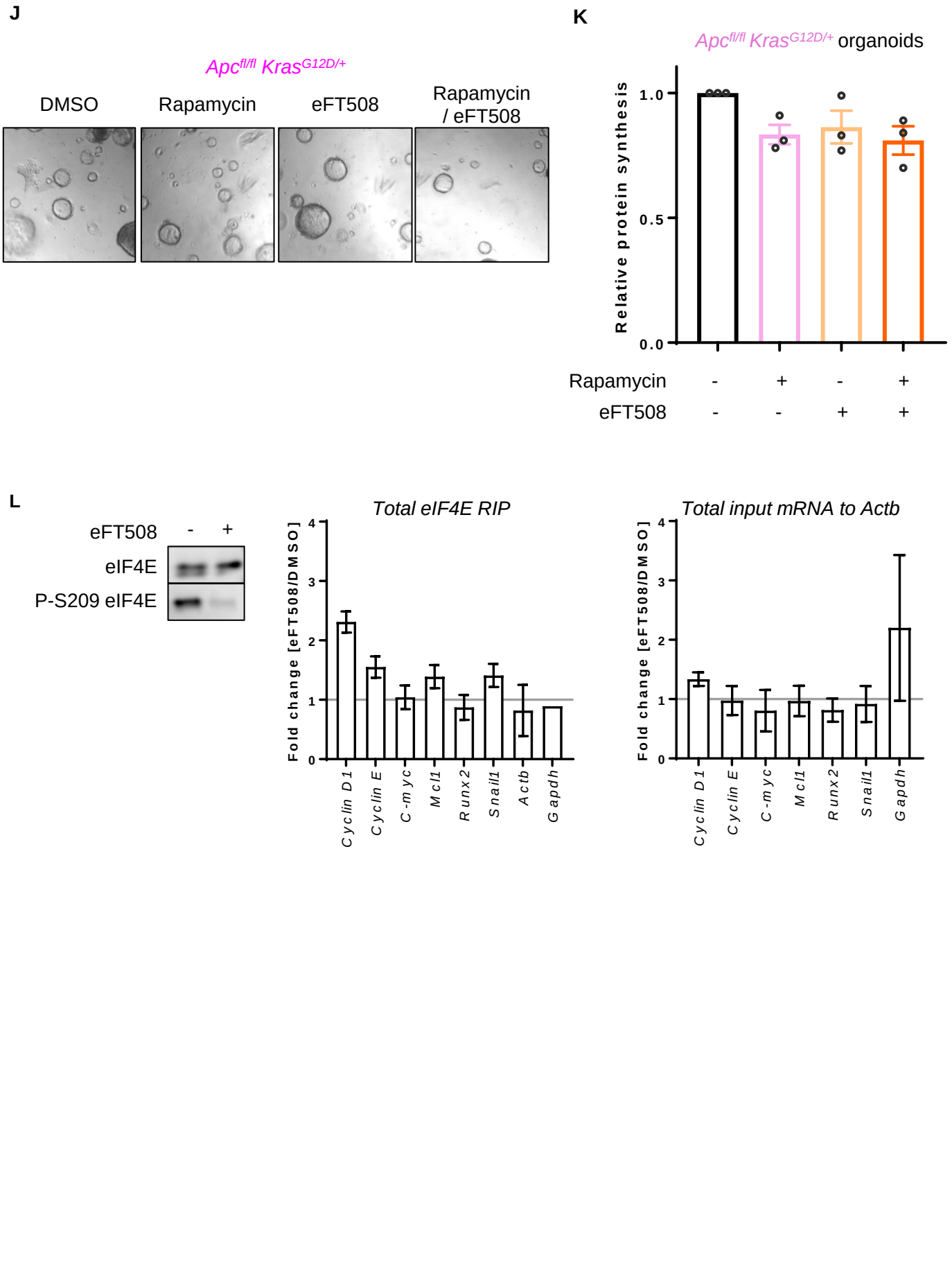

J
Apcfl/fl KrasG12D/+
DMSO
Rapamycin
eFT508
Rapamycin / eFT508
K
Apcfl/fl KrasG12D/+ organoids
Relative protein synthesis
1.0
0.5
0.0
Rapamycin - + - +
eFT508 - - + +
L
eFT508 - +
eIF4E
P-S209 eIF4E
Total eIF4E RIP
Fold change [eFT508/DMSO]
Cyclin D1
Cyclin E
C-myc
Mcl1
Runx2
Snail1
Actb
Gapdh
Total input mRNA to Actb
Fold change [eFT508/DMSO]
Cyclin D1
Cyclin E
C-myc
Mcl1
Runx2
Snail1
Gapdh

