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Supplemental Information

Rocaglates Induce Gain-of-Function

Alterations to eIF4A and eIF4F

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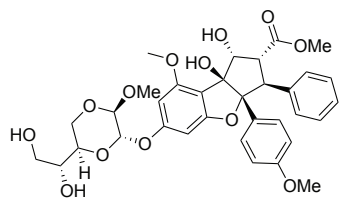
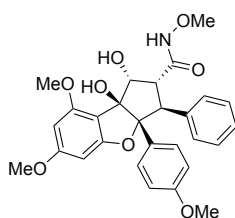
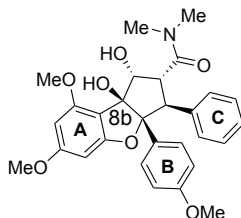
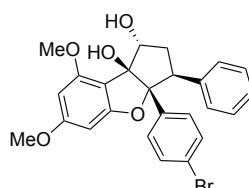
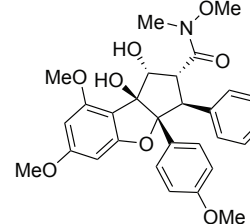
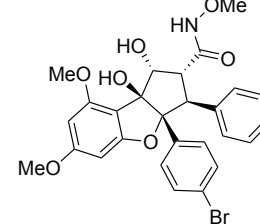
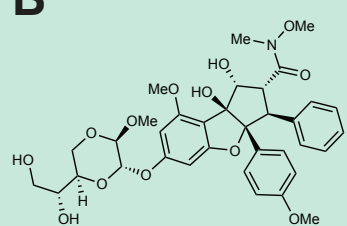
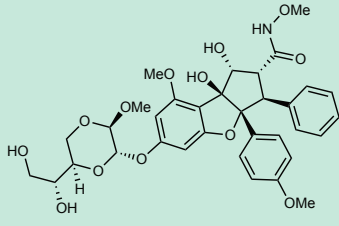
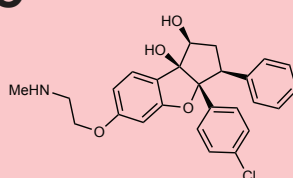
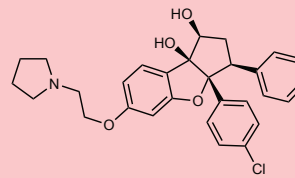
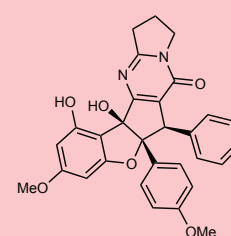
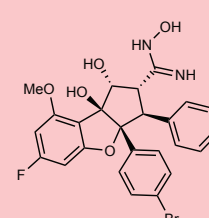
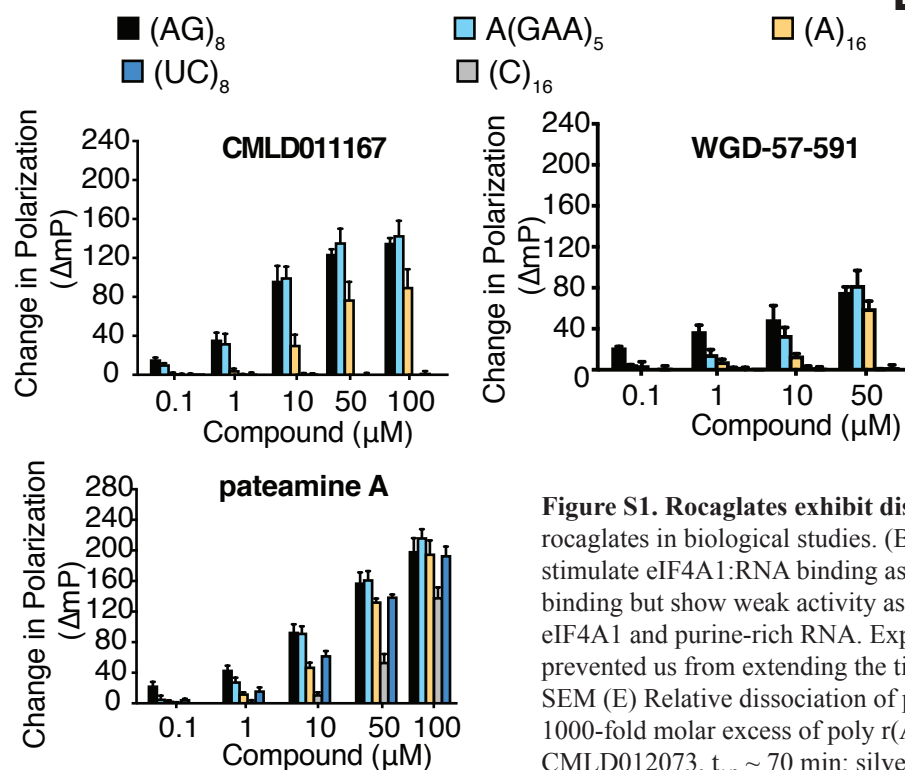
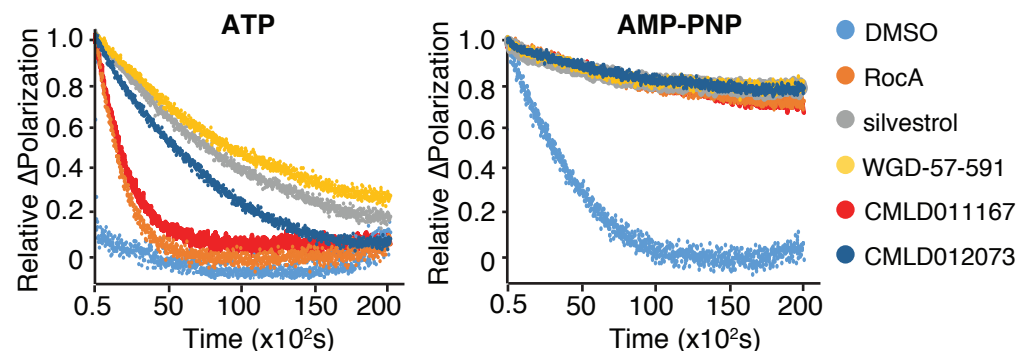
A**silvestrol****CR-1-31-B****RocA****FL3****RHT****SDS-1-021****B****WGD-57-591****WGD-57-591****C****CMLD011167****CMLD011166****CMLD012077****CMLD011352****D****E**

