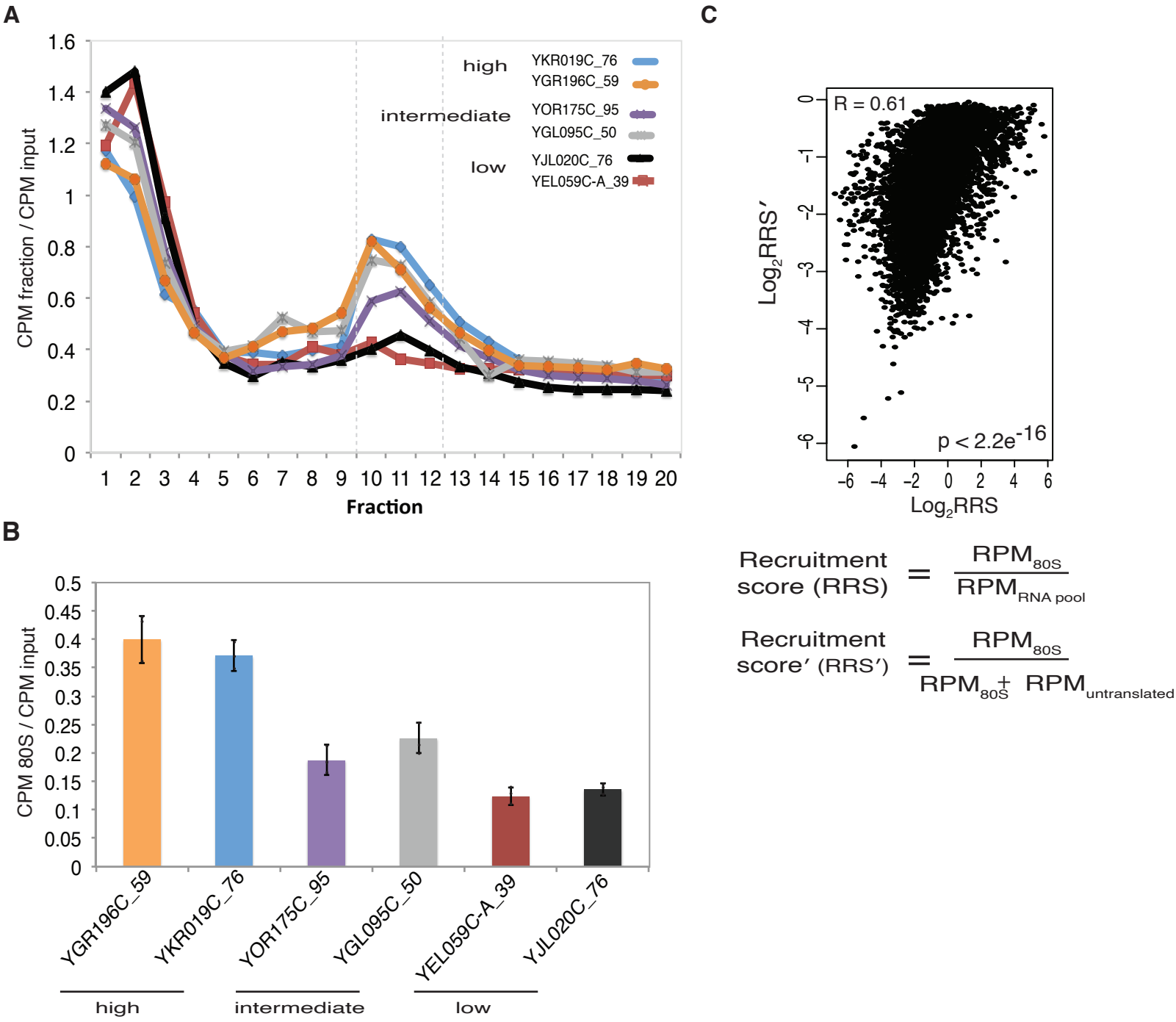