Figure S6: MNK inhibition reduces proliferation in combination with rapamycin

(A) H&E, BrdU and P-eIF4E IHC for eFT508 treated *Apc$^{fl/fl}$ Kras$^{G12D/+}$* short-term mice treated with vehicle, eFT508, or combined eFT508/rapamycin from mice in Figure 6A. Red bars on H&E indicate extent of proliferative zone. (B) Quantification of eFT508 levels in plasma and small intestine of mice treated with eFT508 alone or eFT508 in combination with rapamycin. Each point represents an individual mouse. (C) BrdU incorporation in the small intestine of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* short-term model mice treated with merestinib alone or in combination with rapamycin. Where either or both drugs were not dosed, the equivalent vehicle was administered instead. BrdU incorporation was also measured in *Apc$^{fl/fl}$ Kras$^{G12D/+}$* mice homozygous for the kinase-dead allele of eEF2K (D273A) treated with merestinib and rapamycin. ≥25 half crypts were quantified per mouse, with each point on the graph representing an individual mouse. *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (D) Incorporation in the small intestine of *Apc$^{fl/fl}$ Kras$^{G12D/+}$ Eef2k$^{D273A/D273A}$* mice following daily treatment with rapamycin and eFT508, compared to *Apc$^{fl/fl}$ Kras$^{G12D/+}$* short-term mice treated with the same combination or vehicle. Vehicle and combination treatment data are reused from Figure 6B. ≥25 half crypts were quantified per mouse, with each point on the graph representing an individual mouse. Lack of significant differences was determined by one-way ANOVA test. (E) BrdU incorporation in *Apc$^{fl/fl}$* and *Apc$^{fl/fl}$ Eef2k$^{D273A/D273A}$* short-term model mice treated with or without rapamycin or eFT508. ≥25 half crypts were quantified per mouse, with each point on the graph representing an individual mouse. *P* values are from one-way ANOVA Tukey's multiple comparisons tests. (F) Cytoplasmic c-MYC H-score from the same data presented in in Figure 6D. Dashed line shows the average H-score for nuclear c-MYC in untreated *Apc$^{fl/+}$ Kras$^{G12D/+}$* adenomas. . *P* value is from one-way ANOVA Tukey's multiple comparisons test. (G) *Apc$^{fl/fl}$ Kras$^{G12D/+}$* or *Apc$^{fl/fl}$ Kras$^{G12D/+}$ R26-lsl-MYC* organoids were treated with rapamycin (250nM) and/or eFT508 (30nM) for 24hours then protein lysates analyzed by western blot for the indicated proteins. Red circle indicates an omitted outlier. (H) Wild-type mice were treated with 10mg/kg rapamycin once daily and 1mg/kg eFT508 twice daily for 10days then intestinal tissue harvested 2 hours after final dosing, concurrent with BrdU treatment. Images show intestinal lineages are unchanged by treatment and the successful modulation of mTORC1 and MNK signaling. Animal weight while on treatment was unchanged and similar to vehicle treated animals. (I) Survival of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* mice orthotopically-induced colonic adenomas, plotted as days following treatment with rapamycin, eFT508, or eFT508/rapamycin. Censored mice became sick but did not have colonic tumors. *P* value is from a Log-rank Mantel-Cox test. (J) Bright-field images of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* organoids treated with the indicated drugs for 24hours. (K) Protein synthesis rates measured in *Apc$^{fl/fl}$ Kras$^{G12D/+}$* organoids treated with rapamycin (250nM), eFT508 (30nM) or both drugs for 6hours. Values are represented relative to vehicle treated organoids. Each point represents a biological replicate. (L) RNA immunoprecipitations (RIPs) of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* organoids treated with vehicle or eFT508 (30nM) for 24hours. Left, western blot showing efficacy of eFT508 treatment. Middle, total eIF4E RIP shown as the fold-change in mRNA precipitated from eFT508-treated *Apc$^{fl/fl}$ Kras$^{G12D/+}$* cells compared to DMSO controls. Right, fold-changes in total mRNA abundance for each transcript. All data are represented as mean ± S.E.M. Scale bars, 50μm. See also Figure 6.

Figure S7:

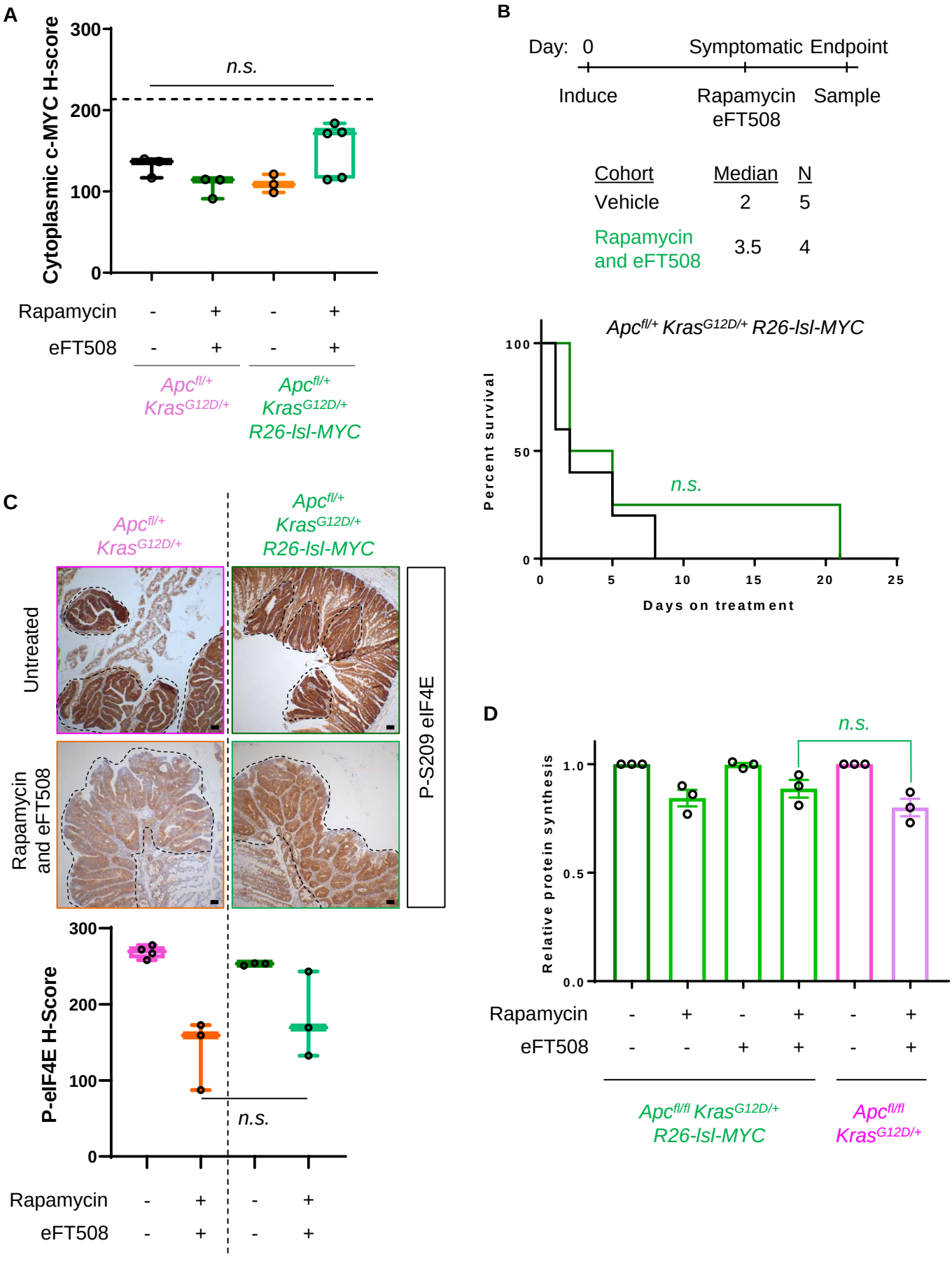
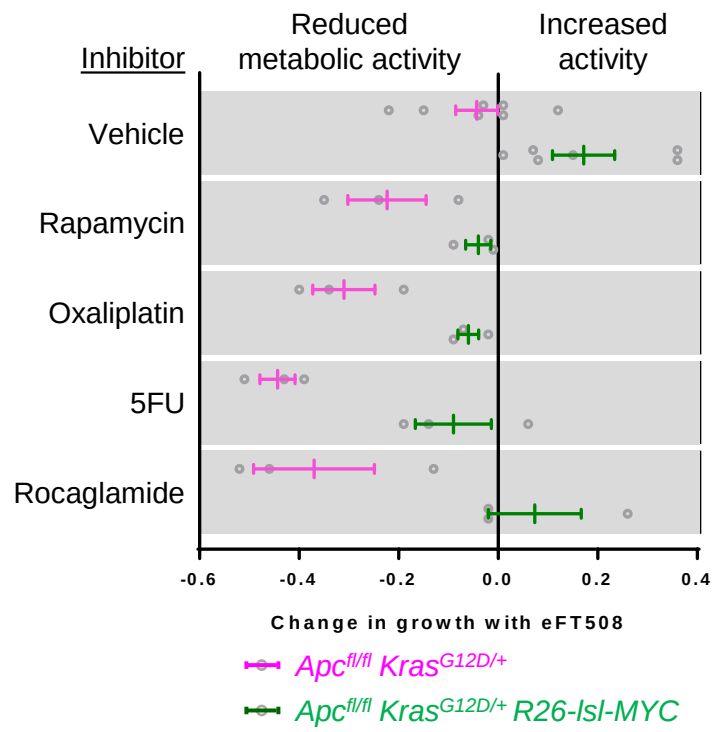

A
Cytoplasmic c-MYC H-score
300
200
100
0
n.s.
Rapamycin - + - +
eFT508 - + - +
Apcfl/+ KrasG12D/+
Apcfl/+ KrasG12D/+ R26-lsl-MYC
B
Day: 0
Symptomatic Endpoint
Induce
Rapamycin eFT508
Sample
Cohort Median N
Vehicle 2 5
Rapamycin and eFT508 3.5 4
Apcfl/+ KrasG12D/+ R26-lsl-MYC
Percent survival
100
50
0
n.s.
0 5 10 15 20 25
Days on treatment
C
Apcfl/+ KrasG12D/+
Apcfl/+ KrasG12D/+ R26-lsl-MYC
Untreated
Rapamycin and eFT508
P-S209 eIF4E
P-eIF4E H-Score
300
200
100
0
n.s.
Rapamycin - + - +
eFT508 - + - +
D
Relative protein synthesis
1.0
0.5
0.0
n.s.
Rapamycin - + - + - +
eFT508 - - + + - +
Apcfl/fl KrasG12D/+ R26-lsl-MYC
Apcfl/fl KrasG12D/+

Figure S7:

E



Inhibitor	*P* value	*Relative protein synthesis* (*Apc$^{fl/fl}$ Kras$^{G12D/+}$*)
Vehicle	*n.s.*	1.00 ± 0.04
Rapamycin	*<0.05*	0.80 ± 0.04
Oxaliplatin	*<0.05*	0.92 ± 0.08
5FU	*<0.05*	0.70 ± 0.02
Rocaglamide	*<0.05*	0.92 ± 0.07