Figure S1. Rocaglates exhibit distinct biological activities (Related to Figure 1). (A) Chemical structures of commonly used rocaglates in biological studies. (B) Structures of two rocaglates that potently inhibit cap-dependent translation but modestly stimulate eIF4A1:RNA binding as measured by FP assay. (C) Structures of four rocaglates that potently stimulate eIF4A1:RNA binding but show weak activity as protein synthesis inhibitors *in vitro*. (D) Rocaglates induce preferential association between eIF4A1 and purine-rich RNA. Experiments were performed as described in Fig 1b. Limitations in WGD-57-591 availability prevented us from extending the titrations to 100 μM . The change in FP obtained relative to vehicle controls is presented. $n = 3 \pm \text{SEM}$ (E) Relative dissociation of pre-assembled eIF4A1:rocaglate:FAM-poly r(AG)₁₀ complexes were measured by the addition of 1000-fold molar excess of poly r(AG)₁₀ and ATP (DMSO, $t_{1/2} < 1$ min; RocA, $t_{1/2} \sim 16$ min; CMLD011167, $t_{1/2} \sim 24$ min; CMLD012073, $t_{1/2} \sim 70$ min; silvestrol, $t_{1/2} \sim 110$ min; WGD-57-591, $t_{1/2} \sim 143$ min) or AMP-PNP (DMSO, $t_{1/2} \sim 33$ min; RocA, $t_{1/2} \sim 580$ min; CMLD011167, $t_{1/2} \sim 591$ min; CMLD012073, $t_{1/2} \sim 832$ min; silvestrol, $t_{1/2} \sim 924$ min; WGD-57-591, $t_{1/2} \sim 928$ min). Relative dissociation was measured as a function of time. $n = 3 \pm \text{SD}$.

B



Figure S2. Preferential inhibition of purine-rich 5' leaders is not a shared property found between small molecules targeting eIF4A1 (Related to Figure 1). (A-C) Dose response of the indicated compounds in Krebs-2 extracts programmed with the indicated mRNAs. $n = 3 \pm \text{SEM}$.