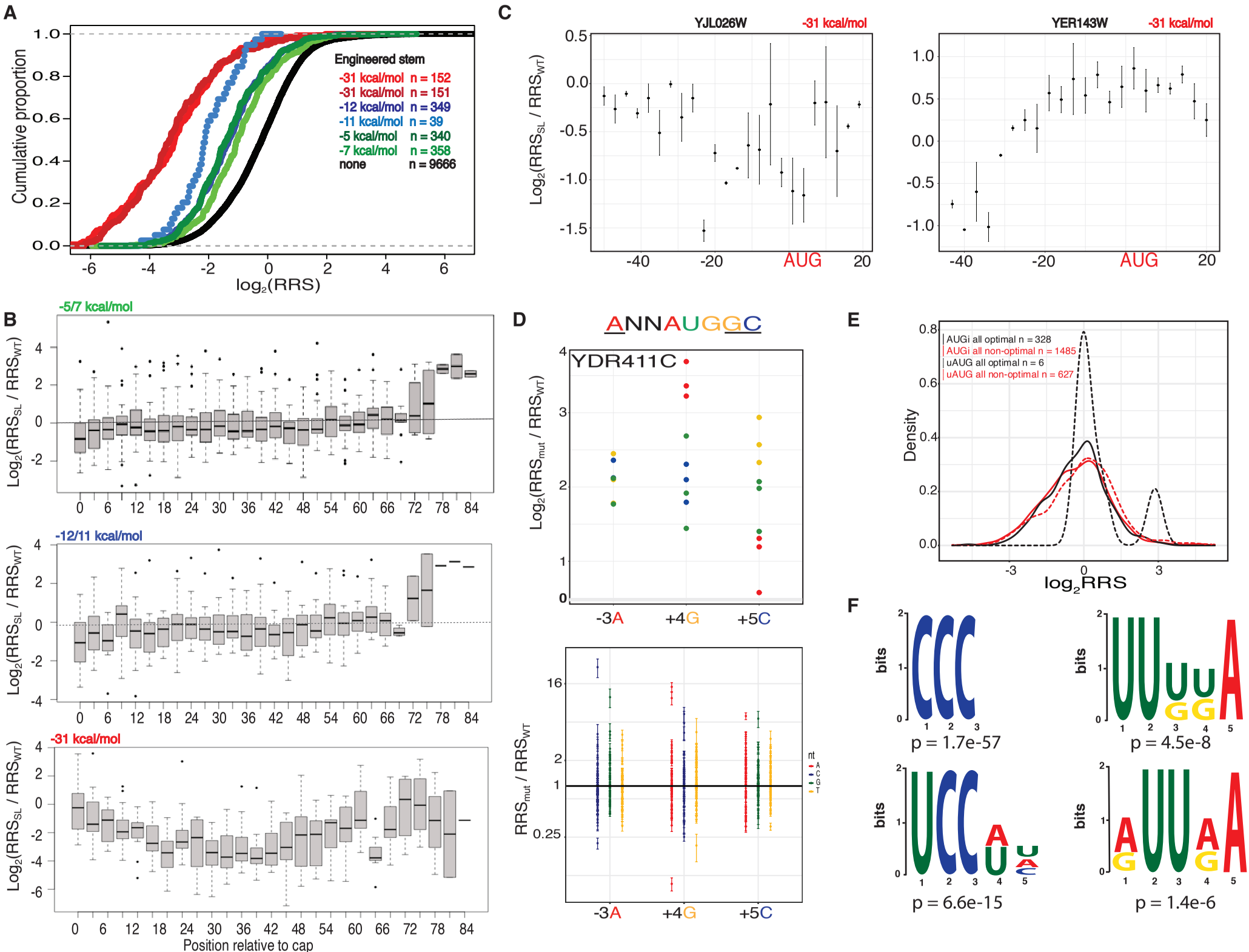
Supplementary Figure 1