F

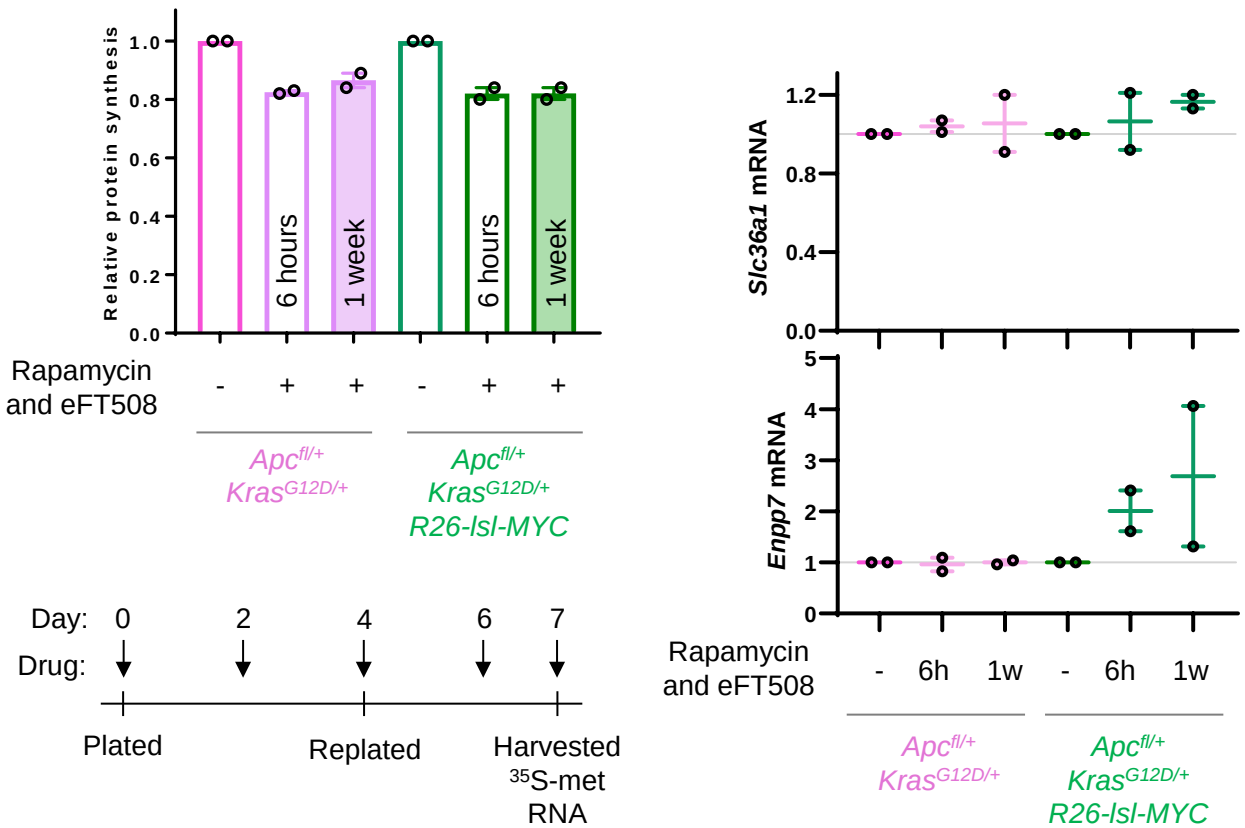
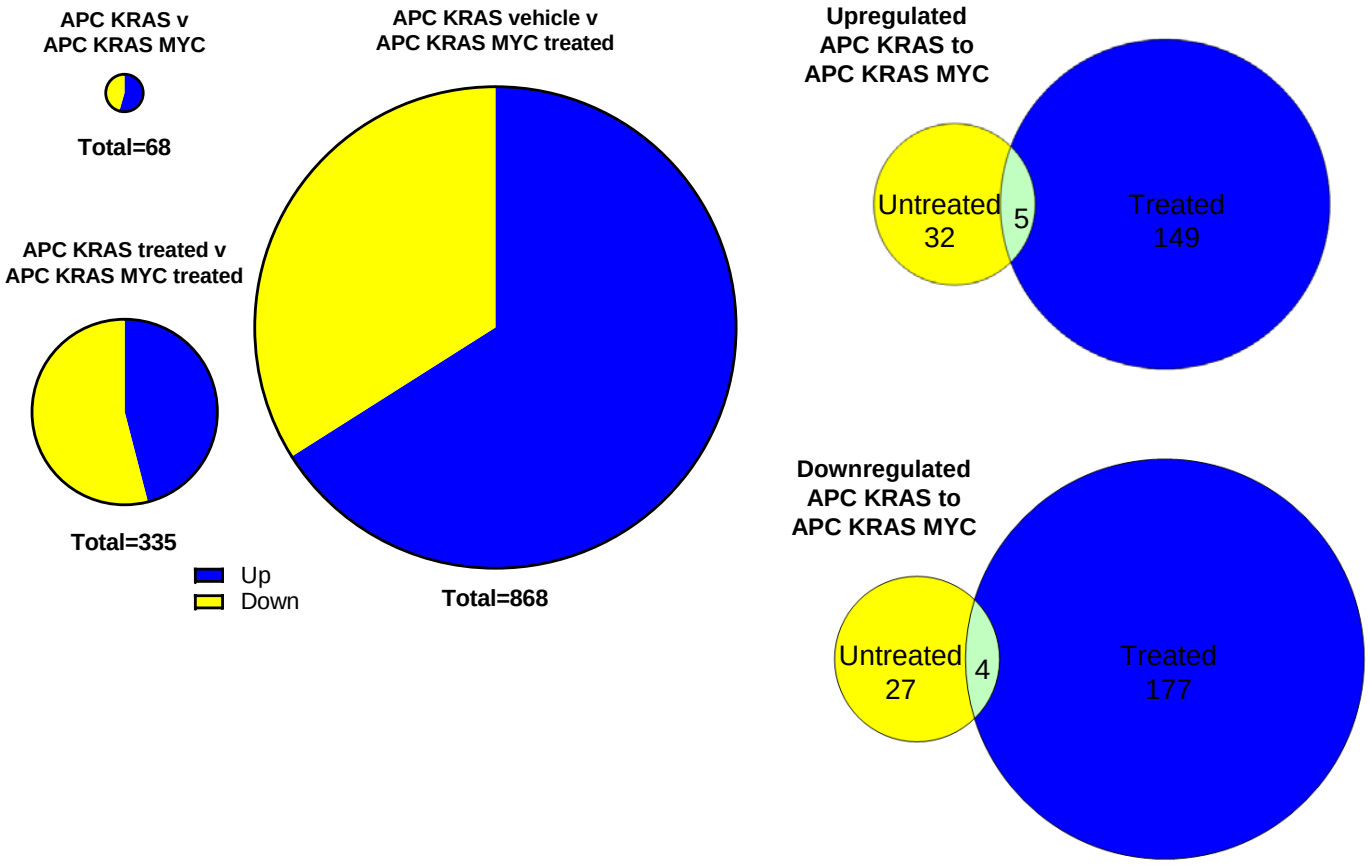