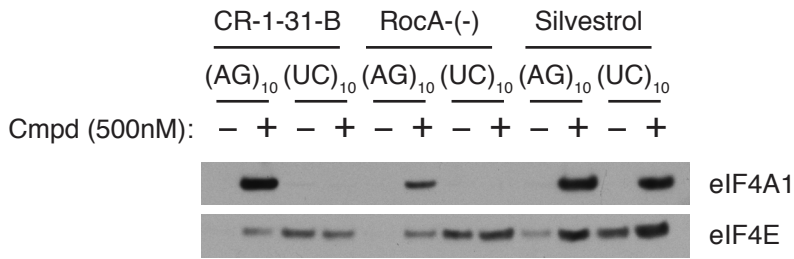
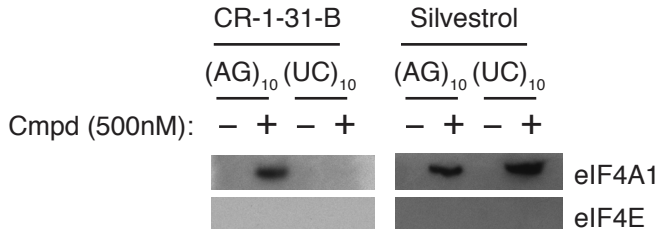
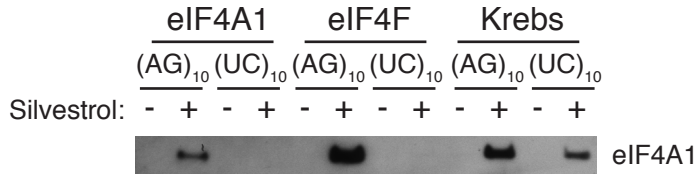
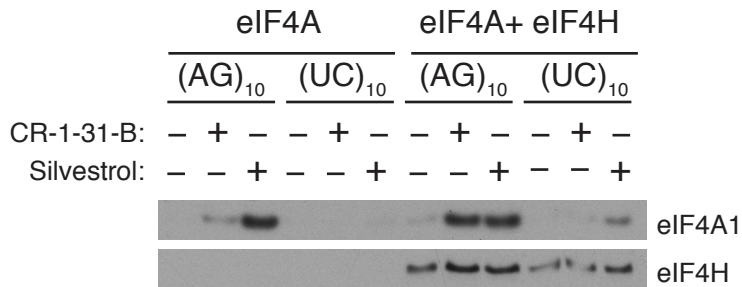
A**B****C****D**

Figure S3. Rocaglates promote association of free eIF4A1 onto biotinylated-RNA probes in a cap-independent manner (Related to Figure 1). (A) RPDs performed with the indicated m⁷GpppG-capped RNA species incubated with retic lysate and either vehicle or 500 nM rocaglate. (B) RPDs performed with the indicated ApppG-capped RNA species incubated with reticulocyte lysate and either vehicle or 500 nM rocaglate. (C) RPDs performed with biotinylated m⁷GpppG-capped RNA incubated in the presence of recombinant eIF4A (125 nM), eIF4F (10 nM), or Krebs-2 extracts and either vehicle or 500 nM silvestrol. (D) RPDs performed with biotinylated m⁷GpppG-capped RNA incubated in the presence of recombinant eIF4A1 (67 nM) and, where indicated, eIF4H (67 nM).