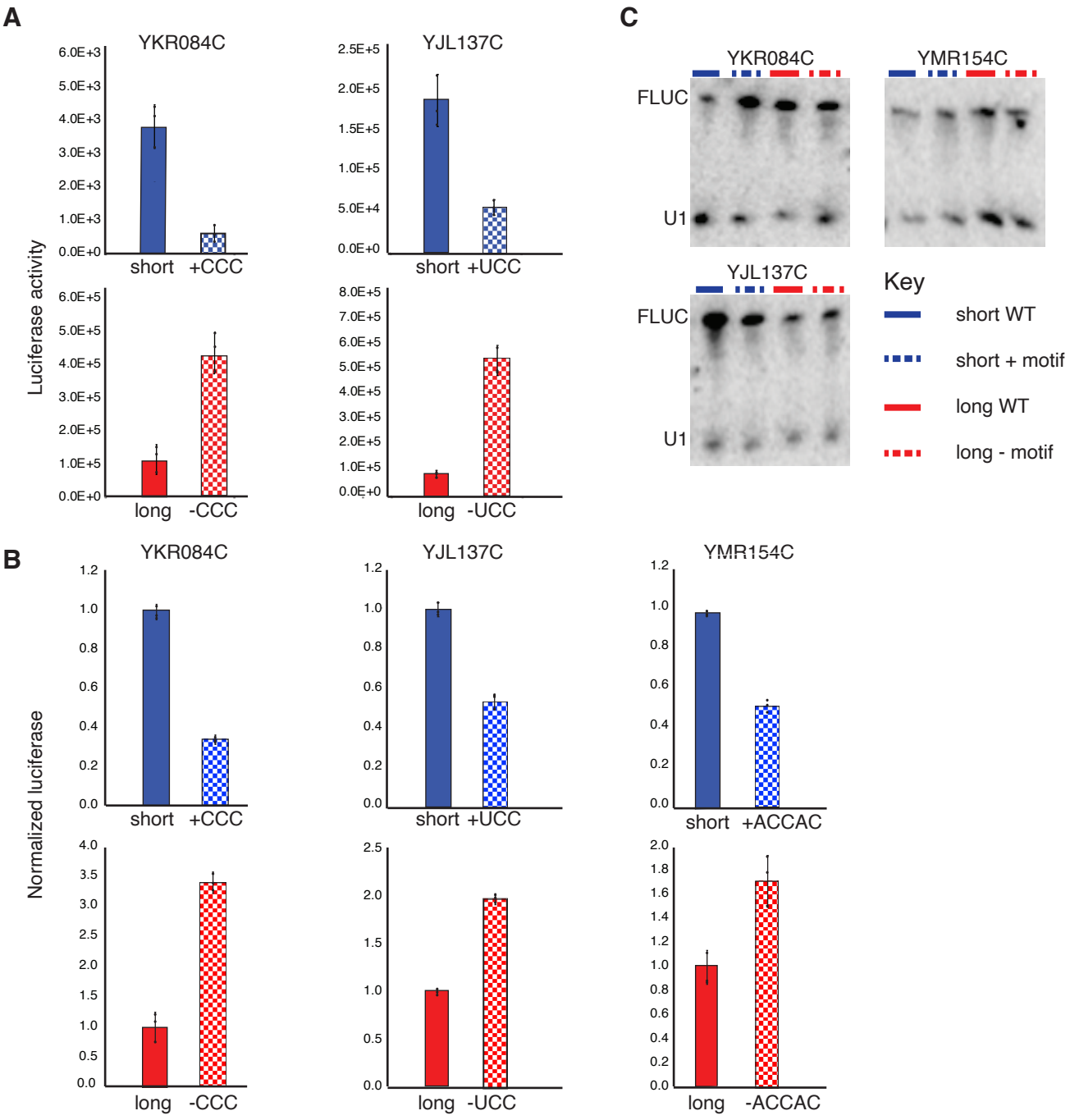
S1. DART validation by low-throughput assays and an alternative metric for ribosome recruitment activity, Related to Fig. 1. (A) 5′ UTR sequences spanning a range of RRS values high (YKR019C_76, 4.9; YGR196C_59, 5.0), intermediate (YOR175C_95, -0.03; YGL095C_50, -0.02), and low (YEL059C-A_39, -4.5; YJL020C_76, -4.5) were radiolabeled and incubated in yeast translation extract in the presence of cycloheximide. Reactions were loaded onto a sucrose gradient. (A) Counts in each fraction are reported relative to counts from 1/10th the input RNA. (B) Quantification of three independent replicates shown. (C) Alternative RRS calculation. Correlation between RRS calculation methods.