Figure S7:

G


APC KRAS v
APC KRAS MYC
Total=68
APC KRAS vehicle v
APC KRAS MYC treated
Total=868
APC KRAS treated v
APC KRAS MYC treated
Total=335
Up
Down
Upregulated
APC KRAS to
APC KRAS MYC
Untreated
32
5
Treated
149
Downregulated
APC KRAS to
APC KRAS MYC
Untreated
27
4
Treated
177

H

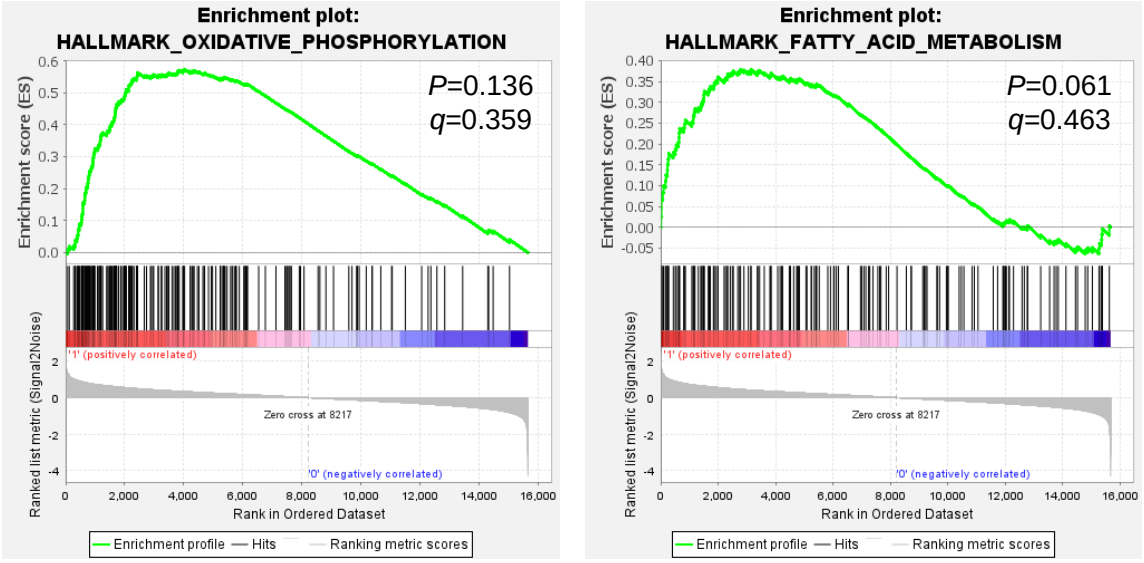
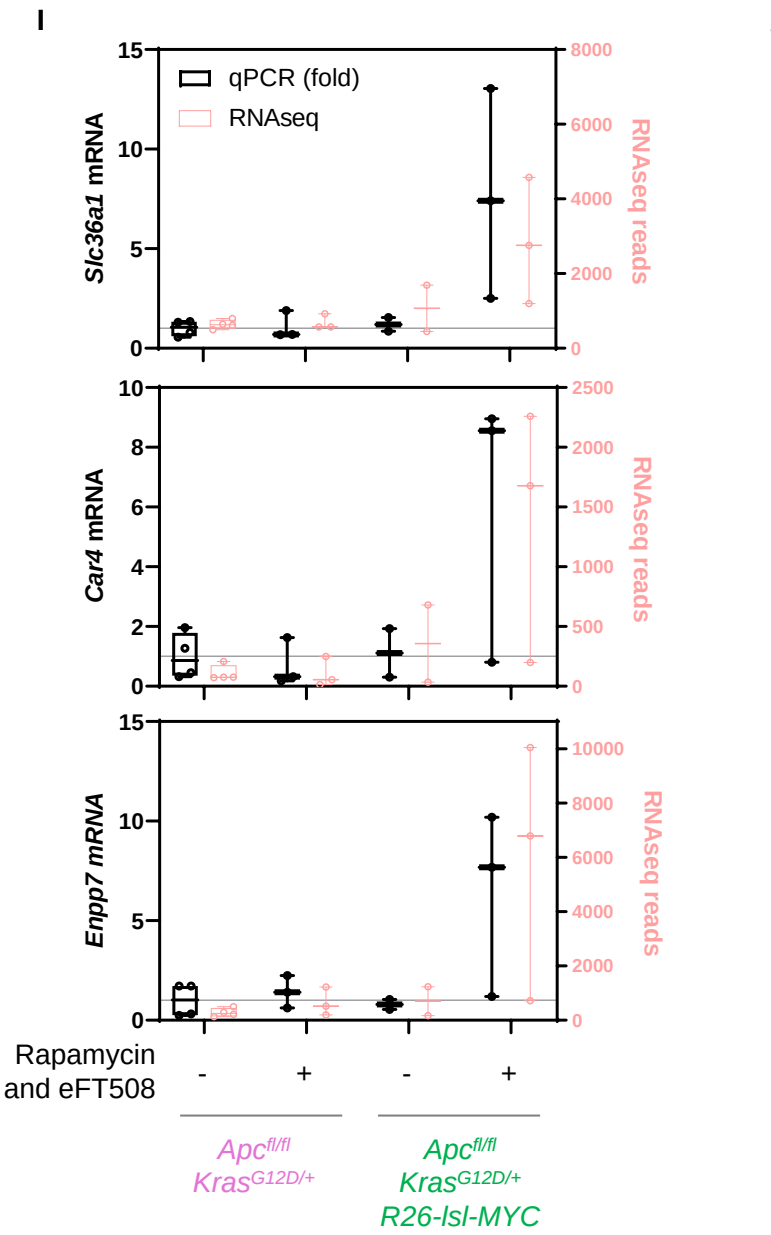