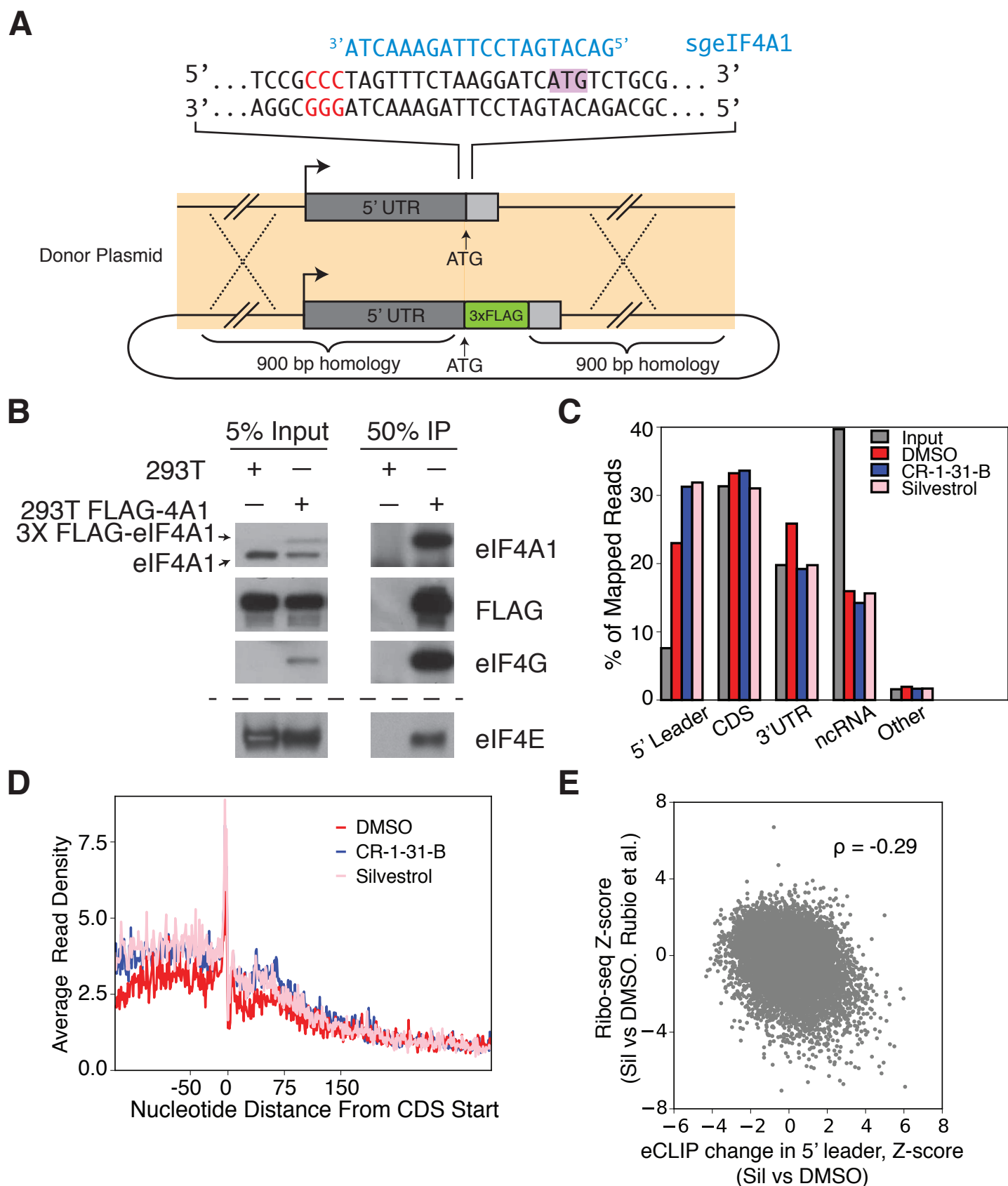


Figure S4. Generation of 3xFLAG eIF4A1 cells using CRISPR/Cas9 mediated gene editing (Related to Figure 2). (A) Schematic of targeting strategy used to knock-in a 3xFLAG epitope tag at the N-terminal end of the eIF4A1 gene in 293T cells using CRISPR/Cas9. The guide RNA sequence (sgeIF4A1) is depicted in blue and the PAM motif of the targeted region is indicated in red. (B) Co-IP experiment using an anti-FLAG epitope antibody in lysates derived from the parental or CRISPR-modified 293T FLAG-4A1 293T cells. Input or immunoprecipitations were probed by Western blotting using antibodies to the proteins indicated to the right. (C) Distribution of eCLIP raw reads to the indicated RNA regions. (D) Metagene profile of aligned reads relative to the CDS start codons (zero coordinate). (E) Correlation between silvestrol enriched 5' leader eCLIP reads and changes in TE from published silvestrol Ribo-seq data. ρ = Pearson correlation coefficient.