Supplementary figure 2



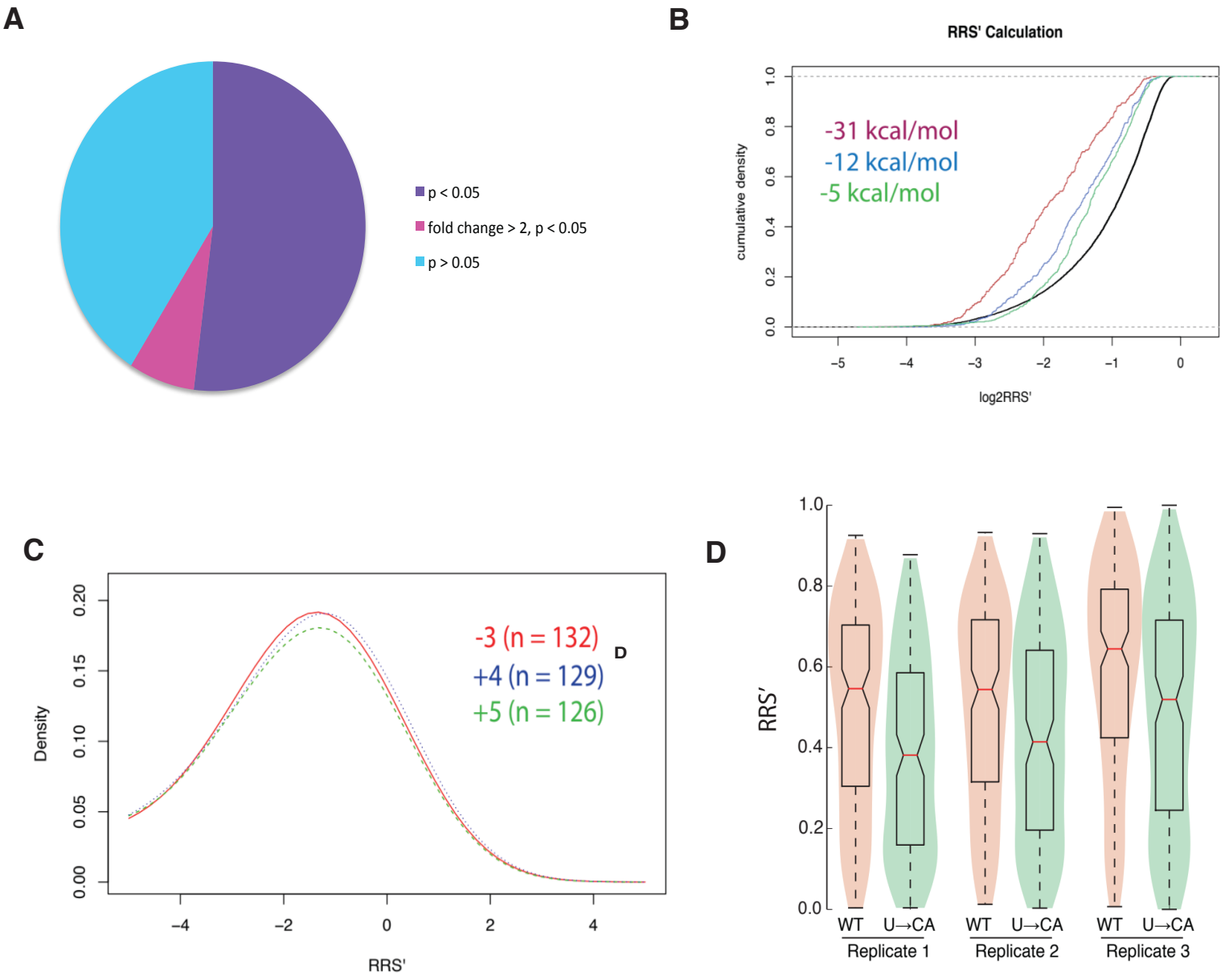
S2. 5′ UTRs show a range of responses to inhibitory elements, related to Fig. 2. (A) Stem loops with equivalent strength repress ribosome recruitment similarly. Reds show 5′ UTRs containing two different sequences with -31 kcal/mol stems. Blues show -11 and -12 kcal/mol stems. Greens show -5 and -7 kcal/mol stems. Black line shows 5′ UTRs without any artificial stems. (B), (C) Designed stems exhibit host 5′ UTR- as well as position-dependent effects on ribosome recruitment. (B) Box plots of RRS for three sets of engineered stem constructs relative to the parent 5′ UTRs with no SL. (C) Stem loops exhibit context-dependent effects on RRS. Ribosome recruitment relative to no stem by position for two individual 5′ UTR contexts (YJL026W and YER143W). (D) Two example 5′ UTRs from YDR411C and YIL052C respond differently to start codon context mutations. RRS values when the position is mutated relative to the RRS from the WT construct. Color indicates mutated nucleotide identity. (E) Comparison of ribosome recruitment between all 5′ UTRs with perfect AUGi context (-3A +4G +5C) and those with all non-optimal nucleotides at all three positions. (F) Bottom (left) and top (right) deciles of RRS for all endogenous 5′ UTRs were searched to identify enriched sequence motifs. Top 2 motifs are shown for each. P values calculated by Fisher's Test.

Supplementary Figure 3



S3. Identified C-rich motifs repress translation, related to Fig. 4. (A) 5′ UTR isoforms tested in vitro. Both UCC (left) and CCC (right) are sufficient to repress translation of luciferase reporter in vitro. (B) 5′ UTR isoforms tested in vivo. Luciferase values are normalized to relative firefly luciferase mRNA levels as monitored by Northern blot (below) and total protein.

Supplementary Figure 4



S4. 5′ UTR features show similar effects on ribosome recruitment when analyzed using alternative RRS′ calculations, related to Figs 1, 2, 3 and 4. (A) Stronger stems show lower RRS′ values. Compare to Fig. 2b. (B) Mutating conserved AUGi context nucleotides frequently reduces RRS′, although the effects are highly variable among the 50 5′ UTRs tested. Compare to Fig. 2e. (C) Mutating oligo(U) sequences, which were previously identified to bind eIF4G1, results in lower RRS′. Compare to Fig. 3b. (D) Most 5′ UTR isoforms tested significantly alter ribosome recruitment levels using the RRS′ calculation. Compare to Fig. 4b. RRS and RRS′ calculations as in Supplementary Fig. 1c.