Enrichment plot:
HALLMARK_OXIDATIVE_PHOSPHORYLATION
P=0.136
q=0.359
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'1' (positively correlated)
Zero cross at 8217
'0' (negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
Enrichment plot:
HALLMARK_FATTY_ACID_METABOLISM
P=0.061
q=0.463
Enrichment score (ES)
Ranked list metric (Signal2Noise)
'1' (positively correlated)
Zero cross at 8217
'0' (negatively correlated)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores

Figure S7:


I
qPCR (fold)
RNAseq
Slc36a1 mRNA
Car4 mRNA
Enpp7 mRNA
RNAseq reads
Rapamycin and eFT508
-
+
-
+
Apcfl/fl KrasG12D/+
Apcfl/fl KrasG12D/+ R26-lsl-MYC

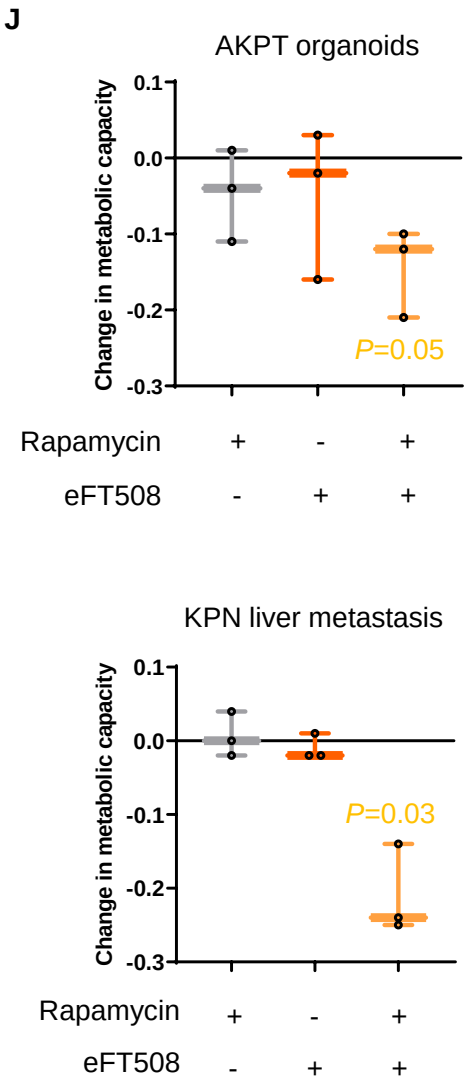
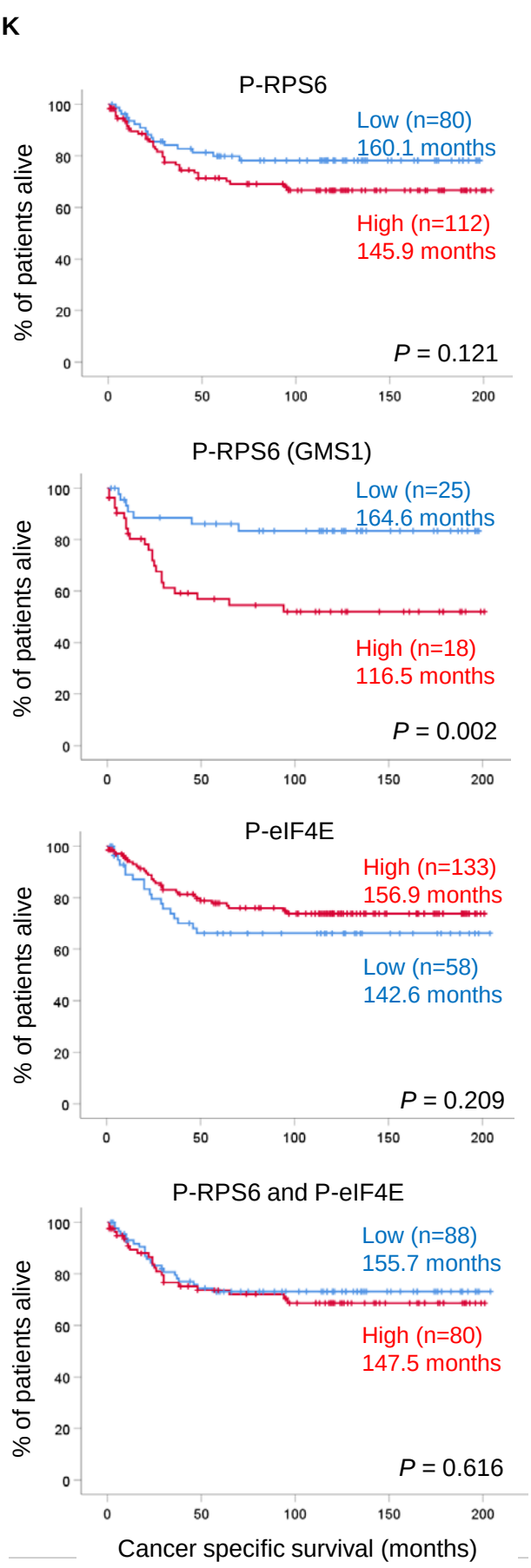
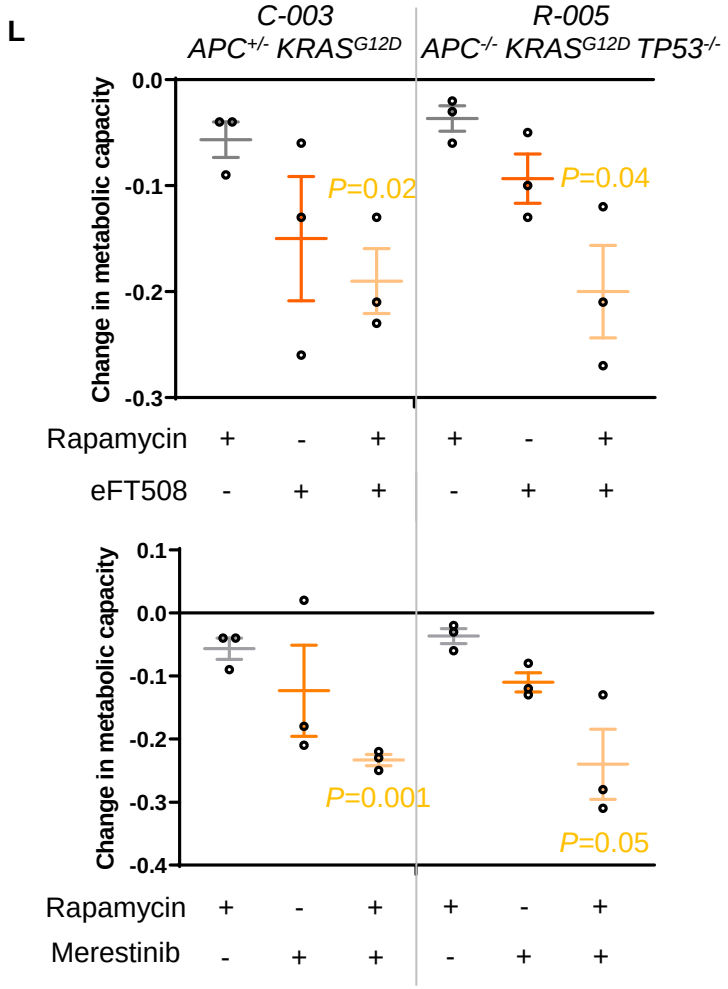