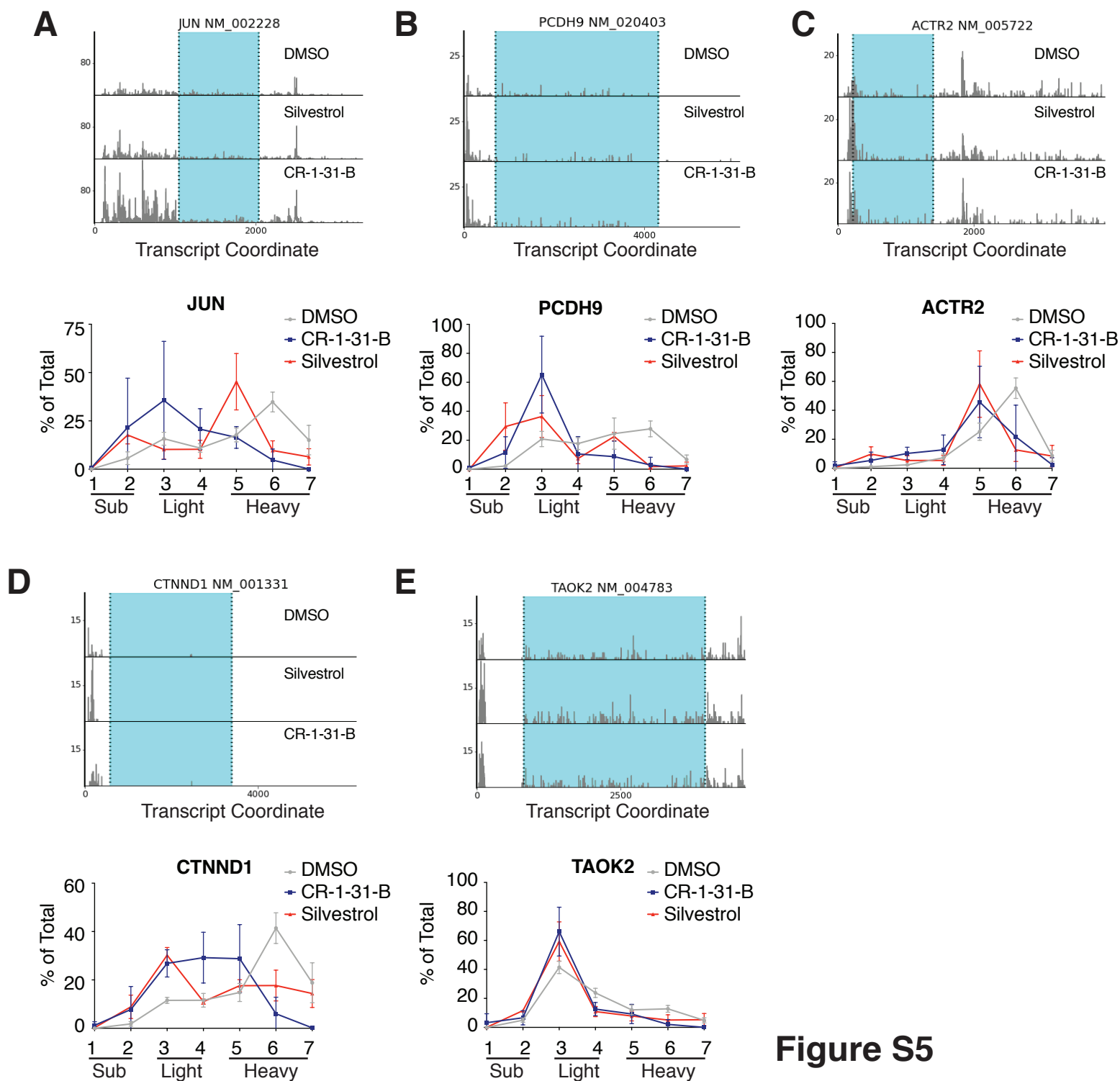


Figure S5

Figure S5. Relationship between increased eIF4A1 binding and translation inhibition (Related to Figure 2). (A-E) eCLIP read density tracks of select targets are shown as top panels. The corresponding distribution of these mRNAs across polysome profile in cells exposed to 20 nM rocaglate is also displayed ($n=3 \pm \text{SEM}$). Sub; sub-polysomal fractions, Light; light polysomes (1-2 ribosomes), heavy; heavy polysomes (≥ 3 ribosomes).