J
AKPT organoids
Change in metabolic capacity
P=0.05
Rapamycin + - +
eFT508 - + +
KPN liver metastasis
Change in metabolic capacity
P=0.03
Rapamycin + - +
eFT508 - + +

Figure S7:


K
P-RPS6
Low (n=80)
160.1 months
High (n=112)
145.9 months
P = 0.121
P-RPS6 (GMS1)
Low (n=25)
164.6 months
High (n=18)
116.5 months
P = 0.002
P-eIF4E
High (n=133)
156.9 months
Low (n=58)
142.6 months
P = 0.209
P-RPS6 and P-eIF4E
Low (n=88)
155.7 months
High (n=80)
147.5 months
P = 0.616
% of patients alive
Cancer specific survival (months)


L
C-003
APC+/- KRASG12D
R-005
APC-/- KRASG12D TP53-/-
Change in metabolic capacity
P=0.02
P=0.04
Rapamycin
eFT508
P=0.001
P=0.05
Merestinib

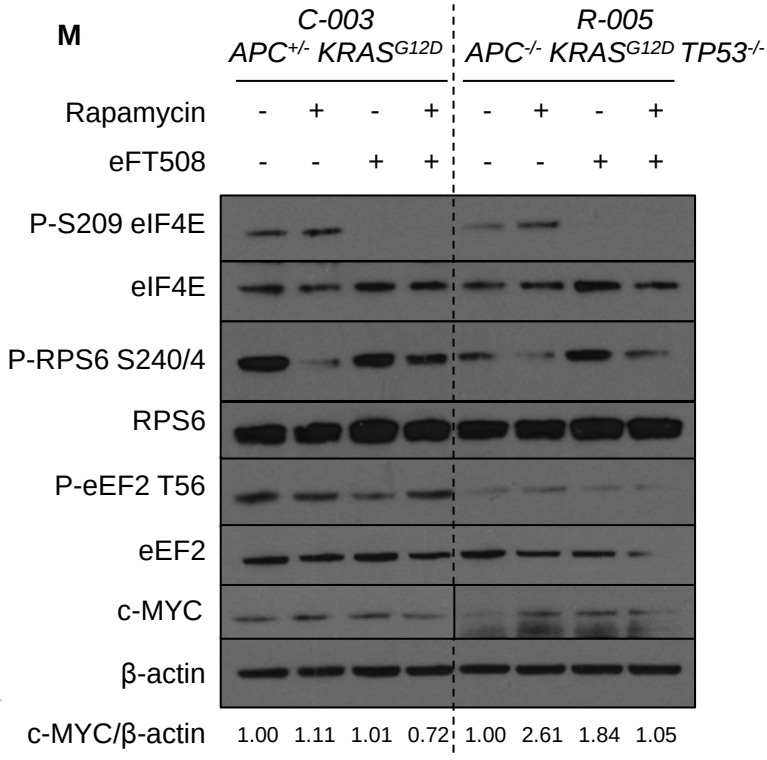

M
C-003
APC+/- KRASG12D
R-005
APC-/- KRASG12D TP53-/-
Rapamycin
eFT508
P-S209 eIF4E
eIF4E
P-RPS6 S240/4
RPS6
P-eEF2 T56
eEF2
c-MYC
β-actin
c-MYC/β-actin
1.00 1.11 1.01 0.72 1.00 2.61 1.84 1.05

Figure S7: *C-MYC* transgene expression reverses the effect of rapamycin/eFT508 combination

(A) Cytoplasmic c-MYC H-score from the same data presented in in Figure 7B. Dashed line shows the average H-score for nuclear c-MYC in untreated *Apc$^{fl/+}$ Kras$^{G12D/+}$* adenomas. Lack of significant differences was determined by one-way ANOVA test. (B) Top, experimental outline. *Apc$^{fl/+}$ Kras$^{G12D/+}$ R26-lsl-MYC* tumor model mice, presenting signs of intestinal adenomas, were enrolled onto either vehicle or rapamycin/eFT508 combination treatment and aged until endpoint. Below, the time from the start of treatment until endpoint is plotted. Lack of significance was determined by Log-rank Mantel-Cox test. (C) Top, representative images of colonic adenomas from Figure 7B stained for P-eIF4E. Below, quantification of P-eIF4E S209 within adenomas of mice with the indicated genotypes. Each point is the average H score from an individual animal. Lack of significant difference was determined by one-way ANOVA test. (D) Protein synthesis rates measured in *Apc$^{fl/fl}$ Kras$^{G12D/+}$ R26-lsl-MYC* organoids treated with rapamycin (250nM) and eFT508 (30nM) or both drugs in combination for 6hours. *Apc$^{fl/fl}$ Kras$^{G12D/+}$* organoids treated with the drug combination are also included. Three independent biological replicates were performed and normalized against vehicle treatment for each cell line. (E) The change in Cell-Titer blue reduction of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* or *Apc$^{fl/fl}$ Kras$^{G12D/+}$ R26-lsl-MYC* organoids following 24-hour treatment with eFT508 (30nM) in combination with the indicated agents. Rapamycin was used at 250nM, oxaliplatin at 300nM, 5FU at 100nM and rocaglamide at 10nM. Experiments were performed in at least biological triplicate with each replicate plotted as a point on the graph. *P* values are from paired student t-tests. To the right are values for the relative change in protein synthesis, measured by ^{35}S methionine incorporation, following treatment of *Apc$^{fl/fl}$ Kras$^{G12D/+}$* organoids (n=3) with rapamycin, oxaliplatin, 5FU or rocaglamide. (F) *Apc$^{fl/fl}$ Kras$^{G12D/+}$* and *Apc$^{fl/fl}$ Kras$^{G12D/+}$ R26-lsl-MYC* organoids were incubated with rapamycin (250nM) and eFT508 (30nM) for 1 week according to the sheme on the bottom left. Left: Protein synthesis was measured by ^{35}S-methionine incorporation compared to untreated and 6hour treated organoids. Right: qPCR for indicated mRNAs normalized to *ActB* expression from RNA purified in parallel to the protein synthesis assay. (G) Left: Proportionally sized pie charts showing significant transcript changes comparing the indicated genotypes and treatments. Right: Proportional Venn diagrams showing overlapping changes between treated and untreated *Apc$^{fl/fl}$ Kras$^{G12D/+}$* and *Apc$^{fl/fl}$ Kras$^{G12D/+}$ R26-lsl-MYC* intestines. (H) Gene Set Enrichment Analysis plots showing positive enrichment for the Hallmarks 'Oxidative Phosphorylation' and 'Fatty Acid Metabolism' in *Apc$^{fl/fl}$ Kras$^{G12D/+}$ R26-lsl-MYC* intestines compared to *Apc$^{fl/fl}$ Kras$^{G12D/+}$*.(I) qPCR analysis for the indicated transcripts, normalized to *ActB*, validating the RNA used for sequencing. Black bars show the qPCR changes, with the RNAseq reads shown side-by-side in red. (J) Organoids derived from the small intestine of *Apc$^{fl/fl}$ Kras$^{G12D/+}$ Tp53$^{fl/fl}$ Tgfbr1$^{fl/fl}$* (AKPT) mice or from the metastatic KPN model were treated with rapamycin (250nM), eFT508 (30nM) or the two drugs in combination for 24hours. The change in metabolic capacity from vehicle for each treatment was calculated and plotted from three biological replicates using the same organoid line. *P* values are from paired student t-tests.

Figure S7: *C-MYC* transgene expression reverses the effect of rapamycin/eFT508 combination continued…

(K) Kaplan-Meier survival curves for associations between cancer-specific survival and levels of P-RPS6 in the full cohort, P-RPS6 in GMS1 patients, P-eIF4E in the full cohort and P-RPS6 and P-eIF4E combined in the full cohort. Median survivals for each group are annotated on the graph. P values were calculated by Log-rank test. (L) Patient-derived metastatic CRC organoids were treated with rapamycin (250nM), eFT508 (30nM), or both drugs combined (top panel) and and with rapamycin (250nM), merestinib (100nM) or both drugs (bottom panel). The relative change in metabolic capacity is plotted compared to vehicle treatment. The status of *APC*, *KRAS* and *TP53* are shown above the plot for each organoid line. Data points represent the average change from 3 independent replicates. *P* value is from paired student t test. (M) Western blot to confirm the efficacy of eFT508 (P- eIF4E) and rapamycin (P-RPS6) following drug treatment of metastatic human organoid lines. β-actin was used as a sample control. All data are represented as mean ± S.E.M. Scale bars, 50μm. See also Figure 7.